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Repeated Stimulation of the Ventral Tegmental Area Sensitizes the Hyperlocomotor Response to Amphetamine

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BEN-SHAHAR, O. AND A. ETTENBERG. *Repeated stimulation of the ventral tegmental area sensitizes the hyperlocomotor response to amphetamine.* PHARMACOL BIOCHEM BEHAV **48**(4) 1005–1009, 1994.—The locomotor-activating effects of amphetamine have been reported to increase with repeated drug administration. Although the precise underlying mechanisms for this behavioral sensitization effect remain unknown, many investigators have suggested a role for the mesolimbic dopaminergic system that emanates from cell bodies in the ventral tegmental area (VTA) of the midbrain. To test this hypothesis, the present study examined the effects of repeated electrical stimulation of the VTA (in place of repeated amphetamine administration) on the hyperlocomotor actions of *d*-amphetamine. Locomotor activity induced by 0.75 mg/kg SC amphetamine was assessed during two 90-min tests, one before and one after a 14-day treatment regimen during which animals experienced daily 15-min sessions of intracranial VTA stimulation. Each session involved the delivery of 600 trains of 0.5 s 60-Hz sine-wave stimulation applied at one of four intensities: 0, 15, 30, or 45 μA. An additional comparison group of rats self-administered 30 μA of VTA stimulation. Data analysis revealed that both the self-stimulation and the high current groups were reliably more active posttreatment compared to pretreatment. No such sensitization-like effects were observed in any of the other treatment groups. These results are consistent with the hypothesis that repeated activation of VTA neurons can produce a sensitization to the behavioral effects of *d*-amphetamine.

Amphetamine VTA Intracranial stimulation Dopamine Locomotor behavior Drug sensitization

SYSTEMIC administration of amphetamine produces dose-dependent elevations in locomotor activity and, at higher doses, the development of stereotyped behaviors. It is generally accepted that the occurrence of amphetamine-induced hyperactivity requires an intact mesolimbic dopaminergic system, while amphetamine-induced stereotypy depends upon the pathways of the nigrostriatal dopaminergic system (6,10,11, 14,18,19). More recent research has demonstrated that repeated administration of amphetamine results in sensitization to its behavioral effects (21,22). This is reflected by successive increases in drug-induced hyperactivity (16,23,25), stereotypy (7,15), and even reinforcement (13), with repeated drug administration.

Robinson and Becker (22) have argued that the behavioral sensitization to amphetamine is mediated, at least in part, by presynaptic changes in the dopaminergic pathways that constitute the mesocorticolimbic system (i.e., neurons emanating from the VTA and project to the nucleus accumbens and to the prefrontal cortex). These authors postulated that the relevant presynaptic changes were characterized by enhanced dopamine (DA) release, a result consistent with more recent reports demonstrating that behavioral sensitization to amphetamine is related to autoreceptor subsensitivity in the VTA (24,26). The presence of a presynaptic DA mechanism underlying amphetamine-induced behavioral sensitization is further supported by the demonstration that repeated intracerebral injections of amphetamine directly into the VTA, but not the nucleus accumbens, resulted in sensitization to drug-induced hyperactivity [(2,8,9) but see (4)].

If, indeed, the VTA represents a primary structure underlying the locomotor sensitization effects of amphetamine, then one might hypothesize that selective and repeated electrical activation of the VTA would produce changes in amphetamine-induced hyperlocomotion similar to those observed fol-

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lowing repeated drug exposure (i.e., behavioral sensitization). Consistent with this hypothesis is the experiment by Druhan et al. (3) which showed that experimenter-administered stimulation of the VTA can facilitate performance on a discrimination task cued by low doses of amphetamine. Thus, VTA stimulation can mimic the stimulus properties of amphetamine. Also relevant are the results of Borowski and Kokkinidis (1) who reported that repeated administration of amphetamine resulted in a sensitization to the drug's stimulatory effects on VTA self-stimulation. The present experiment was, therefore, devised to test the notion that repeated electrical stimulation of the VTA will result in a sensitization to the locomotor hyperactivity produced by an acute application of *d*-amphetamine.

METHOD

Subject

The subjects were 56 male albino Sprague-Dawley rats (400–500 g) obtained from Charles River Laboratories. The animals were individually housed in wire hanging cages located within a temperature-controlled (22°C), 12L : 12D (lights on at 0700 h) vivarium environment. Food and water were made available on an ad lib basis throughout the course of the experiment.

Surgery

Each rat was stereotaxically implanted with a bipolar stimulating electrode (Plastic One, diameter = 0.14 mm) aimed at the VTA (in the no-stimulation control group the electrode was aimed at the VTA but then removed immediately). Surgery was conducted under deep anesthesia produced by 50 mg/kg IP sodium pentobarbital supplemented with 80 mg/kg IP chloral hydrate. An additional 250 μg of atropine sulfate (in a volume of 0.2 ml) was administered IM to alleviate potential respiratory congestion. The toothbar of the stereotaxic instrument was set at 5.0 mm above the interaural line and the VTA electrodes were implanted −2.8 mm posterior to bregma, 1 mm lateral to midline, and 8.8 mm ventral to the skull surface.

Apparatus

Intracranial stimulation apparatus. The chambers in which the intracranial VTA stimulation was delivered were six identical wood-constructed boxes with Plexiglas front doors. Each box measured 26 cm (L) × 26 cm (W) × 66 cm (H). Electrode leads were connected to mercury swivel commutators mounted above each chamber to provide the animal freedom of movement during the stimulation sessions. Some animals were trained to lever press for VTA stimulation and others received computer-delivered stimulation (see the Procedure section below). In each case, the stimulation was generated by a custom-built sine-wave stimulator in conjunction with a TRS-80 Model 4 personal computer linked to a Lafayette System interface. Each animal received 0.5 s trains of 60 Hz stimulation at intensities ranging from 0–45 μA (see the Procedure section). For animals self-administering the stimulation, a metal lever was made accessible midway on the rear wall of each chamber 5.0 cm above the metal grid floor.

Locomotor activity apparatus. Spontaneous locomotion was assessed by placing animals individually into 1 of 16 identical metal wire-hanging cages that each measured 36 cm (L) × 26 cm (W) × 20 cm (H). Each cage contained two sets of infrared emitter-detector photocells positioned along the long axis 1 cm from the floor and 8 cm from the front and back of the cage. Movement within the cages produced photocell interruptions that were automatically recorded at 5-min intervals.

Procedure

All animals were provided 7 days to recover from stereotaxic surgery after which each was administered a single 0.75 mg/kg SC injection of *d*-amphetamine sulfate (Sigma). Five minutes postinjection, the animals were individually placed into one of the locomotor activity cages and a 90-min locomotor pretest was conducted. Following this test, subjects were returned to their homes cages.

Twenty-four hours after the locomotor pretest animals were randomly assigned to one of five groups, each associated with a different treatment regimen. Four of the groups were administered 600 trains of stimulation over a 15-min session each day for 14 days. The groups differed only in the current intensity of the stimulation that they received: 0 μA (n = 13), 15 μA (n = 9), 30 μA (n = 9), or 45 μA (n = 12). A fifth group (n = 13) consisted of animals that were shaped to lever press for 30 μA of VTA stimulation. The self-stimulation group remained in the operant boxes each day until they had earned the equivalent 600 trains of stimulation (mean session duration for theses subjects was approximately 13 min).

Twenty-four hours after the final (14th) treatment session, the animals were again administered a 0.75 mg/kg SC injection of *d*-amphetamine and placed into the locomotor activity apparatus for a 90-min test.

Histology

Upon completion of the experiment the animals were killed by overdose of sodium pentobarbital and perfused through the heart with 0.9% physiological saline followed by a solution of 10% Formalin. The brains were removed and stored in 10% Formalin solution until histological analyses could be conducted. Electrode tip locations within the brain were identified from 50 micron cresyl violet-stained frozen sections.

RESULTS

Figure 1 illustrates the location of electrode sites in or around the VTA for each of the 56 animals in the experiment. Although there was some normal variability, no systematic difference appeared in electrode locations between the groups. Figure 2 illustrates the mean (±SEM) performance of each group during the pretest baseline and test. The panel on the lower right of the figure depicts the mean (±SEM) total activity counts during the 90-min pretest and test.

The data from each of the panels in Fig. 2 were subjected to a separate two-factor ANOVA (test × time). Although all groups showed some increase in activity on the test relative to pretest baseline, these effects reached statistical significance in only two groups: the 45 μA and 30 μA self-stimulation conditions [respective main effects of TEST: $F(1,12) = 20.04$, $p < 0.001$, and $F(1,11) = 16.44$, $p < 0.002$]. All groups exhibited a normal habituation response in which activity gradually decreased during the course of the 90-min test sessions [F scores for 0–45 μA groups and 30 μA self-stimulation group were respectively as follows: $F(17,204) = 14.5$; $F(17,136) = 9.38$; $F(17,136) = 7.88$; $F(17,204) = 9.58$, and $F(17,187) = 9.05$,

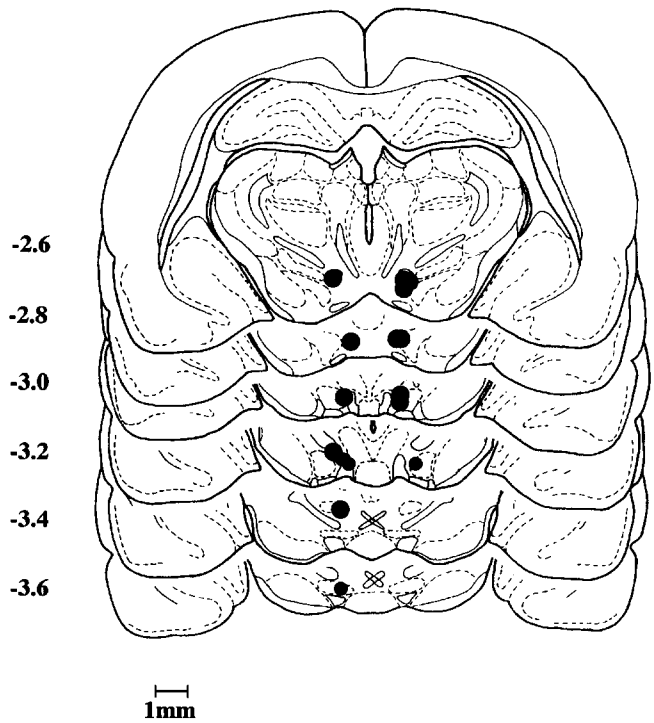

FIG. 1. Histological verification of electrode placements in the VTA. Due to the large number of animals employed in this work, illustration of the specific locations of each electrode tip was not practical. This figure, therefore, represents a summary of the histological results with the black areas designating the regions within which electrode placements were implanted. Illustrations were redrawn from Pellegrino, Pellegrino, and Cushman (17). No systematic group differences in electrode placements existed.

$p < 0.001$]. However, the self-stimulation 30 μA group, $F(17,187) = 1.88$, $p < 0.03$, demonstrated a test × trial interaction, suggesting that the stimulation-induced sensitization worked to alter the pattern with which these animals habituated to the locomotor activity chambers over time. A similar result approached significance in the 45 μA group, $F(17,204) = 1.58$, $p = 0.07$. These effects can be seen in Fig. 2 where test activity (for these two groups) decreased at a lesser rate than the comparable activity of the other three groups.

DISCUSSION

Repeated electrical stimulation of the VTA resulted in a behavioral sensitization to the locomotor-activating effects of amphetamine. The present data are, therefore, consistent with previous reports that amphetamine-induced hyperactivity is mediated by the mesolimbic dopaminergic pathway (10,11, 18,19) and that VTA stimulation can mimic some of the behavioral effects of amphetamine (3). Furthermore, just as the magnitude of the behavioral sensitization produced by repeated amphetamine administration increases with dose (22), so, too, in the present experiment does low levels of VTA stimulation (i.e., current intensities of 15 or 30 μA) produces less sensitization than 14 days of the higher 45 μA stimulation.

It has been suggested that sensitization to amphetamine's locomotor activity effects is due to changes in presynaptic mechanisms within the mesocorticolimbic dopaminergic pathway (22). Consistent with this hypothesis is the report that repeated injections of amphetamine into the VTA, but not the nucleus accumbens, resulted in behavioral sensitization to amphetamine-induced hyperactivity (2,8,9). Clearly, the present data on the effects of repeated VTA stimulation are also consistent with this hypothesis. Also, relevant to these results are the data of Kokkinidis et al. (12) demonstrating that repeated electrical stimulation of either the striatum or the nucleus accumbens can also produce alterations in the sensitivity of subjects to the hyperlocomotor effects of amphetamine. These investigators used a moderate dose of amphetamine (3 mg/kg) which produced both hyperactivity and stereotypy in treated subjects. They reported that their nonstimulation control group showed the most stereotyped behavior and the least hyperactivity. In contrast, animals that received stimulation of the nucleus accumbens showed higher hyperactivity levels and lower stereotypy levels, while subjects administered stimulation of the caudate nucleus showed the highest hyperactivity levels and the lowest stereotypy levels. The Kokkinidis et al. (12) results, therefore, suggest that repeated electrical stimulation of the terminals regions of the mesolimbic and nigrostiatal DA systems may alter the sensitivity of efferent pathways between the circuits underlying stereotypy and locomotor reactions to amphetamine. Their results, nevertheless, remain consistent with those reported here in that both studies observed increases in amphetamine-induced locomotion when either the terminals (12) or cell bodies (present paper) of the mesolimbic system are repeatedly stimulated.

A final comment concerns the results of the self-stimulation animals who demonstrated greater shifts in amphetamine sensitivity than the comparable computer-stimulated 30 μA group. There is considerable evidence that response-contingent stimulation is more effective (i.e., rewarding) than stimulation having identical parameters but passively administered [e.g., (5)]. The explanation for this may be related to the findings of Porrino et al. (20) who reported that self-stimulation of the VTA resulted in a greater elevation in glucose utilization in the terminal fields of the mesolimbic system, compared to comparable experimenter-administered stimulation. Therefore, it may be that the more potent effects of self-administered compared to passively applied 30 μA stimulation in the current experiment resulted from a greater neurophysiological reaction in mesolimbic DA circuitry. Presumably, the act of lever pressing serves to recruit motor circuits whose impact on VTA and/or nucleus accumbens elements sums with the neurophysiological effects of the intracranial stimulation.

In summary then, previous reports have described a behavioral sensitization to the hyperlocomotor effects of repeatedly applied amphetamine (16,23,25). Such effects have been attributed by some to the impact of the drug on dopaminergic circuits within the mesolimbic system (22). The present study provided evidence in support of this hypothesis by demonstrating that repeated electrical stimulation of the cell bodies of origin of the mesolimbic system (i.e., the VTA) can also produce a sensitization to amphetamine's locomotor-activating effects.

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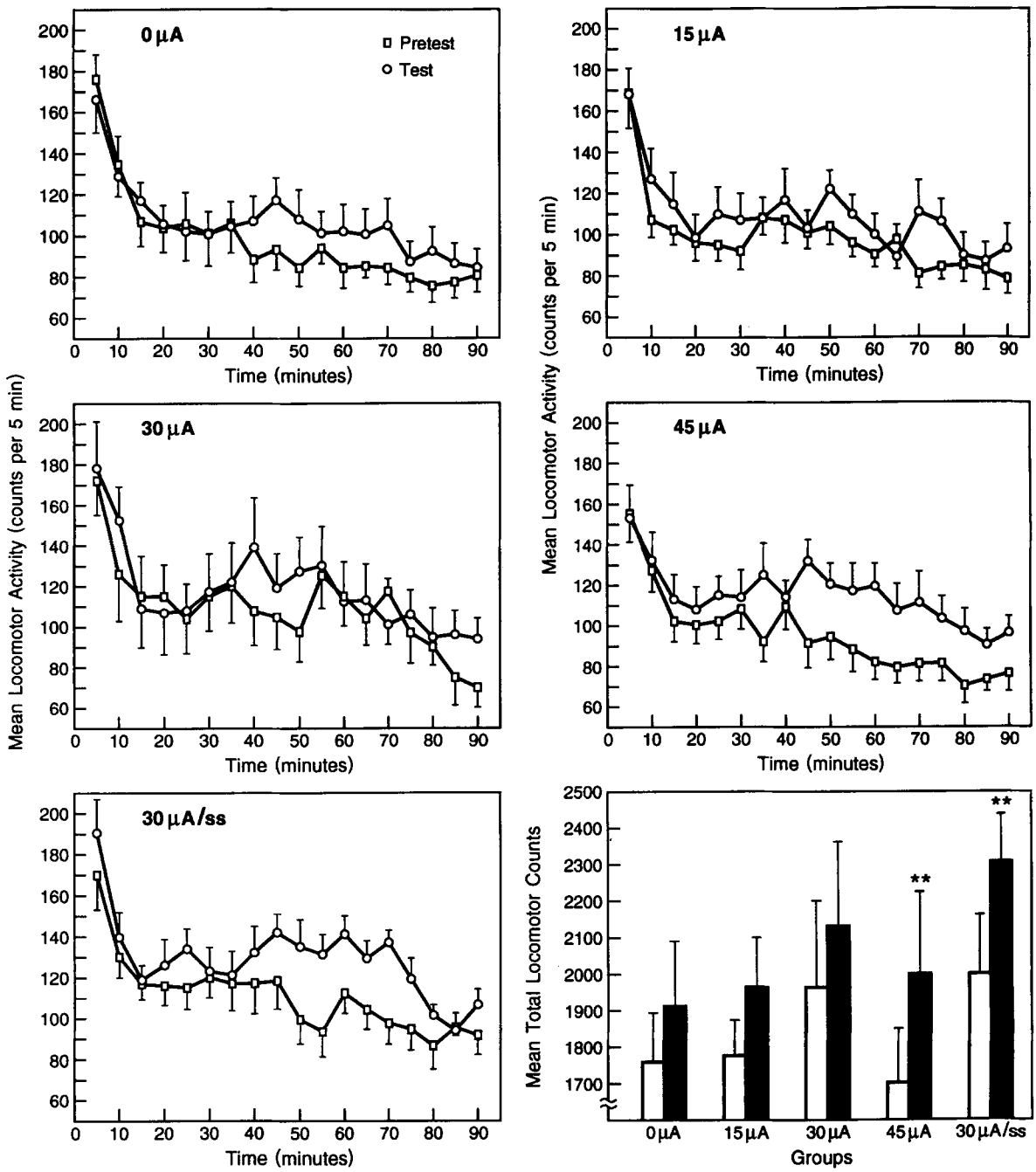

FIG. 2. The individual line drawings each illustrates the mean amphetamine-induced activity (±SEM) for each group of animals before (baseline/pretest) and after (test) 14 days of treatment with 15-min sessions of 0, 15, 30, or 45 μA VTA stimulation. The 30 μA/ss group represents subjects who self-administered the stimulation. Mean total baseline and test responses (±SEM) for each group over the entire 90-min session are depicted in the histogram at the bottom right (**represents significant pretest to test differences with $p < 0.002$). Note that the locomotor response to 0.75 mg/kg *d*-amphetamine was enhanced following repeated VTA stimulation in the 45 μA and 30 μA/ss groups.

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