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Behavioral sensitization: Characterization of enduring changes in rotational behavior produced by intermittent injections of amphetamine in male and female rats

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Abstract. Factors influencing the behavioral sensitization ("reverse tolerance") produced by intermittent amphetamine (AMPH) injections were studied by quantifying rotational behavior in rats that had a unilateral 6-hydroxydopamine lesion of the substantia nigra. The results indicate that: (1) a single injection of a low dose of AMPH enhances rotational behavior induced by a second injection of AMPH for up to 12 weeks; (2) multiple, weekly injections of AMPH produce a progressive enhancement in rotational behavior, over-and-above that produced by a single injection; (3) female rats show more robust sensitization than males following single or multiple injections of AMPH; (4) this sex difference may be due to the suppression of sensitization by an androgen, because removal of testicular hormones potentiates sensitization; (5) the long-lasting sensitization of rotational behavior produced by infrequent injections of AMPH is not due to drug-environment conditioning effects, but perhaps to a persistent AMPH-induced change(s) in brain catecholamine systems; and (6) a simple change in DA receptors is probably not involved, because the sensitization produced by infrequent injections of AMPH does not influence the rotation produced by a subsequent injection of apomorphine. The results illustrate an intriguing example of neuroplasticity that may have clinical relevance.

Key words: Rotational behavior – Amphetamine – Apomorphine – Sensitization – Sex differences – Gonadal hormones – Reverse tolerance – 6-Hydroxydopamine – Amphetamine psychosis – Conditioning – Dopamine – Rat

Introduction

An unusual feature of psychomotor stimulant drugs is that some of their behavioral effects are progressively enhanced with repeated administration; a phenomenon known as sensitization or "reverse tolerance" (Klawans and Margolin 1975; Magos 1969; Segal and Mandell 1974; Wallach and Gerson 1971). In the majority of studies on behavioral sensitization, drugs such as amphetamine (AMPH) have been repeatedly administered and changes in the onset, vigor and/or duration of stereotyped behavior are determined (see Creese 1983 and Post 1981 for recent reviews). However, AMPH produces many different behaviors (e.g., Ungerstedt 1979), and some of these show sensitization, whereas others do not (e.g., Post 1981). It has recently been demonstrated, for example, that not even all the behavioral components of stereotypy show sensitization (Eichler et al. 1980; Rebec and Segal 1980).

One reason that not all AMPH-induced behaviors show sensitization may be because AMPH influences a number of functionally heterogeneous forebrain dopamine (DA) terminal fields (e.g., Iversen 1977; Ungerstedt 1979); and these presumably differ in their response to repeated AMPH (Eichler et al. 1980; Rebec and Segal 1980). Therefore, in attempting to relate changes in discrete neural structures to behavioral sensitization it will be important to know: (1) which neural locus is primarily responsible for the behavior under study, and (2) to what extent the characteristics of behavioral sensitization vary as a function of the behavior under study (i.e., the individual components of stereotypy, locomotion, wall climbing, rotation etc.).

We probably know more about the neural systems responsible for AMPH-induced rotational behavior than for any other DA-mediated behavior (Pycock 1980; Ungerstedt 1971; Ungerstedt and Arbuthnott 1970). For example, it is known that stereotypy and rotational behavior are mediated by different, albeit overlapping, neural systems (Iversen 1977; Ungerstedt 1979). It is therefore surprising that there have been no previous studies on the sensitization of rotational behavior produced by intermittent injections of AMPH in rats with unilateral 6-hydroxydopamine (6-OHDA) lesions of the nigrostriatal system (i.e., using the standard "Ungerstedt" model; Ungerstedt and Arbuthnott 1970). To my knowledge, there have only been four previous studies on the sensitization of AMPH-induced rotation; three with mice (Echols 1977, 1979; Jenner et al. 1978), and one with unlesioned rats (Robinson et al. 1982a). To extend our knowledge of behavioral sensitization, it seemed important to thoroughly characterize the effects of repeated AMPH administration on rotational behavior in rats with a unilateral 6-OHDA lesion. Therefore, studies are reported here on the influence of single vs multiple injections, inter-test interval, sex, gonadal hormones, and conditioning on the sensitization of AMPH-induced rotational behavior, and on the consequences of AMPH sensitization on rotation evoked by apomorphine.

Materials and methods

Subjects

The experiments were conducted with male and female Holtzman (Sprague-Dawley derived) rats (Holtzman Co.,

Madison, WI). All animals (200–300 g at time of surgery) were housed individually in wire-hanging cages with free access to food and water. The animal room was maintained on a reversed light : dark cycle (14 : 10 h) with the lights going off at 06:30 h. All testing was conducted during the "night" portion of the rats' day/night cycle.

Surgical procedures

Thirty minutes after an injection of 15–25 mg/kg desipramine (Merrell-Dow Pharmaceuticals; Breese and Traylor 1971) each rat was anaesthetized with sodium pentobarbital, supplemented with methoxyflurane. A 30-gauge stainless steel cannula was lowered into the right rostral zona compacta of the substantia nigra using standard stereotaxic procedures (coordinates: posterior to bregma, 5.0 mm; lateral to the saggital suture, 2.0 mm; ventral to the skull surface, 7.3 mm – with bregma and lambda horizontal). The cannula was connected to a 10-μl syringe by PE10 tubing filled with a solution of 6-hydroxydopamine hydrobromide (6-OHDA) dissolved in 0.9% saline (0.1 mg/ml ascorbic acid was added to retard oxidation). Each animal received 6–8 μg 6-OHDA in a volume of 4 μl over a 5–8-min infusion period. The cannula was left in place for 2 min following the infusion to minimize diffusion up the cannula track. The animals were allowed to recover from surgery for at least 4 weeks before any behavioral testing began.

Procedures for recording rotational behavior

Rotational behavior was measured in circular automated rotometers similar in principle to those described by Greenstein and Glick (1975). They had a diameter of 30 cm, and flat bottom floors lightly covered with sawdust. The rotometers were interfaced with a microprocessor (AIM-65) that recorded every quarter turn (90°) made to the right or left during each 5-min interval. One complete rotation was defined as four *consecutive* 90° turns in the same direction.

Experimental design and protocol

Experiment 1: Influence of inter-test interval in male and female rats. Following recovery from surgery, 68 female and 70 male rats were tested for AMPH-induced rotational behavior. Each rat was placed in a rotometer, and following a 15-min habituation period received an IP injection of *d*-amphetamine sulfate dissolved in 0.9% saline. Rotational behavior was then recorded for the next 2 h. Half of the animals received a relatively low dose of AMPH (1.0 mg/kg for males and 0.65 mg/kg for females) and half received a higher dose (3.0 mg/kg for males and 2.6 mg/kg for females). Different doses of AMPH were used for males and females because sex differences in liver microsomal enzymes result in higher brain levels of AMPH in females given the same dose of AMPH as males (Becker et al. 1982; Conney 1967; Meyer and Lytle 1978). In previous studies we measured [^{3}H] AMPH levels in whole brain and striatum of male and female rats after various doses of AMPH, and at different points in time after injection (Becker et al. 1982). On the basis of these studies we are confident that the doses used here result in equivalent brain and striatal levels of AMPH in male and female rats.

Following this initial test session the animals were placed back into their home cage and left undisturbed until they were tested for rotational behavior a second time. This second test session was conducted either 24 h, 7 days, 30 days, or 12 weeks (84 days) following the first session (all independent groups), using exactly the same procedures as in the 1st session (e.g., each rat received the same dose of AMPH/kg in each test session).

Experiment 2: Single vs multiple injections. To compare the sensitization produced by single vs multiple injections of AMPH, the animals tested 24 h apart received three additional tests for rotational behavior, each separated by 24 h; and the animals with 7 days between the first two tests also received three additional tests, but with 7 days between each test session. The animals were left undisturbed in their home cages between test sessions.

Experiment 3: Influence of gonadal hormones. To determine the influence of endogenous gonadal hormones on the development of behavioral sensitization, 17 female and 16 male rats received 6-OHDA lesions, as described above. Two weeks later they were gonadectomized under ether anaesthesia and allowed to recover for an additional 2 weeks. These animals were then tested weekly for AMPH-induced rotational behavior for 4 weeks. The same dose of AMPH (3.0 mg/kg) was used in gonadectomized males and females because Becker (unpublished studies; cf Becker et al. 1982) has found that there is no sex difference in the brain levels of AMPH produced by a systemic injection in gonadectomized rats.

Experiment 4: Role of conditioning. Two different experiments were conducted to examine the role of conditioning on the sensitization of rotational behavior by AMPH. Ovariectomized (OVX) female rats were used in both experiments because: (1) Expt. 3 and previous studies (Robinson et al. 1982a) established that OVX females show the same robust sensitization as intact females; and (2) it seemed likely that OVX females would show less inter-test variability than intact cycling females (Becker et al. 1982). The first "conditioning-control" experiment (Expt. 4a) was designed to determine if rotational behavior could be conditioned with relatively infrequent (weekly) injections of AMPH. All animals received 6-OHDA lesions, as described above. Two weeks later they were OVX, and then allowed an additional 2 weeks to recover before being randomly assigned to one of two groups: (1) Animals in the "conditioned" group ($n = 14$) were placed in the rotometers, allowed 15 min to habituate, and then received an IP injection of 3.0 mg/kg AMPH. Rotational behavior was recorded for 2 h. This procedure was repeated weekly for a total of 4 weeks, but on the 4th week each animal received 0.9% saline instead of AMPH. (2) Animals in the control group ($n = 16$) were treated exactly the same as above except they received an injection of saline during each of the four weekly test sessions.

For the second conditioning-control experiment (Expt 4b) animals were surgically prepared, as described above, and then after 4 weeks of recovery were randomly assigned to one of three groups: (1) Animals in the "sensitization" group ($n = 12$) were tested for AMPH-induced (3.0 mg/kg) rotational behavior during four weekly test sessions, as described above. (2) The "saline control" animals ($n = 16$) were treated the same, except they received saline during the first 3 weekly test sessions, and 3.0 mg/kg AMPH

during the fourth. (3) During the first 3 weekly test sessions "pseudoconditioned animals" ($n = 15$) were placed into the rotometers for 15 min, before receiving an IP injection of saline. After 2 h they were removed from the rotometers, given an IP injection of 3.0 mg/kg AMPH, and placed in their home cages until the next test session. During the fourth test session these animals received 3.0 mg/kg AMPH in the rotometer, and rotational behavior was recorded. Thus, all the animals in this experiment received the same treatment during the fourth test session, but differed in terms of their prior history of drug-rotometer pairing.

Experiment 5: Cross-sensitization to apomorphine. This experiment was conducted to determine if the sensitization of rotational behavior to AMPH influences the rotation induced by a subsequent injection of a DA-receptor agonist, apomorphine (APO). OVX female rats were used for the reasons cited above. All animals received a unilateral 6-OHDA lesion of the substantia nigra, as described above. Two weeks later they were OVX and then allowed an additional 2 weeks to recover before being randomly assigned to one of two groups. Animals in the sensitization group received an IP injection of 3.0 mg/kg AMPH every 3–4 days for a total of five injections. Following each injection of AMPH, rotational behavior was recorded for 1 h, as described above. Animals in the saline-control group were treated exactly the same, but received injections of saline instead of AMPH. Starting 1 week after the last AMPH or saline injection, all rats were tested for APO-induced rotation on four different occasions (3–4 days apart), with four different doses of APO (30, 60, 240, and 480 μg/kg). APO was injected subcutaneously in the neck, and the order in which different doses were administered was counterbalanced so that approximately equal numbers of rats received each dose on each test day. Rotational behavior was recorded for 1 h.

Neurochemical methods and procedures. At least 1 week following all behavioral testing the animals were killed by decapitation and the left and right striatum dissected on ice, weighed and placed in tubes containing 400 μl 0.05 N $HClO_4$, with dihydroxybenzylamine (2.5 ng/10 μl) added as an internal standard. The tissue was prepared for determination of DA concentrations using an alumina extraction procedure adapted from Felice et al. (1978), and described by Castañeda et al. (1984). The concentration of DA in the left and right striatum was determined using high performance liquid chromatography with electrochemical detection, as described previously (Castañeda et al. 1984).

Statistical tests. The data were analyzed with one or two-way analyses of variance, or *t*-tests, using software (Stats Plus and ANOVA II) obtained from Human Systems Dynamics (Northridge, CA).

Results

Neurochemistry

For Experiments 1–4 only data from rats that had at least an 80% depletion of right striatal DA (relative to the left) and turned ipsiversive were analyzed (mean DA depletion ± SD = 95.5 ± 4.6%). This was done to reduce variation in rotation rate due to differences in the extent of the 6-OHDA lesion, and to variation in the side of the lesion relative to the "dominant" hemisphere for rotational behavior (Robinson and Becker 1983). Unfortunately, the rats could not be prescreened to determine the dominant hemisphere prior to the 6-OHDA lesion in these experiments, as suggested by Robinson and Becker (1983), because obviously this would presensitize both experimental and control animals. In Expt. 5 only data from rats that had a greater than 95% depletion of right striatal DA, and turned contraversive when given APO were analyzed (mean DA depeletions 98.1–98.4%). This was because although rats turn vigorously in response to AMPH after only a 50% DA depletion, a much more extensive DA depletion is required before animals turn vigorously to low doses of APO (Hefti et al. 1980a, b).

Experiment 1: Influence of inter-test interval on behavioral sensitization produced by a single exposure to AMPH in male and female rats

Inter-test interval. To determine if there was an enhancement in rotational behavior between the first and second test session, difference scores were calculated by subtracting the number of rotations made during the first test session from those made during the second. If on the average a group made the same number of rotations during each test session their difference score should equal zero. A positive number indicates more rotations were made during the second test session than during the first. Single-sample *t*-tests were conducted to determine if the difference scores equaled zero (Table 1: all one-tailed tests because positive difference scores were predicted – Robinson et al. 1982a).

As predicted, all groups had positive difference scores (Table 1). Males treated with the higher dose of AMPH made significantly more rotations during the second test

Table 1. Difference in the number of rotations (mean ± SEM) produced by amphetamine between two test sessions separated by 1, 7, 30, or 84 days (see text)

		24 h	7 days	30 days	12 weeks
High dose	Male	100 ± 32***	148 ± 67**	207 ± 55***	214 ± 46***
	Female	362 ± 121**	522 ± 117***	570 ± 169***	139 ± 111
Low dose	Male	43 ± 38	94 ± 43**	109 ± 40**	70 ± 31**
	Female	118 ± 64*	150 ± 45***	136 ± 88	139 ± 80*

Differs from zero: * $t = 1.7–1.8$, $P < 0.053–0.06$; ** $t = 2.2–2.7$, $P < 0.033$; *** $t = 3.2–4.6$, $P < 0.007$

session than during the first, regardless of whether the two sessions were separated by 1, 7, 30, or 84 days. Males treated with the low dose of AMPH also made significantly more rotations during the second test than during the first, when the two test sessions were separated by 7, 30, or 84 days, but not 1 day ($t = 1.1$). However, there was no significant effect of inter-test interval on the size of the difference scores for either group (high dose, $F = 1.2$, low dose, $F = 0.6$).

In female rats a single injection of the higher dose of AMPH also produced a long-lasting enhancement in the rotational behavior produced by a second injection, when the two test sessions were separated by 1, 7, or 30 days (Table 1). However, females showed more variability in rotational behavior than males (see SEM values in Table 1), probably due to the estrous cycle (Becker et al. 1982). This variability, together with relatively low n's, may explain why there was not a significant difference between two test sessions separated by 84 days ($t = 1.3$, $P = 0.122$; Table 1). Much of the variability in this group was due to one animal, which made 626 rotations during the first test and only 176 during the second. This was an extreme and unusually large decrease in rotational behavior, for which we have no explanation. If this one animal is omitted, the 84-day difference score is significantly different from zero ($t = 2.3$, $P = 0.028$).

Pretreatment with the low dose of AMPH also enhanced rotational behavior in females when the two test sessions were separated by 1, 7, or 84 days, but not 30 days ($t = 1.6$, $P = 0.08$; Table 1). Again, the difference scores did not differ significantly as a function of inter-test interval (high dose, $F = 2.5$, $P = 0.08$; low dose $F = 0.04$).

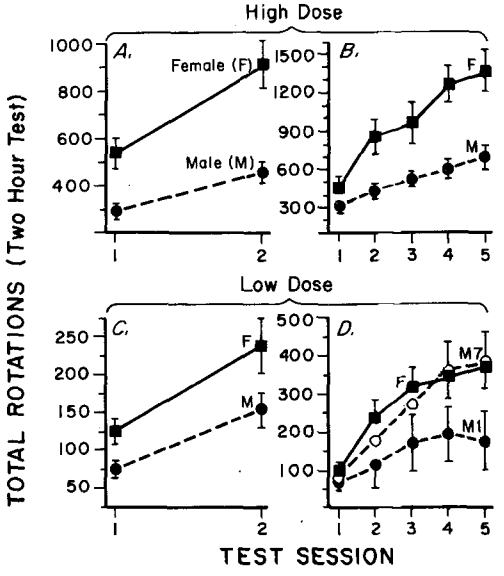

Fig. 1. The sensitization of rotational behavior produced by single or multiple injections of amphetamine in male or female rats. The ordinate shows the average (± SEM) number of amphetamine-induced rotations made during a 2-h test session (note different scales in **A, B, C,** and **D**). See the text for discussion of the time intervals between successive test sessions *(abscissa)*, and the doses of amphetamine used. Abbreviations: *F* females; *M* males; *M1* males with 1 day between each test session; *M7* males with 7 days between each test session

Sex differences. Since analyses of variance revealed no significant effect of the inter-test intervals examined here, the groups were pooled to test for sex differences in the sensitization produced by a single injection of AMPH. Females ($n = 36$) treated with the higher dose of AMPH (Fig. 1A) made significantly more rotations than males ($n = 35$; two-way analysis of variance – influence of sex, $F = 16.9$, $P < 0.001$). Both males and females made more rotations during the second test session than during the first (influence of session, $F = 52.0$, $P < 0.001$). However, a significant sex by session interaction indicates that females showed a more robust enhancement in rotational behavior between the two test sessions than did males (i.e., the lines in Fig. 1A are not parallel, $F = 7.7$, $P = 0.007$).

Figure 1C illustrates that following treatment with the low dose of AMPH, females ($n = 32$) again made more rotations than males ($n = 35$; $F = 6.1$, $P = 0.015$). Both females and males made more rotations during the second test session than during the first ($F = 25.8$, $P < 0.001$); however there was no sex difference in the magnitude of the enhancement in rotational behavior with this dose (interaction, $F = 0.9$).

Experiment 2:
Comparison of behavioral sensitization produced by single vs multiple injections of AMPH in male and female rats

To compare the sensitization produced by single vs multiple injections of AMPH, male and female rats received either the low or higher dose of AMPH over five test sessions separated by either 1 day or 7 days (Figs. 1B and 1D). There was no significant effect of inter-test interval following the higher dose of AMPH in either the males ($F = 0.2$) or females ($F = 0.2$), so the data were pooled in Fig. 1B. It is clear from Fig. 1B that there was a progressive enhancement in rotational behavior following multiple AMPH injections in both male ($n = 16$) and female ($n = 18$) rats, over-and-above that produced by a single

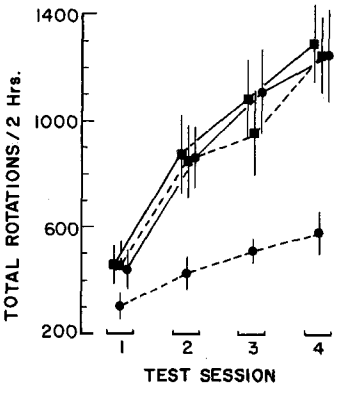

Fig. 2. The effects of gonadectomy on the sensitization of rotational behavior produced by four weekly injections of amphetamine. Note that overiectomized females *(squares, solid line)*, intact females *(squares, dotted line)*, and castrated males *(circles, solid line)* all showed an equivalent progressive enhancement in rotational behavior, which was significantly greater than of intact males *(circles, dotted lines)*. Points represent the average (± SEM) number of rotations in a 2-h test session

injection (two-way ANOVA, influence of test session $F = 36.5$, $P < 0.001$). There was also a sex difference. Females showed a greater enhancement in rotational behavior over the five test sessions than did males, as indicated by a significant sex by session interaction ($F = 6.8$, $P < 0.001$; main effect of sex, $F = 10.5$, $P = 0.003$).

Following the low dose of AMPH, there was no influence of inter-test interval in female rats (1 vs 7 days, $F = 0.08$), and therefore the data were pooled (Fig. 1D). Figure 1D shows that again there was a progressive enhancement in rotational behavior over the five test sessions in female rats ($n = 17$), over-and-above that produced by a single injection ($F = 15.1$, $P < 0.001$). Interestingly, male rats that received five weekly ($n = 8$) test sessions (M7) showed a greater enhancement in rotational behavior than did male rats that received the same low dose, but over five daily ($n = 9$) test sessions (M1; inter-test interval by session interaction, $F = 2.7$, $P = 0.037$, Fig. 1D). However, both of these latter groups (M1 and M7) did show a progressive enhancement in rotational behavior over the five test sessions ($F = 3.8-10.8$, $P < 0.01$).

Experiment 3:
Influence of endogenous gonadal hormones on the development of behavioral sensitization in male and female rats

The sex difference in sensitization observed in experiments 1 and 2, and in previous studies (Robinson et al. 1982a), suggests that endogenous gonadal hormones may influence the development of behavioral sensitization. As a first step in exploring this idea gonadectomized male and female rats were studied. Figure 2 illustrates that castrated males (CAST; $n = 16$) and ovariectomized females (OVX; $n = 17$) showed an almost identical enhancement in rotational behavior over four weekly test sessions (influence of test session, $F = 19.9$, $P < 0.001$; influence of sex and interaction F's < 0.03). For purposes of comparison, data for intact males and females shown in Fig. 1B are replotted on Fig. 2. It is clear that intact females, OVX females and CAST males all showed a greater enhancement in rotational behavior than did intact males (group by session interaction, $F = 4.3$, $P < 0.001$).

Experiment 4:
Role of conditioning in the sensitization of rotational behavior produced by infrequent injections of AMPH

In Expt. 4a we tried to condition rotational behavior by pairing an AMPH injection and a specific test environment (the rotometer) over three weekly test sessions. During the fourth test session all of the animals received saline in the rotometers to determine if conditioned rotational behavior would occur. Three weekly injections of AMPH did produce a progressive enhancement in rotational behavior (Fig. 3; $F = 5.0$, $P = 0.014$). The sensitized animals ($n = 14$) made an average of 941 ± 144 rotations during the third test session. If the sensitization produced by weekly injections of AMPH was primarily due to conditioning one would expect a subsequent injection of saline to produce fairly vigorous rotation. However, during the fourth test session a saline injection resulted in only 35 ± 12 rotations

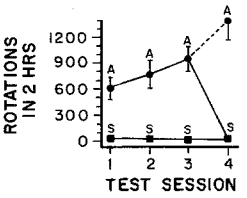

Fig. 3. The average (± SEM) number of rotations produced during four weekly 2-h test sessions. One group of animals received an injection of amphetamine *(A)* during the first three test sessions, and saline *(S)* during the fourth. Another group received four weekly injections of saline *(S)*. A third independent group received four weekly injections of amphetamine, and only the number of rotations made during their fourth session are shown (dashed line to fourth *A*). Where the standard errors are not shown it is because they are smaller than the symbol (see text)

in AMPH-pretreated rats, and 11 ± 4 rotations in saline-pretreated rats ($n = 16$; $t = 1.99$, $P = 0.053$). The dashed line and fourth "A" point in Fig. 3 show the number of rotations made by an independent group of animals that received a fourth injection of AMPH.

Three different treatment groups were compared in Expt. 4b: (1) A sensitized group ($n = 12$) pretreated with AMPH in the rotometers; (2) a saline control group ($n = 16$) pretreated with saline in the rotometers; and (3) a pseudoconditioned control group ($n = 15$) that previously received saline in the rotometers, and AMPH in their home cages. Figure 4 shows the number of rotations made over each 5-min interval of the fourth weekly test session, in which all groups received 3.0 mg/kg AMPH in the rotometers. The number of rotations made by sensitized animals during their first test session is also shown for comparison. Group differences were determined with planned two-way analyses of variance.

As expected, four injections of AMPH at weekly intervals resulted in a robust, progressive enhancement in rotational behavior (Fig. 4). The more rapid onset of rotational behavior during the fourth test session, relative to the first, is reflected by a significant session by time interaction. During the fourth test session saline-pretreated control animals (Cont.) made the same number of rotations as did animals in the sensitization group the first time they received AMPH, but significantly fewer rotations than sensitized animals the fourth time they received AMPH. Again, the more rapid onset of rotational behavior in sensitized animals (S-4), relative to the saline-pretreated control group, is reflected by a significant group by time interaction. Therefore, simply being previously exposed to the rotometers does not significantly contribute to the large progressive enhancement in rotational behavior produced by the weekly administration of AMPH.

Figure 4 also shows that it is not necessary for AMPH administration to be paired with the specific environmental context of the rotometers to produce behavioral sensitization. Pseudoconditioned animals (PC.) made significantly more rotations than did either saline-pretreated control animals, or animals in the sensitization group the first time they received AMPH. Most importantly, pseudoconditioned animals did not differ from animals that had undergone three previous AMPH-rotometer pairings (PC. vs S-4, Fig. 4).

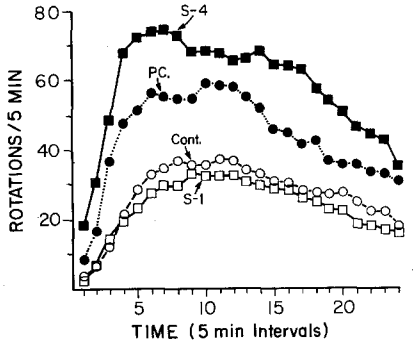

Fig. 4. Average number of rotations during each of 24 5-min intervals following the administration of 3.0 mg/kg amphetamine. The groups differ in their previous history of drug-rotometer pairing. One group received four weekly injections of amphetamine in the rotometers, and the time course of rotational behavior is shown after the first *(S-1)* and fourth *(S-4)* exposure to the drug. Note the significant enhancement in rotational behavior between the first and fourth test (*S-1* vs *S-4*, 2-way ANOVA, effect of session, $F = 26.3$, $P < 0.001$; session by time interaction, $F = 2.2$, $P = 0.001$). Saline-control rats *(Cont.)* received three weekly injections of saline in the rotometers, and then amphetamine during the fourth test session (*Cont.* vs *S-1*, $F = 0.2$; *Cont.* vs *S-4*, effect of group, $F = 9.3$, $P = 0.005$, group by time interaction, $F = 2.1$, $P = 0.002$). During the first three weekly test sessions pseudoconditioned rats *(PC.)* received saline in the rotometers, and amphetamine 2 h later in their home cage. During the fourth test session they received amphetamine in the rotometers, and the time course of rotational behavior is shown. Groups with closed symbols differ from groups with open symbols, but not from each other (*PC.* vs *Cont.*, effect of group, $F = 3.75$, $P = 0.059$, but group by time interaction, $F = 1.59$, $P = 0.04$; *PC.* vs *S-1*, effect of group, $F = 6.35$, $P = 0.017$, interaction $F = 1.82$, $P = 0.011$; *PC.* vs *S-4*, effect of group, $F = 1.94$, $P = 0.17$, interaction $F = 0.5$)

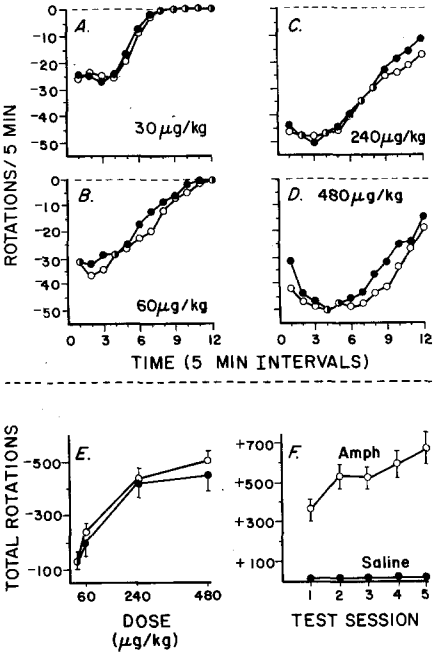

Fig. 5. The influence of AMPH sensitization on the rotational behavior induced by a subsequent SC injection of APO. Positive numbers indicate ipsiversive rotations and negative numbers contraversive rotations. Panel **F** shows that intermittent injections of AMPH produced a progressive enhancement in ipsiversive rotational behavior ($P < 0.001$), whereas repeated saline injections had no effect on rotation (points represent the average number of rotations during each 1-h test session ± SEM). Panels **A–D** show the average number of contraversive rotations made by AMPH-*(open circles)* or saline-*(closed circles)* pretreated rats during each of 12 5-min intervals following an injection of 30, 60, 240, or 480 μg/kg APO, respectively. Note that the lower the number the greater the number of rotations. Panel **E** shows the total contraversive rotations made following each dose of APO over the entire 1-h test session. There was no effect of AMPH pretreatment on APO-induced rotation (see text)

Experiment 5:
The influence of AMPH sensitization on the rotation produced by a subsequent injection of apomorphine

Figure 5 summarizes evidence showing that pretreatment with AMPH has no influence on the rotation induced by a subsequent injection of APO. Panel 5F shows that five injections of AMPH did indeed produce *ipsiversive* rotational behavior that was progressively enhanced with repeated injections (first test differs from fifth, $t = 4.2$, $P < 0.001$), and that saline injections resulted in no change in spontaneous rotational behavior over this period of time ($t = 1.0$, $P = 0.3$). Figure 5 (panels A–D) also shows the time course of the *contraversive* rotational behavior (negative numbers) induced by APO in AMPH and saline-pretreated rats. There were no differences between these groups when tested with 30, 60, 240, or 480 μg/kg APO (F's < 0.7). Panel 5E summarizes the cumulative contraversive rotations made during each 1-h test session, at each dose, and it is clear that although the rate of rotation increases with increasing doses of APO ($F = 29.1$, $P < 0.001$), it does so identically in AMPH- and saline-pretreated rats ($F = 0.9$).

Discussion

The 12-week enhancement in rotational behavior following a single injection of AMPH reported here is a more enduring change in behavior than usually seen in the literature (see Table 3 in Post 1981). It is not known if this represents basic differences in the neurobehavioral substrate of stereotypy and rotational behavior, or whether previous researchers have simply not looked for such long-lasting changes in stereotypy. It is probably the latter, because it is known that a single injection of AMPH may have enduring consequences for a variety of behaviors. Magos (1969) originally reported that a single injection of AMPH facilitated stereotypy for up to 2–5 weeks, and there have been a few subsequent reports that stereotypy (Browne and Segal 1977; Ellison and Morris 1981; Segal and Schuckit 1983), drinking (Antelman and Chiodo 1983), rotational behavior (Echols 1977; Robinson et al. 1982a) and tail pinch-induced behavior (Antelman et al. 1980) are enhanced for weeks after a single injection of AMPH.

It is clear that even moderate exposure to low doses of AMPH may have robust and long-lasting effects on behavior. In fact, our findings in male rats suggest that relatively infrequent multiple injections may be more efficiacious in producing sensitization than those given close together in time – supporting the analogy to kindling previously made (Post 1980; Post and Kopanda 1976; cf Antelman and Chiodo 1981). The results certainly suggest

that the frequently administered (daily or twice daily) high doses of AMPH (5–10 mg/kg) used by many researchers to produce behavioral sensitization (see Table 3, Post 1981) are unnecessary, and perhaps in some cases are even counterproductive.

One reason that very aggressive drug regimens may be used is because it is thought that this more closely mimics the conditions that result in AMPH psychosis (Connell 1958; Griffith et al. 1972; Kramer et al. 1967). It is interesting that both the behavioral sensitization produced by multiple AMPH (or cocaine) injections (Post and Contel 1983; Segal and Janowsky 1978; Segal and Schuckit 1983) and the behavioral changes produced by continuous AMPH administration (Ellison and Eison 1983) have been presented as animal "models" of AMPH psychosis. In many respects these two modes of AMPH administration have opposite effects. For example, continuous AMPH administration destroys striatal DA terminals, resulting in DA depletion (Ellison et al. 1978; Ellison and Ratan 1982), whereas intermittent injections of AMPH do not deplete striatal DA (Kuczenski and Leith 1981), but facilitate striatal DA release (Robinson and Becker 1982; Robinson et al. 1982a).

Whether the continuous administration of AMPH provides a more realistic model of AMPH psychosis than intermittent injections is a matter for further debate and experimentation. However, in regards behavioral sensitization as an animal model of AMPH psychosis, it should be noted we found that intermittent injections of AMPH produced a *progressive* and long-lasting enhancement in rotational behavior, over-and-above that produced by a single injection. The progressive and enduring nature of the change is important for an animal model of AMPH psychosis because: (1) although AMPH psychosis may sometimes result from a single injection of AMPH (Segal and Janowsky 1978), the probability of inducing such behavioral and cognitive changes is thought to increase with repeated exposure to the drug (Ellinwood et al. 1973); and (2) former AMPH addicts are hypersensitive to AMPH even after 1–2 years of abstinence (Sato et al. 1983).

Regardless of the relative merit of intermittent vs continuous AMPH administration as an animal model of AMPH psychosis, the neuroplastic changes responsible for behavioral sensitization require elucidation. In searching for the neural basis of behavioral sensitization, it would seem wise to avoid too agressive drug treatment regimens. This is because the neurobehavioral changes produced by frequent high doses of AMPH, or especially continuous AMPH administration, are probably unrelated to the behavioral sensitization produced by more infrequent exposure to low doses of AMPH (e.g., compare Kuczenski and Leith 1981; Robinson and Becker 1982; Robinson et al. 1982a with Ellison et al. 1978; Ricaurte et al. 1984; Steranka 1983). The experiments reported here establish a minimum set of criteria that must be met before any change identified in the nervous system can be seriously considered as being causally related to the sensitization of rotational behavior: (1) The change should be produced by a single, low dose of AMPH; (2) It should be greater following multiple injections spaced up to 1 week apart than following a single injection; (3) It should persist for very long periods of time (up to 12 weeks); and (4) sex and/or gonadal hormones should influence the magnitude of the change.

At present, there is little consensus as to what causes behavioral sensitization, and no hypothesis satisfies all of the above criteria. One hypothesis is that sensitization is largely due to drug-environment conditioning effects, because the repeated administration of psychomotor stimulants in a unique test environment can result in conditioned locomotion or stereotypy (Pickens and Dougherty 1971; Post et al. 1981; Schiff 1982; Tilson and Rech 1973). However, we found no evidence that the sensitization of rotational behavior produced by weekly injections of AMPH is due to conditioning, consistent with previous reports (Echols 1977; cf Browne and Segal 1977; Segal 1975). This is not to say that it is impossible to condition rotational behavior. Perhaps drug effects must be paired with the rotometer environment much more frequently than once a week for 5 weeks to condition this behavior.

There is also no evidence that behavioral sensitization is due to metabolic or other peripheral effects (Browne and Segal 1977; Kuczenski et al. 1982; Kuhn and Schanberg 1977). Therefore, the sensitization reported here may be due to an AMPH-induced change(s) in the functional activity of brain catecholamine neurons (Klawans and Margolin 1975; Martres et al. 1977; Segal and Mandell 1974). The nature of this putative change has not been well characterized. It is probably not a simple change in DA receptors, as indicated by the lack of an effect of AMPH sensitization on APO-induced rotation (Fig. 5), and the failure to find consistent changes in DA receptor binding (see Conway and Uretsky 1982 and Post 1981 for references). It may involve an enhancement in AMPH-stimulated DA release from striatal terminals (Robinson and Becker 1982; Robinson et al. 1982a; cf Kuczenski and Leith 1981).

Sex differences and gonadal hormones

We found strong and reliable sex differences in behavioral sensitization to AMPH (also see Robinson et al. 1982a). Females show more robust sensitization than males following single or multiple injections of AMPH, and similar sex differences may occur following exposure to cocaine (S.D. Glick, personal communication; Post and Contel 1983). Although there has been one report of greater AMPH-induced sensitization in males than in females (Flemenbaum 1979), this was probably due to a ceiling effect in the females (cf Post and Contel 1983).

The cause of the sex difference in sensitization is not known. It is unlikely that it is due to sex differences in the metabolism of AMPH (Becker et al. 1982). Doses of AMPH were used to ensure that intact males and females had the same brain and striatal levels of AMPH (Becker et al. 1982), as did gonadectomized males and females (J.B. Becker, unpublished observations). Another possibility is that it is related to the well-documented sex difference in AMPH-induced rotational behavior (Becker et al. 1982; Brass and Glick 1981; Robinson et al. 1980). Females show more vigorous rotational behavior than males, and perhaps animals which show vigorous rotational behavior also are more prone to sensitization. However, in the present study there was no correlation between the number of rotations made during the first test session and the magnitude of the increase in rotational behavior between the two test sessions (as indicated by the difference scores, for males,

$r = +0.204$; for females, $r = +0.042$; not significant). In contrast, there was a strong correlation between the number of rotations made during the second test session and the degree of sensitization (for males, $r = +0.67$; for females, $r = +0.75$; $P < 0.01$). Thus, animals that turned the most during the second test session were animals that increased their rate of rotation the most; but they did not necessarily turn vigorously during their initial exposure to AMPH.

The effects of gonadectomy on sensitization (also see Robinson et al. 1982a) suggest that endogenous gonadal hormones influence the development of this form of neuroplasticity. Although removal of the ovaries in females had no effect on sensitization, castrated males showed greater sensitization than intact males, appearing very much like females. A working hypothesis is that the presence of a testicular hormone retards the development of sensitization. It should not be surprising that gonadal hormones might influence the behavioral sensitization produced by psychomotor stimulants, because gonadal hormones are known to normally modulate striatal DA activity (Becker and Ramirez 1981; Robinson et al. 1980, 1982b), to control patterns of neural connectivity during development (Arai 1981; Greenough et al. 1977; Raisman and Field 1973), to influence sprouting and regeneration (Milner and Loy 1982; Yu 1982), and to influence the development of receptor supersensitivity following the repeated administration of neuroleptics (Gnegy et al. 1983; Koller et al. 1980; cf Fujii and Ikeda 1982).

It is interesting to note that there are not only sex differences in this animal model of AMPH psychosis, but also in schizophrenia (Lewine 1981), and other psychopathologies in which brain catecholamine systems have been implicated (e.g., Weissman and Klerman 1977). In female schizophrenics the age of onset and of first hospitalization is later than in males, the symptoms in males and females differ considerably (typical vs atypical, respecitvely; Lewine 1981), and females have a more favorable clinical outcome than males (Watt et al. 1983). It has even been suggested that there may be sex-associated subtypes of schizophrenia (Lewine 1981). Although there has been considerable speculation as to the cause(s) of sex differences in psychopathology, there has been very little substantive research on the role that sex differences in brain organization might play. The experiments reported here suggest that further investigation of sex differences and gonadal hormone modulation in brain DA systems may be very fruitful in this regard.

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