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Chronic Amphetamine: Tolerance and Reverse Tolerance Reflect Different Behavioral Actions of the Drug

NANCY J. LEITH[1] AND RONALD KUCZENSKI

Department of Pharmacology, Vanderbilt University School of Medicine and Tennessee Neuropsychiatric Institute Nashville, TN 37217

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LEITH, N. J. AND R. KUCZENSKI. *Chronic amphetamine: Tolerance and reverse tolerance reflect different behavioral actions of the drug.* PHARMAC. BIOCHEM. BEHAV. 15(3) 399–404, 1981.—Chronic administration of amphetamine (AMPH) has been reported to produce tolerance to the drug's behavioral effects in some paradigms (self-stimulation, discriminative stimulus, self-administration) and an enhanced effect or reverse tolerance when other behaviors are monitored (locomotor activity, stereotypy). The present study investigated whether the two phenomena are, in fact, related to the particular behavior monitored or reflect the marked differences in the injection regimens (1X vs. 3X daily injections) used to produce the phenomena. The effects of chronic AMPH administered once or three times daily on AMPH facilitation of self-stimulation responding and on the locomotor stimulant and stereotypy-producing effects of the drug were assessed. Regardless of the injection regimen used, chronic AMPH resulted in an enhancement of the locomotor stimulant effects of the drug as well as a more rapid onset and greater intensity of the stereotypy produced. In the self-stimulation paradigm, only the 3X daily regimen significantly reduced the effectiveness of a challenge dose of AMPH (tolerance), although the 1X regimen produced effects that were qualitatively similar but quantitatively less. Perhaps behavioral tasks in which tolerance develops reflect the mood-altering properties of the drug in humans whereas a process similar to reverse tolerance may underlie the increased susceptibility to psychoses elicited by the drug with repeated use.

Amphetamine Tolerance and reverse tolerance Chronic administration Behavioral effects

A NUMBER of investigators [4, 5, 14, 18, 20, 22] have reported an enhanced response (reverse tolerance) to the locomotor stimulant and stereotypy-producing effects of amphetamine (AMPH) following chronic administration of that drug. More recently, chronic administration of AMPH has been shown to produce pharmacodynamic tolerance to the facilitating effects on self-stimulation responding [11,13], to the discriminative stimulus properties of the drug [1], and to the reinforcing effects of the drug demonstrated in a self-administration paradigm [15]. This tolerance is in contrast to the reports of other investigators (for example, [17,23]) studying various operant tasks in which the decreased drug effect seen following chronic administration is most parsimoniously interpreted as reflecting behavioral adaptation on the part of the animal to the initially disruptive effect of the drug [3].

Leith and Barrett [11,13] have contended that, in rats, the behavioral tasks in which pharmacodynamic tolerance has been demonstrated reflect the mood-altering properties of the drug in humans to which tolerance also develops. Those behavioral effects of AMPH to which reverse tolerance develops in rats are thought to reflect a different aspect of the drug's action in people, perhaps the ability of the drug to induce psychosis after chronic use [5,19]. Thus, delineating the physiological bases of AMPH's effects on behaviors to which tolerance or reverse tolerance develops would provide potentially important information concerning the mechanism of two major actions of the drug in people.

However, another possibility is that the two phenomena are the result of the markedly different injection schedules that have been used to produce them. Studies demonstrating reverse tolerance have used a wide range of doses but have typically involved single daily drug injections. On the other hand, our work demonstrating tolerance has used multiple daily injections of escalating doses. Therefore it is conceivable that tolerance and reverse tolerance reflect the consequences of these different injection regimens rather than relating to the particular behavior being monitored. The present experiments were undertaken to compare the two dosage regimens on the two types of behavior to determine which interpretation is correct. In addition, these experiments provide parametric behavioral data needed to enable choos-

[1]Send reprint requests to Nancy J. Leith, Ph.D., Tennessee Neuropsychiatric Institute, Central State Hospital, Cooper Bldg., 1501 Murfreesboro Road, Nashville, TN 37217.

ing behaviorally relevant dosage regimens and times after drug challenge for subsequent studies of biochemical changes that underlie the behavioral alterations.

METHOD

Subjects

The subjects were male, Sprague-Dawley rats obtained from Harlan Industries (Indianapolis, Indiana) and housed individually in standard laboratory conditions with constant access to food and water and with a 12 hr light-dark cycle (6:00 a.m.–6:00 p.m. light). All surgery and testing were done during the light phase of the cycle. The animals weighed 250–300 g at the time of testing in the activity studies or at the time of surgery for the brain stimulation studies.

Surgery

The animals were anesthetized with 50 mg/kg sodium pentobarbital and were given 0.2 mg/kg atropine sulfate to reduce respiratory difficulties. They were then mounted in the stereotaxic instrument and implanted with a bipolar electrode aimed at the medial forebrain bundle (MFB). The electrode consisted of two stainless steel wires 0.125 mm in diameter, twisted together and insulated except for the tip, and mounted in a threaded plastic top (purchased from Plastic Products Co., Roanoke, VA). The two tips were separated from each other by a distance of approximately 0.5 mm. The coordinates, corresponding to the König and Klippel [8] atlas, that were used were: 3.75 mm anterior to the interaural line, 1.2 mm lateral to the saggital suture, and 8.5 mm down from the top of the skull. Prophylactic Bicillin (150,000 units) was administered immediately after surgery and again 2 weeks later.

Apparatus—Brain Stimulation

For the self-stimulation study, the animals were tested in three grid-floored operant chambers (24 cm W×24 cm D×17.5 cm H) each equipped with a fluorescent houselight used to illuminate the chamber and a single telegraph key-type lever (5×1.2 cm) located 5 cm above the floor and requiring approximately 15 g of force through an excursion of 3 mm to make an effective bar press. In order to mask extraneous auditory stimuli, the test chambers were housed inside wooden outer chambers containing a speaker for presenting white noise. The behavioral contingencies were programmed using electromechanical equipment housed in an adjacent room.

Apparatus—Activity

Motor activity was monitored in 3 symmetrical Y-mazes with arms 28 cm L×18 cm W×21 cm H and an 18 cm equilateral triangular choice area. Each arm was constructed of black Plexiglas sides, 6 mm diameter stainless steel grid-floor bars spaced 1.9 cm apart on center and a black Plexiglas top. A bulb positioned behind an opaque Plexiglas screen at the end of each arm provided dim illumination of the maze. Photocells mounted 14 cm from the entrance of each arm were used to register an activity count each time the animal entered an arm. Breaking the photocell beam in that same arm could not register another count until the animal had first broken the beam in another arm. Thus, this activity measure reflects locomotion from one arm to another. The mazes were housed in a darkened room adjacent to the programming equipment and white noise was used in the experimental room to mask extraneous auditory stimuli.

Procedure—Brain Stimulation

One week following surgery, the animals were trained in four daily 1 hr sessions to bar press to receive 0.2 sec pulses of 60 Hz AC current. Beginning on the fifth day, the animals were tested during 50 min sessions at 15 current intensities using the following procedure. During the daily session, the intensity was decreased every 5 sec by 5% of the starting value until 15 intensities had been tested, at which point the current was automatically reset to the highest value and the houselight was turned on to signal the reset to the rat. This sequence was repeated continuously throughout the session and the cumulative number of responses at each intensity was recorded. Using this procedure, the rate of responding, when plotted as a function of current intensity, yields a characteristic and relatively stable response curve for each animal. As a rough index of the "reinforcement threshold," for each animal we calculated the current intensity at which responding decreased to 50% of the maximal rate. The highest current intensity was adjusted for each animal so that this 50% value occurred between steps 9 and 5 of the descending series of values, thereby maximizing the sensitivity of the paradigm to detect either increases or decreases in the threshold following our experimental manipulations.

After approximately two weeks of training with the step-down procedure, drug testing was begun. The animals were tested for two successive 25 min sessions with saline injected subcutaneously immediately prior to the first session and AMPH 0.3 mg/kg free base SC (S(+)-amphetamine sulfate obtained from Sigma Chemical Co., St. Louis, MO) immediately prior to the second session, enabling us to assess both baseline responding and drug response each day. The acute effects of this dose of AMPH, which we have found to be approximately the minimal dose that will produce reliable facilitation of responding in all animals, were tested on three successive days prior to the beginning of the chronic treatment. Based on the acute data, the animals were divided into two groups, approximately matched with respect to the maximal current intensities, maximal response rates, shape of the response rate-current intensity functions and magnitude of the drug response. Testing was terminated for 1 week during which one of two chronic drug regimens was administered. The 1X daily group (n=10) received 6 daily injections of 3 mg/kg AMPH free base. The 3X daily group (n=14) received 3 injections each day (8:00 a.m., 2:00 p.m., 8:00 p.m.) beginning on the 3rd day after the last behavioral session and continuing for 4 days. The first dosage was 1 mg/kg AMPH salt and the dosage was increased by 1 mg/kg at each injection with the final dose on the 4th day being 12 mg/kg. For both chronic groups, 2 days after the last injection all animals were again tested for 3 days in an identical manner to that used for assessment of acute drug effects to determine whether the chronic treatment had produced changes in baseline responding and in response to a challenge dose of AMPH 0.3 mg/kg.

Procedure—Activity

The activity of separate groups of rats (n=12) was monitored for 3 hr following the acute administration of 1.0, 2.0 or 3.0 mg/kg AMPH free base. Half of each dosage group then received chronic AMPH administered either 1× or 3× daily according to the regimens detailed above. Two days after the

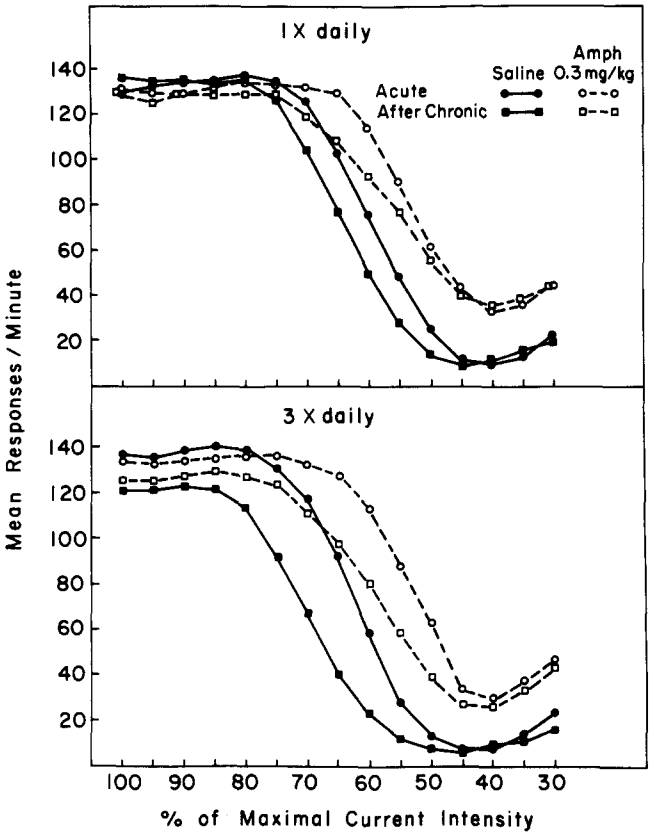

FIG. 1. Changes in self-stimulation behavior and in the effect of AMPH on that behavior as the result of chronic treatment with 3 mg/kg daily for 6 days (1X group) or increasing doses (1–12 mg/kg salt) for 4 days (3X group). Current intensity was decreased by 5% of the starting value every 5 sec for 15 steps and then was automatically reset to the starting value. Each curve represents the mean responding for 3 days prior to or following chronic treatment.

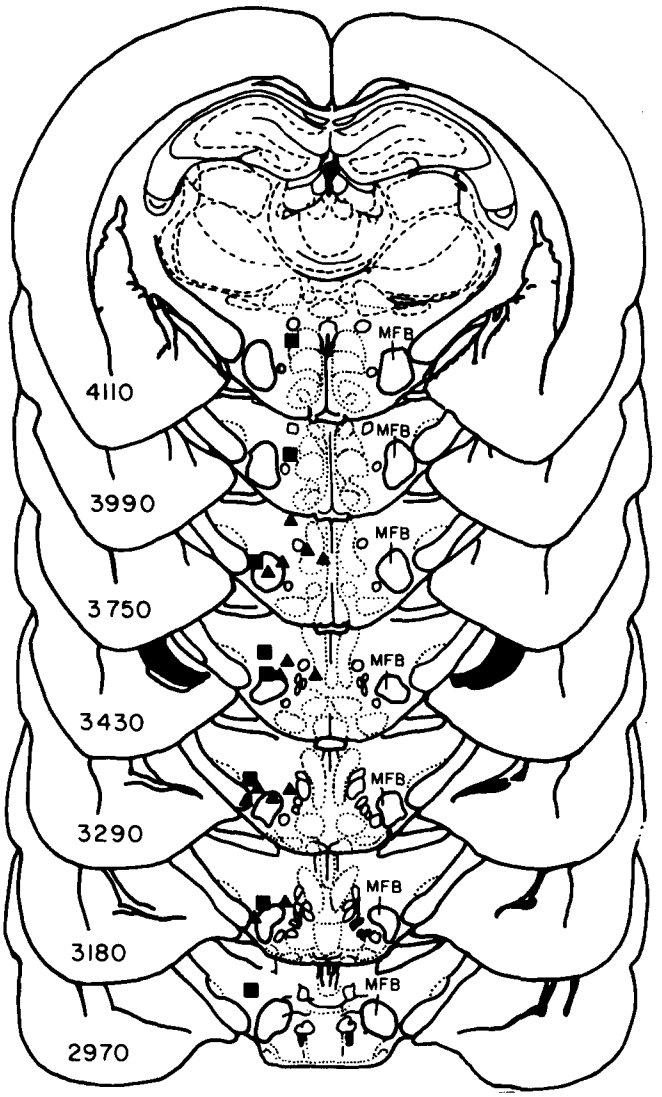

FIG. 2. Diagram of coronal sections of the rat brain indicating the approximate location of the electrodes. Squares designate placements of the 1X group and triangles placements of the 3X group. The numbers on the left of the sections correspond to the plate numbers from the atlas of König and Klippel [8]. MFB=medial forebrain bundle.

last injection, the rats were again administered their test dose of the drug and activity was monitored for 3 hr.

Histology

Following the completion of brain stimulation testing, all animals were given an overdose of sodium pentobarbital and perfused intracardially with 60 ml 0.9% saline followed by 100 ml of 10% Formalin. The brains were removed and stored in Formalin for several days prior to being blocked in the plane of the electrode track. The brain section containing the track was then frozen with liquid CO_2 and 60 μ sections were made using a freezing microtome. Photomicrographs, made directly from these slices, were used to locate the electrode placements on plates taken from the König and Klippel [8] atlas without knowledge of the behavioral results obtained.

Statistical Analyses

For the brain stimulation portion of the study, the baseline and drug response data were separately averaged for the last 3 sessions prior to chronic treatment and the first 3 sessions following. These data then were analyzed using a 2 (1X vs 3X) × 2 (SAL vs AMPH) × 2 (Acute vs Chronic) × 15 (current intensities) analysis of variance. Similarly, the activity data were analyzed using a 2 (1X vs 3X) × 3 (Doses) × 2 (Acute vs Chronic) × 15 (12 min intervals) analysis of variance.

RESULTS

The effects of acute and chronic AMPH on self-stimulation responding are presented in Fig. 1. Each curve, in which response rates are plotted as a function of current intensity, represents the group mean of 3 days prior to or following chronic AMPH administration. Statistical analysis showed that there was a significant interaction between the particular injection regimen and the effects of chronic AMPH, $F(1,22)=4.6, p<0.05$. That is, only with the 3X daily regimen was there a significant decrease in baseline responding and in the response to AMPH, although the 1X daily

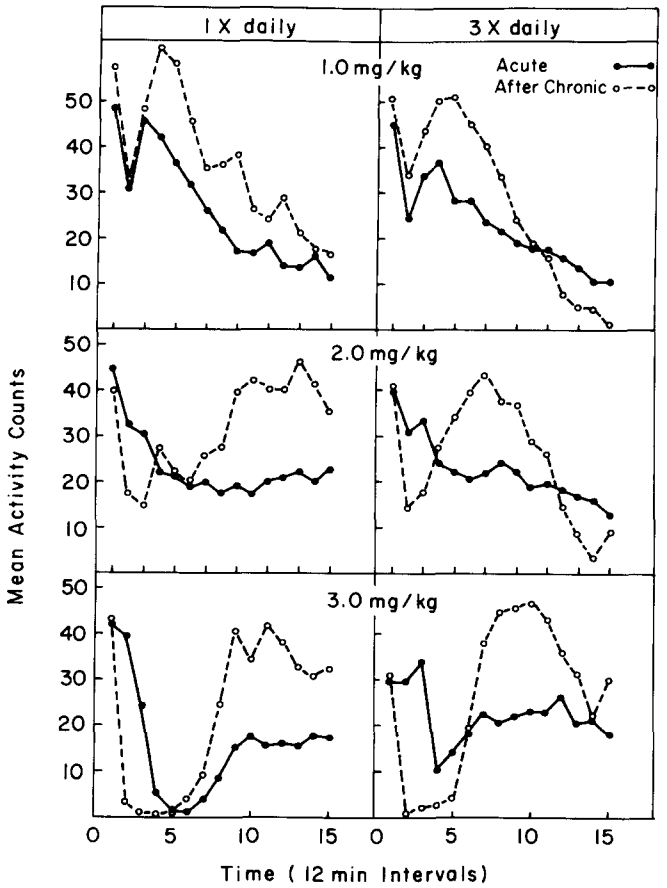

FIG. 3. Effects of 3 doses of AMPH on a measure of locomotor activity following acute injection or chronic treatment with 3 mg/kg daily for 5 days (1X group) or increasing doses (1–12 mg/kg salt) for 4 days (3X group).

regimen produced qualitatively similar changes but of a lesser magnitude. In addition, there was a significant difference between baseline and drug responding, $F(1,22)=49.1$, $p<0.001$, a significant effect of current intensity, $F(14,308)=56.1$, $p<0.001$, and a significant interaction of current intensity with the drug effect, $F(14,308)=2.6$, $p=0.002$. This interaction reflects the fact that AMPH produces little or no change in responding at the highest current intensities but markedly increases responding at intensities that are only marginally reinforcing in the absence of the drug. Figure 2 presents the histological verification of the location of the electrodes and shows that there were no notable differences in the placements of the two treatment groups. Two brains from the 1X group were damaged prior to obtaining histological data.

The effects of acute and chronic AMPH on locomotor activity as a function of time and dosage regimen are presented in Fig. 3. Both treatment regimens resulted in significant changes in response to a challenge dose of AMPH after chronic treatment, $F(1,30)=12.5$, $p=0.002$, and these changes interacted with dose and time, $F(28,420)=4.7$, $p<0.001$, and with regimen and time, $F(14,420)=4.3$, $p<0.001$. As can be seen in Fig. 3, following chronic treatment, there was a marked increase in locomotor activity with the 1 mg/kg challenge dose. With 2 mg/kg, there was an initial decrease in activity, indicative of mild stereotypy, followed by marked hyperactivity. With 3 mg/kg, which produces decreased activity (i.e., stereotypy) following acute administration, there was more rapid onset of the stereotypy and the period of intense stereotypy that is reflected in the near zero levels of locomotor activity was followed by a period of marked hyperactivity. Since our measure of stereotypy in this study was an indirect one, i.e., absence of locomotion, we periodically observed the animals during periods of zero activity to confirm that they were in fact staying in one arm of the maze and engaging in intense and persistent head bobbing and sniffing. The two injection regimens produced qualitatively similar effects with two exceptions. At each dose, the duration of AMPH's action following the 3X regimen was significantly shortened. In addition, with the 2 and 3 mg/kg doses, the onset of the post-stereotypy hyperactivity occurred at an earlier time point in the 3X daily groups.

DISCUSSION

The present data replicate the previously reported findings that, following chronic administration of AMPH, tolerance develops to the facilitating effects of AMPH on self-stimulation responding [11,13] whereas an enhanced drug effect or reverse tolerance is seen to the locomotor stimulant and stereotypy-producing effects of the drug [18,20]. In the latter case, the pattern of changes as a function of dose and time is nearly identical to that originally reported by Segal and his co-workers [2, 18, 20]. Furthermore, these opposite kinds of changes that occur in the two behavioral tasks are not related to the particular pattern of chronic administration (1X vs 3X daily) that had been used in previous studies. This conclusion is warranted since a direct comparison of the two regimens in the two behavioral tasks showed that the critical factor determining whether tolerance or reverse tolerance is seen is the behavior monitored, not the dosage regimen. In fact, we were surprised that the two regimens produced such strikingly similar qualitative effects despite the marked difference in the total amount of drug the two groups received (18 mg/kg vs 59 mg/kg free base).

Although the particular dosage regimen used in the present study was not critical in determining the qualitative changes that chronic AMPH produces, it did affect the quantitative aspects of the changes. Thus, the 1X daily regimen did not produce a statistically significant change in self-stimulation responding, although the trend was in the same direction as that seen with the 3X regimen. Presumably the greater effect of the 3X regimen is related to the greater amount of drug which that group received rather than the multiple daily dosage aspect of the regimen, since we have shown in other work [12] that similar changes can be produced with single daily injections of higher doses (5–10 mg/kg). However, it should be noted that recent work reported by Kokkinidis and Zacharko [6,7] has demonstrated reverse tolerance rather than tolerance to AMPH in a self-stimulation paradigm. Their first report demonstrating such sensitization was in animals with stimulating electrodes in the substantia nigra, suggesting that the differences between their data and our repeated findings of tolerance [11,13] might be related to differences in electrode placements. But these authors have now reported sensitization to AMPH effects with electrode placements comparable to ours in the lateral hypothalamus [6]. At this point, the most reasonable

explanation to account for the differences between their work and ours is that Kokkinidis and Zacharko [6] administered high doses of AMPH (2×7.5 mg/kg/day) for considerably longer periods of time (15 days) than we did, suggesting that with some dosage regimens, qualitative changes in the effects of chronic AMPH can be observed. It does seem likely, though, that the sensitization observed by Kokkinidis and Zacharko [6,7] in a self-stimulation paradigm is unrelated to the reverse tolerance seen in the present work which occurs with much lesser amounts of chronic drug treatment.

In the case of the locomotor activity data there were also some differences between the two regimens. Most notable is the shortened duration of action of the drug effect seen in the 3X daily group. A similar effect obtained with multiple daily injections has recently been reported by Segal *et al.* [21] suggesting that it is the multiple daily injection aspect of the regimen rather than the total dosage that is important for this behavioral change. Additionally, the 3X group showed a more rapid onset of post-stereotypy hyperactivity (at the 2 and 3 mg/kg doses) than did the 1X group, an effect also reported by Segal *et al.* [21]. In this case, however, other unpublished data from our laboratories suggests that the total amount of drug is the important factor. Thus, a comparison of animals that received 6 vs 9 daily injections of 3 mg/kg showed that the only difference between the two groups was a more rapid onset of post-stereotypy hyperactivity in the 9 injection group.

Our data, as well as that of Nelson and Ellison [16], indicate that, depending on the particular regimen administered, a variety of behavioral changes occurs following chronic AMPH. However, whether these changes, in general, resemble tolerance or reverse tolerance depends primarily on the particular behavior monitored. Despite the fact that AMPH facilitates a wide variety of behaviors, including locomotor activity and self-stimulation responding, clearly the brain mechanisms mediating the facilitation of the two types of behaviors must be different since they respond to chronic AMPH in opposite ways. Leith and Barrett [11,13] have previously suggested that those behavioral tasks in which tolerance develops following chronic AMPH (self-stimulation, discriminative stimulus and self-administration) are ones which reflect the mood-altering properties of AMPH to which tolerance also develops in people [9]. Additional support for this suggestion comes from the finding in the present study as well as in previous work [11,13] that, following termination of the chronic AMPH treatment, there is a marked depression of self-stimulation responding, reflected primarily as an elevation of the reward threshold, perhaps corresponding to the withdrawal depression experienced by humans [24]. In fact, it seems likely that the elevation of baseline reward thresholds is the major factor underlying the finding that the drug did not reduce the threshold to the same absolute level after chronic treatment as after acute (see [13] for further discussion of this point).

Segal and co-workers [18–21], on the other hand, have suggested that the reverse tolerance seen with AMPH may be related to the development of amphetamine psychosis in humans since the human phenomena occurs after chronic administration of the drug and seems to reflect a heightened sensitivity to the drug. Thus, determining the brain mechanisms mediating tolerance and reverse tolerance should provide important information concerning the effects of AMPH in humans. The present study provides the basic parametric behavioral data necessary to determine the appropriate dosage regimens and times after drug challenge for subsequent biochemical studies aimed at determining the bases for the various behavioral changes. The first of such biochemical data are reported by Kuczenski and Leith in the next paper in this volume.

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REFERENCES

1. Barrett, R. J. and N. J. Leith. Tolerance to the discriminative stimulus properties of d-amphetamine. *Neuropharmacology* **20:** 251–255, 1981.
2. Browne, R. G. and D. S. Segal. Metabolic and experiential factors in the behavioral response to repeated amphetamine. *Pharmac. Biochem. Behav.* **6:** 545–552, 1977.
3. Campbell, J. C. and L. S. Seiden. Performance influence on the development of tolerance to amphetamine. *Pharmac. Biochem. Behav.* **1:** 703–708, 1973.
4. Hitzemann, R. J., L. F. Tseng, B. A. Hitzemann, S. Sampath-Khanna and H. H. Loh. Effects of withdrawal from chronic amphetamine intoxication on exploratory and stereotyped behaviors in the rat. *Psychopharmacology* **54:** 295–302, 1977.
5. Klawans, H. L. and D. I. Margolin. Amphetamine-induced dopaminergic hypersensitivity in guinea pigs. *Archs gen. Psychiat.* **32:** 725–732, 1975.
6. Kokkinidis, L. and R. M. Zacharko. Enhanced lateral hypothalamic self-stimulation responding after chronic exposure to amphetamine. *Behav. Neural Biol.* **29:** 493–497, 1980.
7. Kokkinidis, L. and R. M. Zacharko. Response sensitization and depression following long-term amphetamine treatment in a self-stimulation paradigm. *Psychopharmacology* **68:** 73–76, 1980.
8. König, J. F. R. and R. A. Klippel. *The Rat Brain.* Baltimore: Williams and Wilkins, 1963.
9. Kosman, M. E. and K. R. Unna. Effects of chronic administration of the amphetamines and other stimulants on behavior. *Clin. Pharmac. Ther.* **9:** 240–254, 1968.
10. Kuczenski, R. and N. J. Leith. Chronic amphetamine: Is dopamine a link in or a mediator of the development of tolerance and reverse tolerance? *Pharmac. Biochem. Behav.* **15:** 405–413, 1981.
11. Leith, N. J. and R. J. Barrett. Amphetamine and the reward system: Evidence for tolerance and post-drug depression. *Psychopharmacologia* **46:** 19–25, 1976.
12. Leith, N. J. and R. J. Barrett. Effects of chronic amphetamine or reserpine on self-stimulation responding: Animal model of depression? *Psychopharmacology* **72:** 9–15, 1980.
13. Leith, N. J. and R. J. Barrett. Self-stimulation and amphetamine: Tolerance to d and l isomers and cross tolerance to cocaine and methylphenidate. *Psychopharmacology*, 1981, in press.
14. Magos, L. Persistence of the effect of amphetamine on stereotyped activity in rats. *Eur. J. Pharmac.* **6:** 200–201, 1969.

15. McCown, T. and R. J. Barrett. Development of tolerance to the rewarding effects of self-administered S(+)-amphetamine. *Pharmac. Biochem. Behav.* **12:** 137–141, 1980.
16. Nelson, L. R. and G. Ellison. Enhanced stereotypies after repeated injections but not continuous amphetamines. *Neuropharmacology* **17:** 1081–1084, 1978.
17. Schuster, C. R. and J. Zimmerman. Timing behavior during prolonged treatment with *dl*-amphetamine. *J. exp. Analysis Behav.* **4:** 327–330, 1961.
18. Segal, D. S. Behavioral and neurochemical correlates of repeated d-amphetamine administration. In: *Neurobiological Mechanisms of Adaptation and Behavior, Advances in Biochemical Psychopharmacology, Vol. 13,* edited by A. J. Mandell. New York: Raven Press, 1975, pp. 247–262.
19. Segal, D. S. and D. S. Janowsky. Psychostimulant-induced behavioral effects: Possible models of schizophrenia. In: *Psychopharmacology: A Generation of Progress*, edited by M. A. Lipton, A. DiMascio and K. F. Killam. New York: Raven Press, 1978, pp. 1113–1123.
20. Segal, D. S. and A. J. Mandell. Long-term administration of amphetamine: Progressive augmentation of motor activity and stereotypy. *Pharmac. Biochem. Behav.* **2:** 249–255, 1974.
21. Segal, D. S., S. B. Weinberger, J. Cahill and S. J. McCunney. Multiple daily amphetamine administration: Behavioral and neurochemical alterations. *Science* **207:** 904–907, 1980.
22. Short, P. H. and L. Schuster. Changes in brain norepinephrine associated with sensitization to d-amphetamine. *Psychopharmacology* **48:** 59–67, 1976.
23. Sparber, S. B. and H. A. Tilson. Tolerance and cross-tolerance to mescaline and amphetamine as a function of central and peripheral administration. *Psychopharmacologia* **23:** 220–230, 1972.
24. Watson, R., E. Hartmann and J. J. Schildkraut. Amphetamine-withdrawal: Affective state, sleep patterns, and MHPG excretion. *Am. J. Psychiat.* **129:** 263–269, 1972.