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Effects of Chronic Intermittent and Continuous Amphetamine Administration on Acoustic Startle

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KOKKINIDIS, L. *Effects of chronic intermittent and continuous amphetamine administration on acoustic startle.* PHARMACOL BIOCHEM BEHAV **20**(3) 367–371, 1984.—Acoustic startle was evaluated after mice were exposed to two different schedules of long-term amphetamine treatment. Under one schedule, mice received two daily subcutaneous injections of d-amphetamine for 7 consecutive days, whereas the other consisted of continuous administration of amphetamine via a subcutaneously implanted minipump. The enhanced acoustic startle observed after a test dose of d-amphetamine (3.0 mg/kg) was further facilitated when animals were exposed to long-term intermittent amphetamine administration. In contrast, the enhanced startle response to amphetamine was attenuated when mice received chronic continuous exposure to amphetamine. Possible behavioral and neurochemical mechanisms that may be involved in the development of tolerance after continuous amphetamine administration, and reverse tolerance after intermittent amphetamine treatment were discussed.

Acoustic startle Intermittent amphetamine treatment Continuous amphetamine administration
Dopamine receptors Conditioning factors Tolerance Sensitization

THE behavioral outcome of long-term administration of a variety of drug treatments is dependent upon a number of variables, including dosage, testing environment, and experiential factors [9, 11, 20]. In addition to these factors, it has become apparent that the temporal characteristics associated with long-term drug administration are paramount in determining the behavioral consequences of repeated drug treatments [20]. For example, administration of morphine at intervals of several days potentiated morphine-induced locomotor activity [24]. A more continuous regimen of long-term drug treatment, however, resulted in an attenuated locomotor response to morphine [24]. Chronic intermittent injections of cocaine augmented cocaine-induced seizure activity, whereas tolerance to the convulsion-inducing effects of cocaine were reported after animals were exposed to a long-term continuous regimen of cocaine administration (for review see [20]).

It is well established that certain components of the stereotypic response to amphetamine become sensitized after long-term amphetamine treatment [10,11]. For example, focused stereotypies such as repetitive limb movements and head bobbing occurred sooner after drug injection, and with a greater intensity after animals were exposed to repeated intermittent injections of amphetamine [22]. Similarly, amphetamine-induced sniffing had a shorter onset, heightened intensity, and longer duration after drug injection, when rats were exposed to long-term intermittent administration of amphetamine [4]. Although the enhanced stereotypic response to amphetamine after chronic intermittent amphetamine treatment is well established, recent evidence indicates that the behavioral outcome of chronic amphetamine administration is not dependent so much on the amount of drug administered, but rather the interval between drug injections during the chronic drug regimen appear to be critical in the development of behavioral sensitization [18]. Thus, rats exposed to daily intraperitoneal injections of amphetamine for 28 days showed an enhanced stereotypic response to amphetamine, whereas animals exposed to the same amount of drug via a subcutaneous implanted silicone pellet showed an attenuated response to amphetamine [5].

The present study was designed to evaluate the effects of long-term intermittent and continuous amphetamine administration on acoustic startle. In particular, it is well documented that animals show a measurable startle response after exposure to a loud acoustic stimulus, and the acoustic startle is facilitated by amphetamine treatment (for review see [3]). As is the case with amphetamine-induced stereotypic behaviors, the facilitated startle response ordinarily observed after amphetamine administration, was exacerbated when animals had a prior history of amphetamine treatment [12]. Since the sensitized startle after long-term amphetamine treatment was observed when animals were exposed to a chronic intermittent drug schedule, it was of interest in the present study to determine whether a similar behavioral sensitization would be observed after chronic continuous amphetamine administration.

The effects of long-term amphetamine administration on acoustic startle were evaluated in two experiments. In Experiment 1, the effects of amphetamine on acoustic startle were assessed after mice were chronically exposed to an

intermittent schedule of d-amphetamine sulfate, and in Experiment 2 the effects of amphetamine on acoustic startle were evaluated after mice received a chronic continuous drug regimen via an implanted Alzet minipump containing d-amphetamine.

METHOD

Subjects

Eighty Swiss mice procured from the Animal Resources Centre, University of Saskatchewan, served as subjects. Mice were housed individually in standard polypropylene cages, and allowed free access to food and water. Mice weighed 30–35 g at the time of testing and behavioral testing was carried out during the light portion of the light/dark cycle.

Apparatus and Procedure

Startle behavior was recorded in two acoustically insulated (styrofoam, 2.0 cm thick) circular chambers 28.0 cm in diameter and 21.0 cm high. The styrofoam floor of each chamber was positioned on an 8-W speaker (28.0 cm in diameter). Voltages produced by movements on the floor were fed to a Commodore PET Series 2001 Computer. The analogue signal from the speaker was amplified and digitized by an 8 bit A/D converter. The digitized output from the PET was printed out on a Data Terminal Mart printer. Only responses made during the tone presentation were measured and startle scores could vary from 1–5,000 units. The 2700 Hz tone (700 msec in duration, 5-msec rise-fall time) was generated by a Piezo Crystal Audio Transistor (Projects Unlimited, Dayton, OH) situated in the centre of the styrofoam roof of each chamber. The intensity of the tone in the chambers was 97 dB and background noise in the chambers was 44 dB. Sound intensity measurements were made with a Bruël Kjaer sound level meter (model 2203; A-scale).

Experiment 1—Intermittent Chronic Drug Schedule

Forty mice served as subjects in Experiment 1. Half of the subjects received two subcutaneous injections (10:00 a.m. and 4:00 p.m.) of 7.5 mg/kg of d-amphetamine sulfate (10 ml/kg volume) daily for 7 consecutive days, whereas the remaining half were treated with an equivalent volume of saline. On test day (Day 8), mice in each group were subdivided (N = 10 per group) and injected intraperitoneally with either saline or 3.0 mg/kg of d-amphetamine. Immediately after this injection, mice were placed into the startle chambers and allowed a 10 min adaptation period. Following the adaptation period mice were exposed to 160 tone presentations with a 10 sec intertone interval.

Experiment 2—Continous Chronic Drug Schedule

Forty mice were placed under light ether anesthesia and implanted subcutaneously in the upper back region with an ALZET osmotic minipump (model 2001; ALZA Corporation, CA), containing either 20 mg/ml of d-amphetamine sulfate or an equivalent volume of saline. The minipumps (200 μl resevoir volume) had a steady output for 7 days at which time the pumping rate declined rapidly to zero. The mean pumping rate was 0.971 microliters per hour, allowing for a total administration of 3.26 mg of d-amphetamine during the chronic phase of the experiment. This was comparable to the total amount of drug administered during the chronic phase of Experiment 1 (3.15 mg). On test day (Day 8), half of the mice in each group (N = 10 per group) were tested for acoustic startle after an intraperitoneal injection of 3.0 mg/kg of d-amphetamine sulfate, whereas the remaining subjects were tested after a saline injection. Minipumps were removed immediately after behavioral testing, and residual levels of amphetamine were measured. All other particulars concerning the testing procedure were identical to those described in Experiment 1.

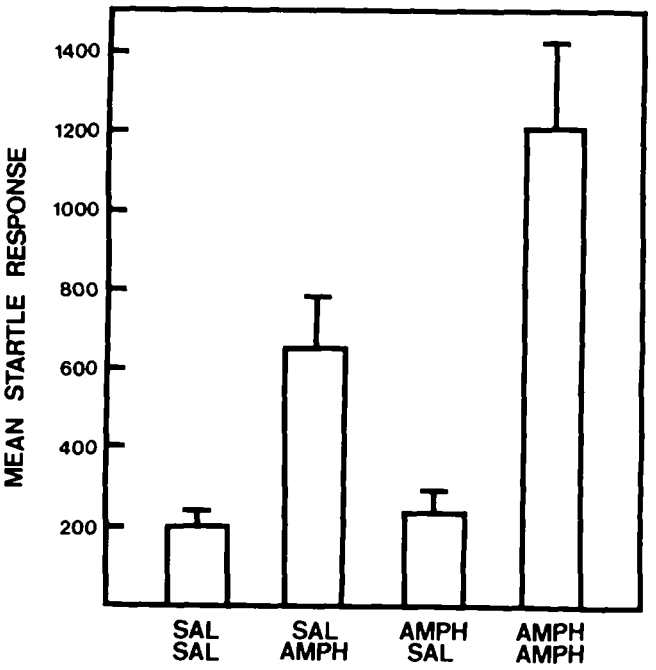

FIG. 1. Mean startle response (±S.E.M.) as a function of long-term intermittent amphetamine administration and test day drug treatment (saline or 3.0 mg/kg of d-amphetamine).

RESULTS

Experiment 1

Mean startle scores (±S.E.M.) over 160 tone presentations as a function of acute and chronic amphetamine treatment are shown in Fig. 1. Analysis of variance of the startle scores yielded a significant Chronic Drug Treatment × Test Day Drug Treatment interaction, $F(1,36)=4.2$, $p<0.05$. Newman-Keuls multiple comparisons ($\alpha=0.05$) of the simple main effects involved in the interaction, revealed that mice chronically exposed to daily intraperitoneal injections of saline and tested with 3.0 mg/kg of d-amphetamine showed a heightened acoustic startle response relative to mice chronically treated and tested with saline. As depicted in Fig. 1, long-term amphetamine treatment had no effect on acoustic startle when mice were tested with saline. That is, performance of mice in the amphetamine-saline group was comparable to that of mice in the saline-saline group. Repeated daily administration of amphetamine, however, substantially modified the facilitative effects of amphetamine on acoustic startle. Specifically, mice chronically treated and tested with amphetamine had significantly higher startle scores than mice in the saline-amphetamine group.

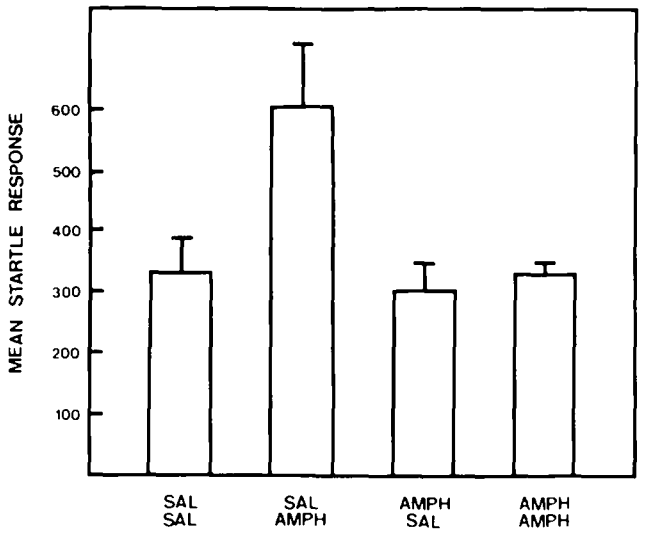

FIG. 2. Mean startle response (±S.E.M.) as a function of chronic continuous amphetamine treatment and test day drug treatment (saline or 3.0 mg/kg of d-amphetamine).

Experiment 2

Analysis of variance of the mean startle scores over 160 tone presentations yielded a significant Minipump Treatment × Test Day Drug Treatment interaction, $F(1,36)=5.11$, $p<0.05$. Consistent with the results of Experiment 1, 3.0 mg/kg of d-amphetamine enhanced acoustic startle among animals implanted with a minipump containing saline. This was not the case, however, when performance of mice that received long-term continuous exposure to amphetamine was considered. As can be seen in Fig. 2, mice implanted with a minipump containing d-amphetamine and tested with either saline or 3.0 mg/kg of amphetamine, had startle scores that were comparable to that of mice in the saline-saline group. Thus, in marked contrast to the results of Experiment 1, in which behavioral sensitization was observed after long-term intermittent treatment with amphetamine, continuous exposure to amphetamine via an implanted minipump attenuated the facilitative effects of amphetamine on acoustic startle.

DISCUSSION

Several aspects of the results of this study are consistent with previous reports involving the acute and chronic effects of amphetamine on acoustic startle. In particular, it is well documented that catecholamine stimulants, in general, have an excitatory effect on acoustic startle (for review see [3]). In addition to d-amphetamine, enhanced startle was observed after administration of l-amphetamine, apomorphine and L-Dopa [3,12]. Moreover, we recently reported that repeated daily administration of amphetamine for 10 consecutive days was sufficient to potentiate the facilitative effects of d-amphetamine and L-Dopa on acoustic startle [12].

The novel finding in this study involves the effects of a chronic continuous amphetamine schedule on the enhanced acoustic startle response to amphetamine. Unlike the effects of long-term intermittent amphetamine administration, which resulted in behavioral sensitization, the facilitative effects of amphetamine on acoustic startle were attenuated when animals received chronic continuous exposure to the drug. The development of behavioral sensitization after long-term intermittent amphetamine treatment, and tolerance after chronic continuous amphetamine administration, cannot be accounted for by the amount of drug administered during the chronic drug schedules, since under both chronic drug regimens comparable amounts of amphetamine were administered.

It could be argued, however, that since the minipumps were not removed prior to behavioral testing, continued release of amphetamine during the test session may have modified the enhancing effects of the drug on acoustic startle. Approximately 80–85% of the contents of the minipumps were released, thus it is possible that there was residual amphetamine released at the time of testing. However, it is unlikely that the low levels of the drug released at this time could account for the development of tolerance. Since it is well documented that amphetamine facilitates acoustic startle in a dose dependent manner [3], any residual release of amphetamine should have facilitated the enhancing effects of amphetamine on acoustic startle.

One possibility that may account for the development of tolerance after chronic amphetamine administration and sensitization after repeated intermittent administration of the drug, involves the disposition of amphetamine in brains of animals exposed to the two chronic drug regimens. After continuous exposure to amphetamine for 5 days, a gradual decline in brain levels of amphetamine was observed [8]. In contrast, studies involving chronic intermittent exposure to the drug have shown that brain levels of amphetamine were higher in animals exposed to the drug chronically, than in animals that received an acute injection of the drug [14,16]. It is likely that the increased brain levels of amphetamine resulted from the accumulation of the drug in inactive tissue. Specifically, it has been suggested that with repeated administration of amphetamine the drug accumulates in adipose tissue and is released upon subsequent amphetamine administration [23]. Recent reports, however, have shown that the accumulation of amphetamine in fat stores is not involved in the development of sensitization to the effects of amphetamine on locomotor activity, stereotypy and self-stimulation responding after repeated administration of the drug [13,15]. Consistent with these findings, sensitization was observed to the stereotypic response to amphetamine after exposure to only one previous injection of the drug [2].

It is unlikely, then, that changes observed in acoustic startle after long-term amphetamine treatment reflect the dispositional effects of amphetamine in peripheral or brain tissue. Rather, the possibility should be considered that the effects of amphetamine on neuronal substrates governing acoustic startle may vary according to the schedule of drug administration. Considerable attention has been focused on the effects of long-term amphetamine treatment on dopamine receptors [10, 11, 19], and since dopamine is an important modulator of acoustic startle [3], it might be the case that the development of tolerance and reverse tolerance may be subserved by parallel changes in dopamine receptor activity. However, the available dopamine receptor binding studies do not provide a clear picture of the relationship between the behavioral consequences of long-term amphetamine treatment and changes in dopamine receptor binding. For example, Ellison and Morris [5] reported that 5 days of continuous

amphetamine treatment resulted in decreased ^{3}H-spiroperidol accumulation in the caudate nucleus, substantia nigra and the nucleus accumbens. Long-term intermittent amphetamine administration also decreased ^{3}H-spiperone binding sites in the striatum [1, 6, 7]. In the nucleus accumbens, however, an increase in the number of dopamine binding sites was observed after 14 days of daily amphetamine administration [1]. Similarly, in the limbic system increased ^{3}H-spiperone binding sites were observed after 4 days of daily amphetamine administration, but not after 20 days of intermittent drug treatment [7]. Robertson [21] found increased ^{3}H-spiroperidol binding in both limbic and striatal areas after long-term intermittent amphetamine treatment. Given this state of affairs, it is difficult to make conclusions concerning the role of dopamine post-synaptic receptors in the development of tolerance after chronic continuous amphetamine treatment and reverse tolerance after long-term intermittent amphetamine administration. There is some evidence to suggest that the behavioral sensitization observed after exposure to a chronic intermittent schedule of amphetamine may involve a decreased number of dopamine pre-synaptic receptors [17]. However, there is a lack of similar research on the effects of continuous amphetamine administration on dopamine autoreceptors.

Although the neurochemical substrates subserving the development of tolerance and reverse tolerance are not well understood, it should be stressed at this point that the effects on behavior observed after long-term amphetamine administration appear to be dependent upon the interval between successive drug injections. That is, in the presence of continuous low levels of the drug, the organism appears to adapt to the behavioral consequences of amphetamine, whereas intermittent stimulation with high doses of the drug sensitized animals to later amphetamine administration. Similar observations involving the behavioral effects of continuous and intermittent stimulation have been made with a variety of other manipulations. For example, intermittent electrical stimulation of the amygdala resulted in behavioral seizures, whereas seizures were not observed after continuous amygdaloid stimulation (for review see [19,20]). Intermittent application of a stressor will lead to a number of well documented neurochemical changes and behavioral deficits, whereas neurochemical and behavioral adaptation is observed after prolonged stress [19].

One contributing factor to the development of tolerance and reverse tolerance after chronic amphetamine administration may involve the presence or absence of sensory cues during the chronic drug schedule [5]. There is some evidence to suggest that stimulus factors associated with drug administration play a role in the development of behavioral sensitization. Specifically, rats injected with amphetamine and tested for self-stimulation responding immediately after drug treatment showed enhanced rates of responding. When this procedure was carried out daily for 10 consecutive days, rats showed increased response rates to a saline injection [13]. Increased response rates to a saline injection were not observed when rats received daily behavioral testing followed by amphetamine administration [13]. Similar results were reported with respect to the effects of long-term amphetamine administration on locomotor activity [25]. These findings suggest that the contiguity between the systemic effects of the drug and the stimulus array associated with the injection procedure, contribute to the development of behavioral sensitization after intermittent amphetamine treatment. It might be the case that the absence of sensory cues associated with the drug experience during chronic continuous amphetamine administration, may play a role in the development of tolerance.

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