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Metabolic and Experiential Factors in the Behavioral Response to Repeated Amphetamine[1]

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BROWNE, R. G. AND D. S. SEGAL. *Metabolic and experiential factors in the behavioral response to repeated amphetamine.* PHARMAC. BIOCHEM. BEHAV. 6(5) 545–552, 1977. – Previous studies have shown that repeated administration of d-amphetamine results in a progressive augmentation of locomotor activity and stereotypy. The present studies demonstrate that rats also exhibit an enhanced behavioral response following multiple daily injections of l-amphetamine and methylphenidate. Furthermore, behavioral augmentation is shown to persist for at least six days after a single injection of d-amphetamine. These results demonstrate the generality of the reverse tolerance phenomenon and indicate that metabolic factors, such as the formation of false neurotransmitters, do not account for the enhanced behavioral responsiveness observed with multiple injections of these drugs. The role of experiential factors in the behavioral augmentation was studied by (1) varying the amount of continuous exposure to the experimental environment prior to d-amphetamine administration, and (2) examining the effects of repeated injections of saline or d-amphetamine in different environments prior to testing in the experimental chambers. The results, which revealed a behavioral augmentation independent of pretreatment condition, indicate that neither acclimation to the test chamber nor state-dependent conditioning to external stimuli accounts for the enhanced locomotor activity and stereotypy observed with repeated administration of psychomotor stimulants.

Locomotor activity Stereotypy l-Amphetamine d-Amphetamine Methylphenidate Behavioral augmentation

PREVIOUS studies [20,23] demonstrated that repeated administration of d-amphetamine produced a progressive augmentation of locomotion and of more restricted behaviors, i.e., stereotypy. Similar results have been reported more recently for locomotion in mice [25] and for stereotypy in guinea pigs [10]. A number of different mechanisms may contribute to this behavioral augmentation, including the possible involvement of amphetamine metabolites. In the rat d-amphetamine is primarily metabolized by liver microsomes to para-hydroxy-amphetamine (POA) [1, 3, 9] which in turn is converted to the alleged false neurotransmitter para-hydroxy-norephedrine (PONE) in peripheral and central noradrenergic neurons by dopamine-β-hydroxylase (DBH) [4, 7, 8, 11, 15]. Although the disappearance rate of amphetamine and POA from brain is relatively rapid, reaching undetectable amounts in 12 hr [14,15], brain levels of PONE decline only slightly over a 24 hr period [3,15]. Thus, this metabolite may contribute, at least in part, to the enhanced behavioral effects observed during repeated d-amphetamine administration. For example, the reduction in brain norepinephrine (NE) levels corresponding to the accumulation of PONE, and the alleged consequent decline in central adrenergic tone, was suggested as one potential mediator of a compensatory increase in catecholamine (CA) biosynthesis and/or receptor sensitivity [20]. Such compensatory changes might then be responsible for the chronic d-amphetamine-induced behavioral augmentation. Therefore, the possible involvement of PONE was examined in the present studies.

In addition to metabolic variables, situational and experiential factors are known to play a role in the behavioral response to single or repeated administration of d-amphetamine. Indeed, recent evidence indicates that conditioned locomotor activity (i.e., motor activity which is conditioned to neutral stimuli attending drug administration) can result from multiple injections of d-amphetamine [26]. However, while in our studies the test chamber is routinely utilized as the home environment, a procedure which appears to minimize such conditioning, there exists the possibility that factors associated with acclimation to the test chamber, e.g., isolation and/or state-dependent conditioning, may influence the behavioral response to repeated d-amphetamine. In this regard Sahakian *et al.* [19]

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have recently reported that rats socially deprived from weaning exhibit more intense stereotyped behavior in response to amphetamine as adults. Hence, the present studies further examine the role of situational and experiential variables in the behavioral response to repeated amphetamine administration.

METHOD

Animals and Drugs

Male Sprague-Dawley rats (300–350 g) obtained from Hilltop Laboratories were housed under standard laboratory conditions for at least 1 week prior to experimentation. All drugs were administered in a volume of 1 ml/kg and the doses are expressed as free base. D- and l-amphetamine sulfate were obtained from Smith, Kline and French Laboratories, and were administered subcutaneously. Methylphenidate (Ritalin, Ciba-Geigy Pharmaceuticals, Inc.) was administered intraperitoneally to avoid the necrotic lesions produced by subcutaneous injection of this drug.

Apparatus and General Procedures

The behavioral chambers and data recording systems previously described [20,23] allow for continuous automatic recording of locomotion as crossovers from one portion of the activity chamber to another; and as rearings detected when the rat makes contact with touchplates located 13 cm above the floor. Both measures are continuously monitored with the use of a NOVA 1200 computer. Stereotypy, including continuous sniffing, licking, chewing, gnawing and repetitive head and limb movements, concurrent with the absence of forward locomotion, is characterized by regular observations through a viewing lens located in the door of the chamber and by detailed analysis of videotapes made of representative animals.

Unless otherwise indicated, the animals were housed in the experimental chambers for the duration of an experiment. Food and water were continuously available and lighting was maintained on a 12 hr bright light/12 hr dim light cycle. The animals were injected with drugs or vehicle control at about 10 a.m. each day and at the same time, the chambers were serviced, i.e., food and water replenished and the internal chambers cleaned. The whole process required about 1–2 min per box and was the only time during the day when the animals were disturbed.

METABOLIC FACTORS: EXPERIMENT 1

In order to determine the generality of the augmentation phenomena, and also the possible involvement of specific amphetamine metabolites, we examined the effects of repeated administration of l-amphetamine and methylphenidate on locomotion and stereotypy in rats. The observation of behavioral augmentation during repeated administration of l-amphetamine would rule out PONE as mediating the enhanced responsiveness since the l-isomer of amphetamine is a poor substrate for DBH [4,15] and thus, is not converted to a false neurotransmitter [4,15]. Similarly, there is no evidence that methylphenidate and/or its metabolites act as false neurotransmitters [6,24].

Procedures

Rats were placed in the experimental chambers up to 7 days prior to the initiation of drug treatment, during which time they received daily injections of isotonic saline. The rats were then randomly assigned to the experimental groups and received 5 or 6 daily injections of either d-amphetamine (2.5 mg/kg), l-amphetamine (6, 8, or 12 mg/kg), or methylphenidate (3, 9, or 18 mg/kg). Alterations in the behavioral response were assessed by comparing the results obtained on the first and last days of drug administration. Unless otherwise indicated, the data for this and subsequent experiments was analyzed by matched pairs *t*-test.

Results and Discussion

Figure 1 depicts the typical pattern of behavioral augmentation which occurs after 5 daily injections of d-amphetamine. A single dose of 2.5 mg/kg d-amphetamine produces a multiphasic response pattern [20,23], consisting of an early and late period of enhanced locomotion and an intermediate phase of continuous stereotypy during which locomotion is absent. Following the fifth injection of d-amphetamine, the typical pattern of augmentation is apparent in the form of a more rapid onset of stereotypy reflected by fewer crossovers during the first hour (19 ± 6 for Day 5 vs 70 ± 14 for Day 1, $p<0.01$). Visual observations verified that with repeated d-amphetamine administration the animals more rapidly engaged in continuous sniffing, licking and repetitive head movements with correspondingly reduced ambulation. Another component of the augmentation observed with repeated d-amphetamine is an elevation in post-stereotypy hyperactivity. However, although we have previously shown that 15 days of d-amphetamine (2.5 mg/kg) results in a significant increase in locomotion following the stereotypy phase, the apparent increase after only 5 days did not achieve statistical significance (Fig. 1).

Following an acute low dose of methylphenidate (3.0 mg/kg, Fig. 1), the predominant response exhibited is enhanced locomotion. After 5 daily injections of this dose the response was not significantly different from that produced by the first injection. This result is similar to previous findings with 0.5 mg/kg d-amphetamine which required up to 15 days of injections before a significant increase in locomotion was observed. However, as with d-amphetamine, administration of higher doses of methylphenidate resulted in a significant augmentation after only 5 daily injections. Thus, a dose of 9.0 mg/kg of methylphenidate, which after the first injection produced a prolonged period of locomotion, after the fifth injection elicited episodic periods of stereotypy reflected by significantly fewer crossovers during the first hour (221 ± 43 vs 77 ± 12, $p<0.01$). This behavioral pattern characteristic of augmentation is further exemplified with repeated administration of a higher dose of methylphenidate (Fig. 1). During the first hour after an acute injection of 18 mg/kg of methylphenidate, the primary effect was enhanced locomotion. By the fifth injection locomotion was replaced by continuous stereotypy as reflected by the marked reduction in locomotion (221 ± 43 vs. 13 ± 2, $p<0.01$). These findings demonstrate that repeated administration of methylphenidate, like d-amphetamine, produces an augmented behavioral response as indicated by the replacement of locomotion with stereotypy during the early time intervals.

Figure 2 illustrates the effects of 6 daily injections of either 6, 12 or 18 mg/kg of l-amphetamine. Although the

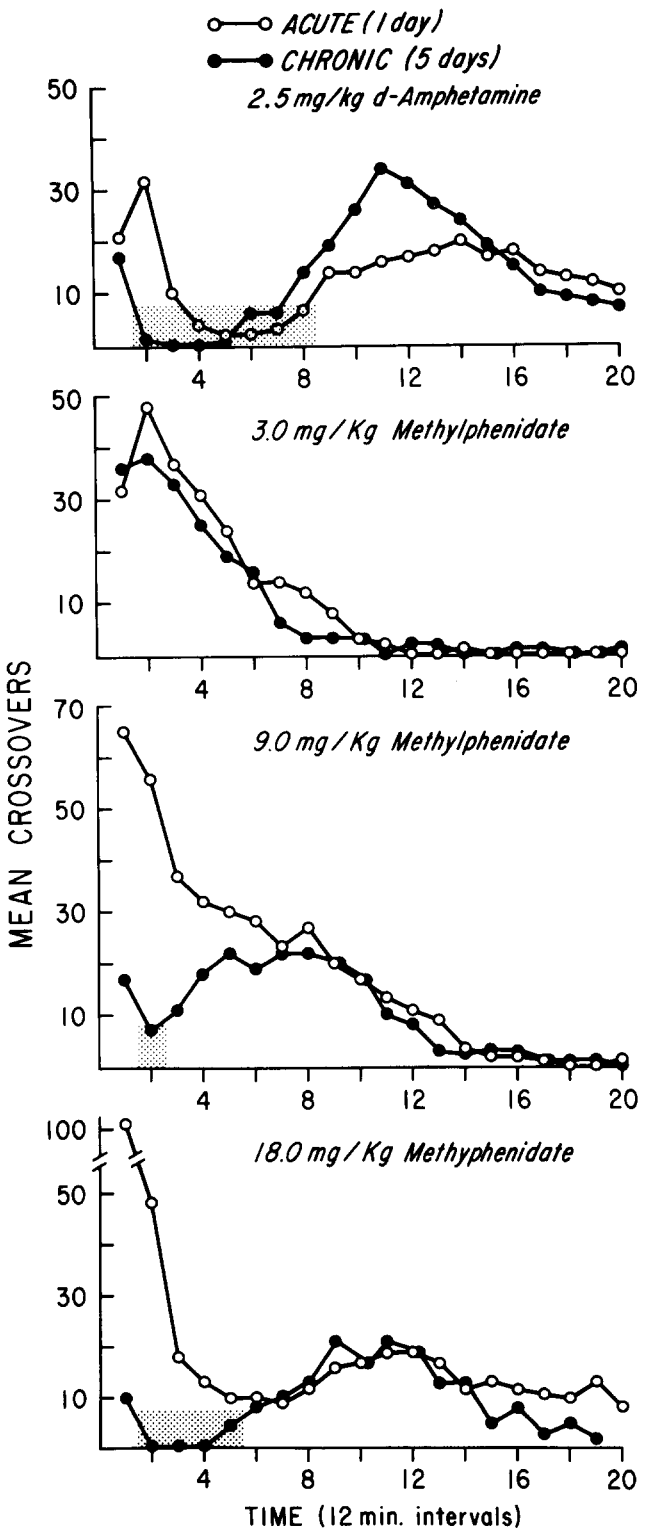

FIG. 1. Mean crossovers during successive 12 min intervals following the first and fifth injections of either d-amphetamine (2.5 mg/kg) or various doses of methylphenidate. Shaded areas indicate the period of focused stereotypy. Repeated injections of d-amphetamine and 18 mg/kg of methylphenidate resulted in augmentation characterized by the more rapid onset of stereotypy. The replacement of locomotion with stereotypy is apparent by the fifth injection of 9 mg/kg methylphenidate. N = 9 in each group.

well-documented potency differences are apparent, the patterns of augmentation for d- and l-isomers of amphetamine are similar. For the lower dose of d-amphetamine (6.0 mg/kg), there is both an increase in locomotion (121 ± 20 vs. 68 ± 17, $p<0.01$ for the second hour) and the progressive appearance of episodic stereotypy reflected by the transient decline in locomotion during the early intervals. With the intermediate dose of l-amphetamine (12 mg/kg), the onset of stereotypy (12 min intervals 2–4) was significantly more rapid following repeated injection (114 ± 44 vs. 21 ± 18, $p<0.02$; Wilcoxon matched pairs test used because of nonhomogeneity of variance). The peak increase in crossovers (12 min interval 12) was also significantly enhanced by the sixth injection of l-amphetamine (54 ± 10 vs. 23 ± 5, $p<0.02$).

The results obtained with multiple injections of l-amphetamine and methylphenidate demonstrate that the behavioral augmentation is not unique to d-amphetamine. Furthermore, these studies appear to rule out the possibility that a false neurotransmitter such as PONE is involved in the progressive behavioral effects.

METABOLIC FACTORS: EXPERIMENT 2

The possible involvement of metabolites in the behavioral augmentation can be further examined by characterizing the effects of a single injection of d-amphetamine on the response to subsequent administration at a time when brain levels of d-amphetamine and its metabolites are known to be undetectable. After an injection of 20 mg/kg of d,l-amphetamine, brain levels of PONE achieve a maximal concentration by 3 hr, but decline very slowly with a half-life of about 24 hr [14]. With the high dose employed in this study, PONE had not completely disappeared until four to six days after injection [14]. We have previously observed that with moderate doses of amphetamine, augmentation in the form of a more rapid onset of stereotypy can be demonstrated 24 hr after a single injection. Therefore, if the behavioral augmentation after a single injection could be shown to persist at a time when PONE is not detectable, then the presence of this false neurotransmitter could be excluded as a necessary condition for the enhanced response.

Procedures

To compare the carry-over effects one and six days after the initial d-amphetamine administration, one group of rats received 4 daily injections of saline followed by two consecutive daily d-amphetamine injections (2.0 mg/kg) on Days 5 and 6. Another group received their first 2.0 mg/kg d-amphetamine injection on Day 1 followed by 4 daily saline injections and a second 2.0 mg/kg dose of d-amphetamine on Day 6. This design allows all animals to be tested on the same day while minimizing the differences in handling and number of injections received prior to the second injection of amphetamine.

Results and Discussion

Figure 3 shows that a single 2.0 mg/kg injection of d-amphetamine produced hyperactivity as the predominant behavioral response. Rats receiving a second injection of d-amphetamine 24 hr later exhibited an augmented response as evidenced by the pronounced phase of continuous stereotypy during which locomotion was markedly

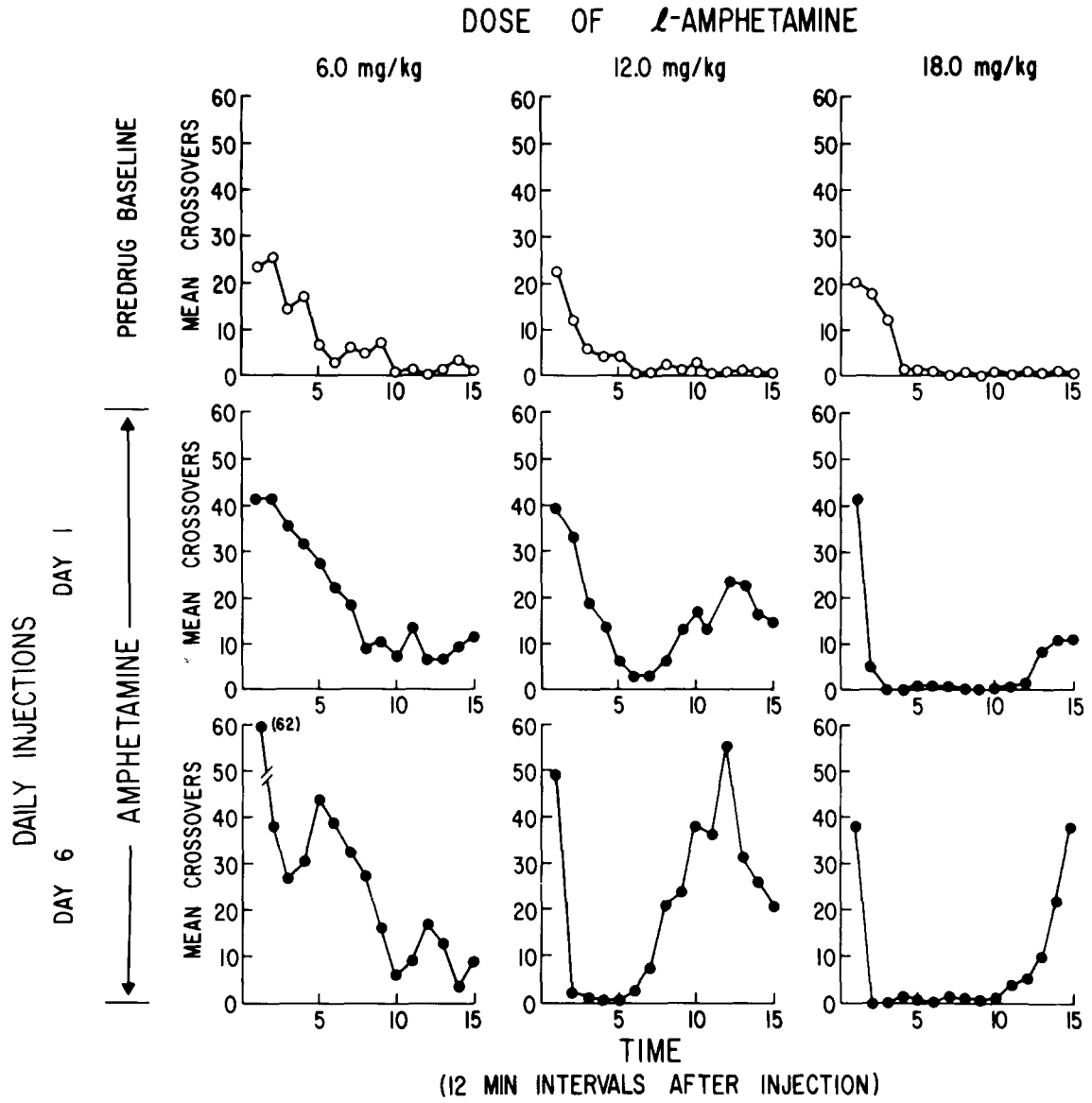

FIG. 2. Mean crossovers during the 3 hrs following the first and sixth daily injections of various doses of l-amphetamine. Repeated administration produced an augmentation in both locomotor activity and stereotype. N = 7 in each group.

reduced (crossovers for 12 min intervals 2–8, first vs. second injection: 163 ± 40 vs. 65 ± 27, $p<0.01$). Similarly, rats receiving their second injection 6 days later also responded with a significant increase in stereotypy (183 ± 40 vs 57 ± 25, $p<0.02$). The two groups did not differ in their respective first and second responses to amphetamine. The results of this experiment further negate the possibility that behavioral augmentation is mediated by the presence of a false neurotransmitter such as PONE since the brain levels of this compound are significantly different at 24 hr and 6 days following injection [14,15], while the behavioral response to a second injection of d-amphetamine is similar at these two time intervals.

EXPERIENTIAL FACTORS: EXPERIMENT 1

Mice and rats isolated for prolonged periods of time are more sensitive to the toxic effects of stimulant drugs [2,27]. Furthermore, Segal *et al.* [22] have demonstrated that after only four to six days of isolation rats exhibit enhanced spontaneous and d-amphetamine-induced locomotor activity. Thus, because in our experiments the rats are maintained individually in the test chambers, it is conceivable that factors such as social isolation may account for the enhanced effects produced by repeated administration of d-amphetamine and related psychomotor stimulants.

Procedures

Animals were placed in the chambers three days preceding 4 daily injections of either saline or d-amphetamine (2.5 mg/kg). On the fifth injection day all rats received a dose of 2.5 mg/kg of d-amphetamine. If factors related to acclimation, social isolation, or repeated injections are responsible for the enhanced response to d-amphetamine, then the behavioral pattern resulting from injection of d-amphetamine on Day 5 should be similar for both groups,

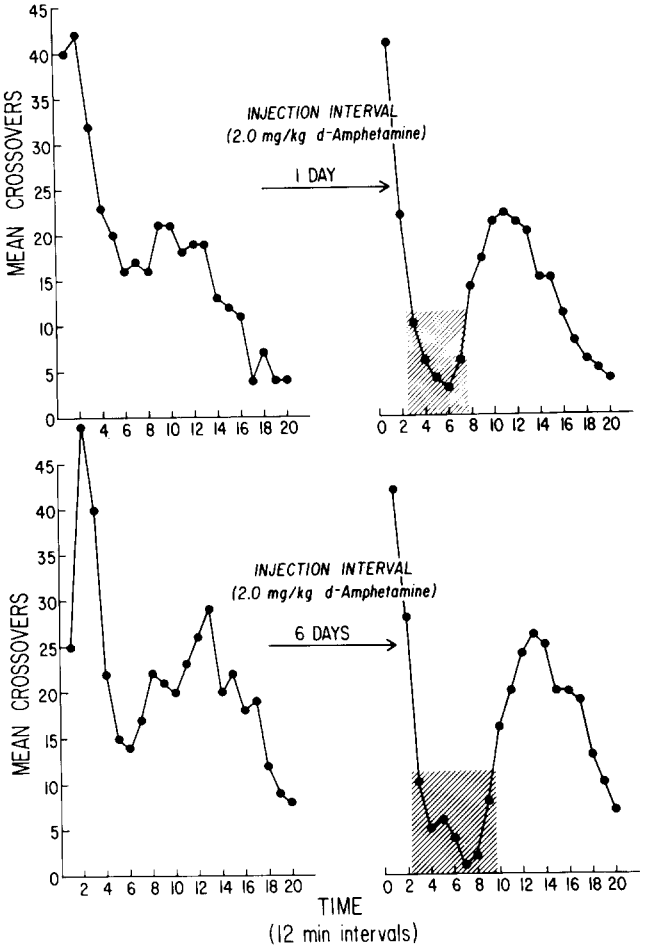

FIG. 3. Mean crossovers following the first and second injections of d-amphetamine. The second d-amphetamine injection was administered either 1 day (top panel) or 6 days (bottom panel) after the first drug administration. Augmentation occurs after a single injection and persists for at least 6 days. N = 8 in each group.

that is, independent of prior amphetamine treatment.

Results and Discussion

The response of the two groups to amphetamine on the fifth day (Fig. 4) is significantly different with respect to both onset of stereotypy (49 ± 11 vs 10 ± 2 for the first 3 12 min intervals, $p<0.01$) and post-stereotypy hyperactivity (18 ± 4 vs 36 ± 5 for the peak effect during the thirteenth 12 min interval, $p<0.01$). In contrast, the response to d-amphetamine by the saline pretreated group is not significantly different from the initial response of the amphetamine pretreated group. These results indicate that the intervening four days of acclimation to the test chambers, handling and stimuli associated with repeated injections are not sufficient to account for the behavioral augmentation.

EXPERIENTIAL FACTORS: EXPERIMENT 2

Conditioned locomotor activity has been demonstrated by Tilson and Rech [26] to account for the increased responsiveness they observed with repeated d-amphetamine administration. In that study concordance between d-amphetamine exposure and the test chamber was shown to be necessary in order to produce conditioned locomotion. In contrast, animals receiving repeated d-amphetamine in the home environment did not show enhanced responsiveness to saline in the test chamber. The results presented in Fig. 3 therefore are not indicative of such a conditioning effect since, although a behavioral augmentation is produced after a single injection of d-amphetamine, there was no evidence of elevated activity in response to saline during the five days separating the first and second d-amphetamine injections. Moreover, saline tests administered following long-term repeated d-amphetamine injections also failed to demonstrate such a conditioning effect [20,23]. However, the possibility exists that state-dependent conditioning to environmental stimuli unique to the test chamber might account for the enhanced effects observed with chronic administration of d-amphetamine.

Procedures

To assess state-dependent conditioning to the test environment, rats received 4 daily injections of either saline or 2.5 mg/kg of d-amphetamine in one of three different environments: (1) singly housed in the standard test chambers; (2) singly housed in plastic cages; or (3) grouped eight per cage in plastic cages. Six hr after the fourth injection all animals were placed in the standard experimental chambers and on the following day the behavioral effects of 2.5 mg/kg of d-amphetamine were assessed.

Results and Discussion

The response of d-amphetamine after repeated injections in different environments is depicted in Fig. 5. For all three environmental conditions, pretreatment with d-amphetamine produced a more rapid onset of stereotypy reflected as a diminished number of crossovers during the first three 12 min intervals after injection: standard, 78 ± 20 vs 19 ± 3, $p<0.02$; isolated, 99 ± 24 vs 18 ± 5, $p<0.01$; grouped, 75 ± 15 vs 23 ± 6, $p<0.01$. These results indicate that the behavioral augmentaion is not the result of conditioning to environmental stimuli unique to the test chamber since animals receiving repeated amphetamine in different environments consistently show an enhanced response.

GENERAL DISCUSSION

D-amphetamine administration produces a prolonged reduction of peripheral and brain NE which appears to result from the accumulation of PONE in noradrenergic neurons [13,15]. This amphetamine metabolite which is alleged to act as a false neurotransmitter has been implicated in the development of tolerance to amphetamine-induced anorexia and hyperthermia [16]. It has also been proposed that a PONE-mediated reduction in central noradrenergic tone might result in a compensatory increase in NE biosynthesis and/or receptor sensitivity [20]. This contention, however, is not supported by our findings that repeated administration of l-amphetamine and methylphenidate also results in enhanced behavioral effects (Figs. 1 and 2) since neither of these drugs appear to be metabolized to a false neurotransmitter substance. Moreover, the results presented in Fig. 3 demonstrate that behavioral augmentation can be demonstrated for at least six days after injection of a single moderate dose of

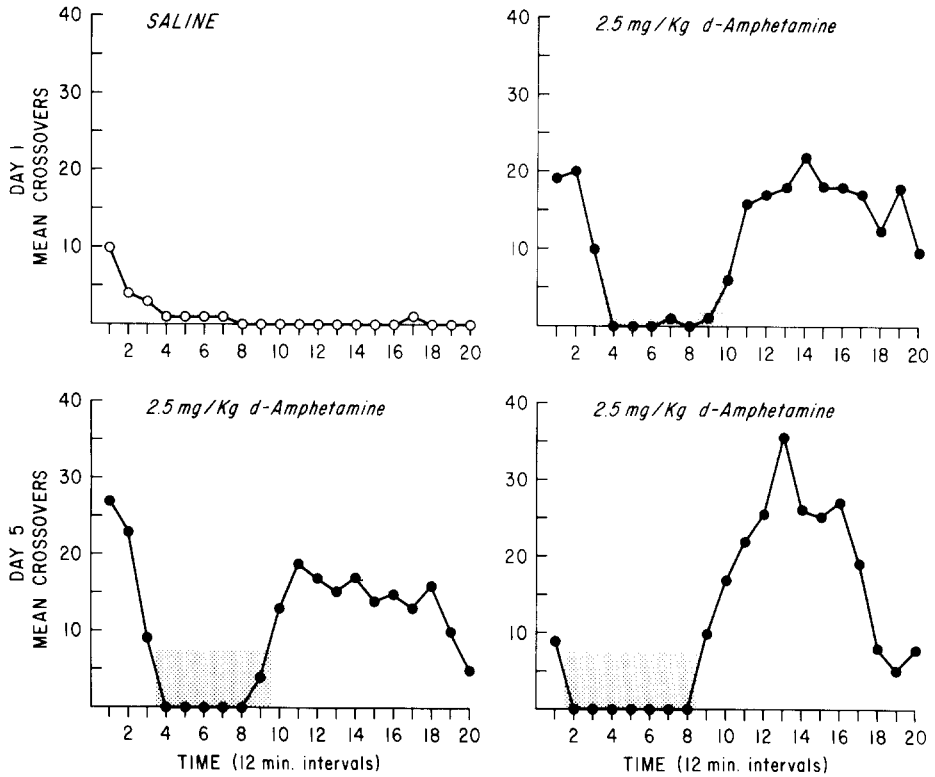

FIG. 4. Response to 2.5 mg/kg d-amphetamine after pretreatment with 4 daily injections of either saline (left) or 2.5 mg/kg (right). Effects indicate that factors associated with exposure to the experimental chambers prior to drug administration are not responsible for the augmentation seen with repeated injections. N = 8 in each group.

amphetamine, at which time brain levels of PONE are no longer detectable.

The present studies do not, however, exclude the possibility that amphetamine administration may induce a persistent effect on dispositional factors such that the accumulation of amphetamine increases with its repeated injection. In this regard previous reports indicate that in the CNS there is no difference or perhaps even an increase [17] in the disappearance rate of d-amphetamine with chronic administration. Nevertheless, interpretation of many of these studies is limited because of the use of relatively insensitive measuring techniques as well as the failure to determine amphetamine levels in localized brain regions. That whole brain determinations may mask significant alterations in regional amphetamine accumulation is indicated by recent evidence which reports enhanced accumulation of POA in striatum and olfactory tubercles but not in hippocampus and hypothalamus [5]. Moreover, in preliminary studies in collaboration with Dr. A. Cho examining regional brain amphetamine pharmacokinetics, we hve found, using isotopic variants and gas chromatography-mass spectrometry techniques, persistent residual traces of d-amphetamine which appear to be released by subsequent injection of d-amphetamine. Although the residual pool of amphetamine is relatively small, it may be localized in functionally significant sites and thus contribute to the enhanced behavioral response to repeated amphetamine.

Experiential factors such as social grouping conditions have been reported to influence the behavioral response to amphetamine [19,22]. However, our results (Fig. 4) indicate that isolation is not responsible for the behavioral augmentation. Furthermore, conditioning to external stimuli attending the injection (Figs. 1 and 2 [20,23]) or a conditioned drug response (Fig. 5) does not appear to be implicated in the enhanced responsiveness. It is, however, possible that conditioning of interoceptive stimuli may account for the behavioral augmentation. Consistent with this possibility is the observation that d-amphetamine can serve as a discriminative stimulus in the control of behavior [12].

A number of other mechanisms may be implicated in the enhanced behavioral effects resulting from repeated administration of psychomotor stimulants. The possible involvement of serotonergic systems has been examined [21] and, although the amphetamine response appears to be modulated particularly by the serotonergic system originating in the median raphe, any alterations in brain serotonin alone do not appear to be sufficient to account for the behavioral augmentation.

A compensatory alteration in biosynthetic capacity and receptor sensitivity has also been proposed [20]. Klawans and Margolin [10] recently reported that following chronic amphetamine pretreatment, guinea pigs display a hypersensitivity to the dopamine receptor agonist apomorphine. However, our studies show that whereas changes in the intensity and latency to onset of stereotypy are consistent with an increased receptor responsivity, the duration of the stereotypy phase is not correspondingly increased and, in fact, may be slightly decreased with prolonged administration. An increase in the duration of stereotypy, as occurs with increasing amphetamine dose, might be expected if amphetamine administration resulted in receptor sensi-

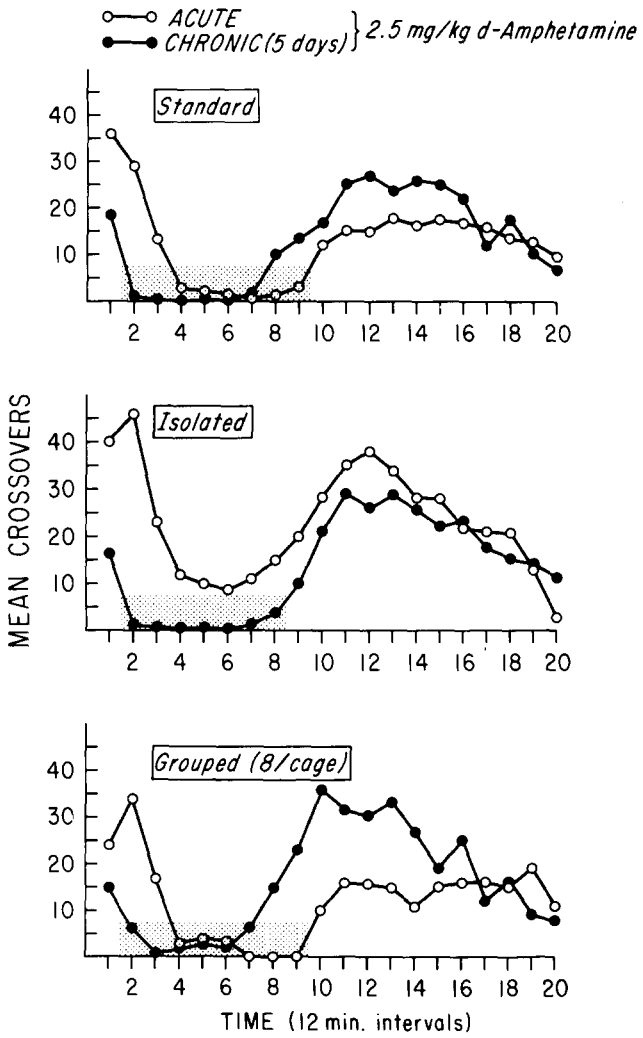

FIG. 5. Effects of 2.5 mg/kg d-amphetamine in rats pretreated with either saline or d-amphetamine in one of three different environments. The results suggest that behavioral augmentation is independent of pretreatment conditions. N = 8 in each group.

tization. Also inconsistent with a receptor supersensitivity model is the finding that CA-stimulated cyclic AMP accumulation, frequently used as an index of CA receptor sensitivity, is reduced after chronic amphetamine [18]. We have replicated this observation in neocortex and caudate-putamen with the use of a dosage regimen which produces behavioral augmentation (i.e., 2.5 mg/kg d-amphetamine daily for 7 days [Skolnick and Segal, unpublished observation]).

With respect to presynaptic alterations, we have not found any change in CA biosynthetic capacity as measured by tyrosine hydroxylase activity or synaptosomal conversion of tyrosine to dopamine (Segal, Kuczenski and Bullard, unpublished observation). Similarly, Short and Shuster [25] have reported that there is no alteration in whole brain tyrosine hydroxylase activity after chronic amphetamine in mice. It is apparent, therefore, that although several factors warrant further examination, the mechanisms responsible for psychomotor stimulant-induced behavioral augmentation remain to be elucidated.

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