Pharmacology Biochemistry and Behavior, Vol. 53, No. 4, pp. 899–902, 1996

0091-3057/96 $15.00 + .00

SSDI 0091-3057(95)02059-4

Fimbria-Fornix Lesions Do Not Block Sensitization to the Psychomotor Activating Effects of Amphetamine

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Received 25 March 1995; Revised 20 June 1995; Accepted 6 July 1995

BROWMAN, K. E., A. BADIANI AND T. E. ROBINSON. *Fimbria-fornix lesions do not block sensitization to the psychomotor activating effects of amphetamine.* PHARMACOL BIOCHEM BEHAV **53**(4) 899–902, 1996. – The repeated, intermittent administration of amphetamine produces a long-lasting sensitization to its behavioral activating effects. Excitatory amino acid receptors in the striatum have been implicated in the development of amphetamine sensitization, and one source of excitatory amino acid input to the striatum is the hippocampus. The purpose of this experiment, therefore, was to determine if an intact hippocampal system is necessary for either the development or expression of sensitization to the psychomotor activating effects of amphetamine. Rats received either fimbria-fornix lesions or sham lesions and approximately 2 weeks later received 10 injections of 3.0 mg/kg *d*-amphetamine or saline (IP) every other day. Rotational behavior was quantified as an index of amphetamine's psychomotor stimulant effects. Animals with a fimbria-fornix lesion were hyperresponsive to an acute injection of amphetamine, but animals with a fimbria-fornix lesion and control animals did not differ in the *development* of sensitization (i.e., the rate of sensitization). Furthermore, both groups *expressed* comparable sensitization (relative to their respective saline-pretreated control groups) when given a challenge injection of amphetamine. These results suggest an intact hippocampal system is not necessary for the development or expression of amphetamine sensitization.

Hippocampus Environment-specific sensitization Mesostriatal dopaminergic system 6-OHDA lesions Rotational behavior Rat

THE REPEATED intermittent administration of amphetamine produces a progressive enhancement (sensitization) in its psychomotor stimulant effects, and this is accompanied by neuroplastic adaptations in the mesostriatal DA system (8,16). Amphetamine sensitization is a very long-lasting phenomenon (13), suggesting that neural changes produced by a chronic intermittent amphetamine treatment may be similar to neural changes associated with other forms of experience-dependent plasticity (18). Consistent with this, excitatory amino acid receptors, which have been implicated in associative learning, have also been implicated in the development of drug sensitization (9,10,19). One of the neural structures known to provide excitatory amino acid inputs to the striatum is the hippocampus (5). It is possible, therefore, that projections from the hippocampus to the striatum modulate the development and/or expression of behavioral sensitization. In support of this hypothesis, some researchers have reported that bilateral lesions of the hippocampal projections to the ventral striatum (via the fimbria-fornix) block the development (20), but not the expression (21), of amphetamine sensitization in the rat.

The present experiment was designed to further investigate the role of the hippocampal system in the development and expression of sensitization using a paradigm that is known to produce robust sensitization, and a behavioral measure that permits the accurate quantification of the rate and degree of behavioral sensitization: rotational behavior in unilateral DA-denervated rats (15). The quantification of rotational behavior offers a number of advantages over measures of locomotor activity. For example, a progressive increase in drug effect during sensitization results in a progressive increase in rota-

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tional behavior, whereas a progressive increase in drug effect is not necessarily characterized by a progressive increase in locomotor activity (2,15). The purpose of the present experiment was twofold: 1) to investigate whether damage to the hippocampal system (fimbria-fornix lesions) affects the *development* of amphetamine sensitization; and 2) to investigate whether fimbria-fornix lesions disrupt the *expression* of sensitization on a challenge test day, relative to saline-pretreated control animals.

METHODS

Subjects

Male Sprague–Dawley rats (Harlan Co., Indianapolis, IN), weighing 200–250 g at the beginning of the experiment, were housed two per cage in stainless steel wire hanging cages in a temperature-controlled room with a 14 L : 10 D cycle (lights on from 0600 to 2000 h). The rats had free access to standard rat chow and water.

Surgical and Screening Procedures

After approximately 7 days of habituation to the animal colony 55 animals received a unilateral 6-OHDA lesion of the mesostelencephalic DA system using procedures described previously (15). One week after the 6-OHDA lesion the rats were screened for apomorphine-induced rotation (0.05 mg/kg) to determine the degree of DA denervation (7). At least 1 day later, 24 rats that passed the apomorphine screen received a bilateral anodal electrolytic lesion of the fimbria-fornix (FIMBRIA-FORNIX), or received a sham surgery (SHAM; $n = 20$). The lesion was made using Nichrome electrodes insulated with Teflon to within approximately 0.8 mm of the tip, which were lowered bilaterally in three locations, coordinates measured from bregma in mm: i) anterior/posterior (AP), −0.85; medial/lateral (ML), ±1.4; dorsal/ventral (DV), 5.1; ii) AP, −0.85; ML, ±0.8; DV, 5.1; iii) AP, −0.85; ML, 0; DV, 5.1. Lesions were made by passing 1 mA of constant current for 20 s at each site via a rectal cathode. After recovery from anesthesia the animals were returned to individual cages in the animal room.

Protocol

Two weeks after surgery FIMBRIA-FORNIX and SHAM animals received 10 IP injections (one injection every other day) of either 3.0 mg/kg of amphetamine or saline. On testing days the animals were transported to a testing room, placed in cylindrical plastic cages (see below), tethered to a rotometer by an elastic harness, and given an injection of either amphetamine or saline. Three days after the last pretreatment all rats (i.e., including saline pretreated) received a challenge injection of 3.0 mg/kg of amphetamine in this apparatus. Rotational behavior was quantified for 90 min following each injection using automated rotometers previously described (11). The test cages were cylindrical plastic buckets with ground corn cob bedding on the floor of the cages, and white noise present during all test sessions.

Histology

After completion of the behavioral studies the animals were given an overdose of sodium pentobarbital and transcardially perfused with physiological saline, followed by 10% buffered formalin. Brains were postfixed in formalin for 1 day following perfusion, and then for an additional 2 days to 1 week in sucrose formalin. They were then frozen and cut into 40-μm-thick sections. Every other section through the fimbria-fornix was stained with cresyl violet and every 10th section through the hippocampus was stained for acetylcholinesterase using a procedure modified from Butcher (3).

Drugs

6-OHDA (2,4,5-trihydroxyphenethylamine hydrobromide) was dissolved (2 mg/ml) in a saline-ascorbate solution. Desipramine hydrochloride was given IP (15 mg/kg in 1 ml/kg distilled water). Apomorphine hydrochloride was dissolved in a saline-ascorbate solution and 0.05 mg/kg was injected SC in the neck. *d*-Amphetamine sulfate was administered IP (3.0 mg/kg, weight of the salt, in 1 ml/kg saline). All these drugs were purchased from Sigma Chemical Company (St. Louis, MO). Sodium pentobarbital (The Butler Company, Columbus, OH) was given IP (52 mg/kg in 0.8 ml/kg of a 10% ethanol solution).

RESULTS

A total of seven amphetamine-pretreated animals and eight saline-pretreated animals had a complete lesion of the fimbria-fornix, as assessed by *both* examination of the cresyl violet stained sections and an absence of acetylcholinesterase staining in the hippocampus. The criteria for including animals were i) all areas of the fimbria-fornix were completely destroyed bilaterally (medial to lateral and dorsal to ventral) at one level along the rostrocaudal axis in the cresyl violet stained sections, and ii) there was no detectable acetylcholinesterase staining in the dorsal hippocampus of the same animal. Indeed, a few animals appeared to have a complete lesion when the cresyl violet-stained sections above were examined, but had detectable acetylcholinesterase staining, and were therefore eliminated. On the other hand, all animals with a complete loss of acetylcholinesterase staining in the hippocampus had a complete lesion, as assessed by examination of the cresyl violet-stained section. Figure 1 illustrates a representative lesion on plates taken from Paxinos and Watson (14).

Figure 2A shows the number of amphetamine- and saline-induced rotations across test days in FIMBRIA-FORNIX and SHAM animals. Figure 2A illustrates three major findings. i) There was a greater acute effect of amphetamine on rotational behavior in the FIMBRIA-FORNIX group than in the SHAM group (see figure legend for statistics). ii) Repeated treatment with amphetamine produced progressively greater rotational behavior with successive injections (i.e., sensitization) in both groups, as indicated by slope coefficients that were greater than zero. iii) The rate of sensitization was the same in FIMBRIA-FORNIX and SHAM animals, as indicated by a comparison of the slope coefficients in the two groups (see Fig. 2A).

Figure 2B shows the mean number of rotations on the challenge test day when both amphetamine- and saline-pretreated animals received an injection of amphetamine. The amphetamine challenge produced significantly greater rotational behavior in amphetamine-pretreated animals than in saline-pretreated controls, regardless of whether or nor animals had a fimbria-fornix lesion.

DISCUSSION

Two major findings are reported here. i) Although fimbria-fornix lesions enhanced the acute psychomotor response to amphetamine (rotational behavior in rats with a unilateral 6-OHDA lesion of the mesostriatal dopamine system), they

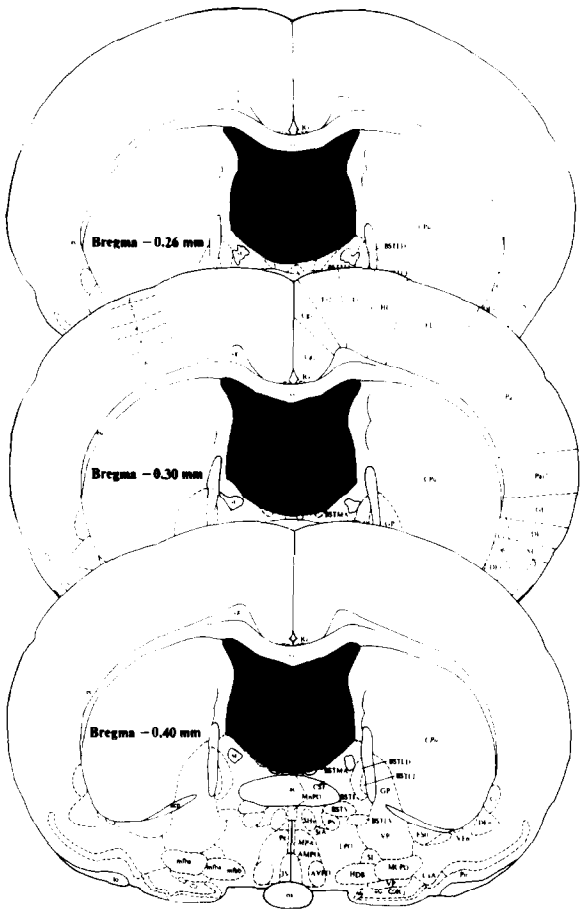

FIG. 1. Illustrations of coronal sections of the rat brain showing the extent of a representative lesion of the fimbria-fornix region. Animals were included in the analysis only if i) examination of cresyl violet-stained sections revealed complete bilateral destruction of the fimbria-fornix (medial to lateral and dorsal to ventral), and ii) acetylcholinesterase staining in the dorsal hippocampus was nondetectable, relative to animals with a sham lesion. Plates are taken from Paxinos and Watson (14).

did not effect the rate of amphetamine sensitization (i.e., the *development* of sensitization). ii) There was no effect of a fimbria-fornix lesion on the *expression* of sensitization.

Consistent with the data reported here, Wolf and her colleagues (4) have reported that fimbria-fornix lesions do not block the expression of behavioral sensitization to the locomotor response to amphetamine. In contrast, Yoshikawa and colleagues (20,21), reported that fimbria-fornix lesions disrupt the development of sensitization, but not the expression of sensitization to methamphetamine. It is not clear what accounts for the apparent discrepancy between the current findings and those by Yoshikawa and colleagues (20,21). One difference between the two studies is that in the current study rotational behavior was quantified, whereas Yoshikawa and colleagues quantified locomotor activity (20,21). It is unlikely that this accounts for the difference, however, because Wolf and colleagues (4) measured locomotor activity and obtained results similar to those reported here. Another difference between these studies is that Yoshikawa and colleagues (20) did not find that animals with fimbria-fornix lesions were hyperresponsive to the acute effects of methamphetamine. In the present study, however, animals with fimbria-fornix lesions were clearly hyperresponsive to the acute psychomotor effects of amphetamine, as has been reported by a number of authors (1,4,6,12). It is possible this reflects a difference between methamphetamine and *d*-amphetamine, but this is unlikely because Mittleman and colleagues (12) reported recently that heightened locomotion occurs in animals with hippocampal damage in response to a variety of direct or indirect dopamine agonists.

In conclusion, the current results do not support the notion that fibers passing through the fimbria-fornix are essential for either the development or expression of sensitization to the psychomotor activating effects of amphetamine [also see (4)]. It is still possible, however, that the hippocampus plays a role in context-specific sensitization, because contextual stimuli can gate the expression of sensitization [for a recent review see (18)], and the hippocampus is thought to be involved in

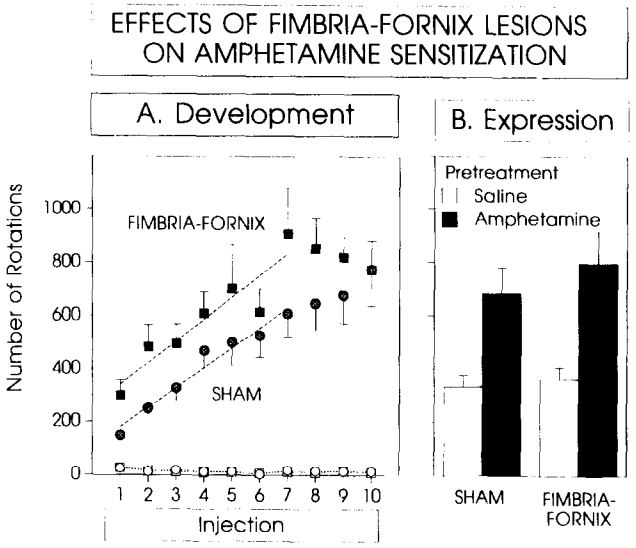

FIG. 2. The effects of fimbria-fornix lesions on the development and expression of amphetamine sensitization. (A) The mean ± SE number of rotations in response to ten injections (one every other day) of 3.0 mg/kg amphetamine IP in SHAM (●) or FIMBRIA-FORNIX (■) animals, or saline in SHAM (○) or FIMBRIA-FORNIX (□) animals. There was a significant difference in the acute response to amphetamine between SHAM and FIMBRIA-FORNIX animals (unpaired *t*-test, $t = 2.87$, $p = 0.012$ on data from injection one). Sensitization was quantified by calculating regression lines from data of individual animals over the first seven injections. The first seven injections were used because the average increase in drug response was relatively linear over that period of time in both groups, for SHAM ($r^2 = 0.958$) and for FIMBRIA-FORNIX ($r^2 = 0.857$). A slope coefficient significantly greater than zero indicates a progressive increase in drug effect (i.e., sensitization). Both groups given amphetamine had slope coefficients that were significantly greater than zero FIMBRIA-FORNIX (mean slope coefficient, 81.5 ± 18.33, $t = 4.45$, $p = 0.004$), SHAM mean slope coefficient (74.84 ± 16.09, $t = 4.65$, $p = 0.001$). There was no group difference in slope coefficients (unpaired *t*-test, $t = 0.27$, $p = 0.79$). (B) The mean total number of rotations in response to an amphetamine challenge given to both amphetamine- and saline-pretreated animals. A two-way ANOVA indicated a significant effect of pretreatment, $F(1, 31) = 23.05$, $p < 0.0001$, but no effect of lesion, $F(1, 31) = 0.742$, $p = 0.4$, and no pretreatment by lesion interaction, $F(1, 31) = 0.263$, $p = 0.61$.

contextual learning. Moreover, in all of the experiments on the effects of fimbria-fornix lesions on sensitization published thus far, drug administration was paired with a specific test environment, and this procedure does not allow one to assess the involvement of contextual factors in sensitization.

ACKNOWLEDGEMENTS

This research was supported by a grant from the NIDA (04294 to T.E.R.). We would like to thank Charla Ewing, Megan Clancy, and Hans Crombag for their help in conducting this experiment.

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