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Involvement of the Fimbria Fornix in the Initiation But Not in the Expression of Methamphetamine-Induced Sensitization

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YOSHIKAWA, T., A. WATANABE, H. SHIBUYA AND M. TORU. *Involvement of the fimbria fornix in the initiation but not in the expression of methamphetamine-induced sensitization.* PHARMACOL BIOCHEM BEHAV **45**(3) 691–695, 1993.—To put forward our previous finding that the lesion of the fimbria fornix, a hippocampo-accumbal pathway, blocked the development of behavioral sensitization induced by repeated methamphetamine (MAP) administrations, we examined the role of the fimbria fornix in the expression of sensitization in this study. After rats had shown locomotor augmentation following repeated drug injections, they received either the fimbria fornix lesion or a sham operation. Both groups of rats still exhibited a similar sensitized locomotor response to MAP as before the surgeries. In addition, we evaluated dopamine metabolism in the nucleus accumbens of these rats following a challenge injection of MAP after the behavioral study. The data obtained correspond to the results of behavioral experiments in that 3-methoxytyramine, one of the dopamine metabolites, increased significantly after MAP challenge only in the groups of sensitized animals. These findings further support the recent concept that there may exist different neural mechanisms in the initiation and expression of sensitization phenomenon.

Methamphetamine Behavioral sensitization Locomotor activity Hippocampus Dopamine Nucleus accumbens

THE subchronic intermittent administration of low doses of methamphetamine (MAP) produces enhanced locomotor responses to the drug in rats. This phenomenon is called behavioral sensitization and has been extensively studied as an animal model of the relapse liability in amphetamine psychosis and schizophrenic disorders [for review, see (15)]. While there is general agreement on increased response of mesolimbic dopamine (DA) neurotransmission following MAP challenge in sensitized animals (11), the precise mechanisms underlying sensitization are still unknown. For example, it seems difficult at present to satisfactorily explain the production of long-lasting changes characteristic of this phenomenon with reference to the process of the intradopaminergic system alone.

Recently, several lines of evidence indicated a role of excitatory amino acid systems in the induction of neural plasticity including behavioral sensitization (3,8). We supposed a possible involvement of the hippocampus (HPC) in behavioral sensitization because the HPC is shown to coordinate exploratory behavior probably via its glutamatergic efferents to the nucleus accumbens (NAC) (24), a critical substrate for mediating locomotion (13), and the HPC plays an important role in the memory/learning process [for review, see (1)] that may relate to some aspects of sensitization (17). Indeed, we recently demonstrated that the destruction of the fimbria fornix, a hippocampo-accumbal pathway (10), blocked the development of locomotor sensitization induced by repeated injections of MAP (27).

Other results of sensitization studies now suggest that different neural mechanisms may be involved in the initiation and expression of sensitization phenomenon [for review, see (7)]. The present study was therefore undertaken to gain further insight into our previous finding by exploring the effects of the fimbria fornix lesion on the expression of behavioral sensitization and evaluating DA metabolism in the NAC associated with behavioral manifestation.

METHOD

Behavioral Measurements

Thirty-two male Wistar rats, weighing 180–200 g on arrival, were allowed free access to food and water and maintained on a 12 L : 12 D cycle that corresponded with the day/

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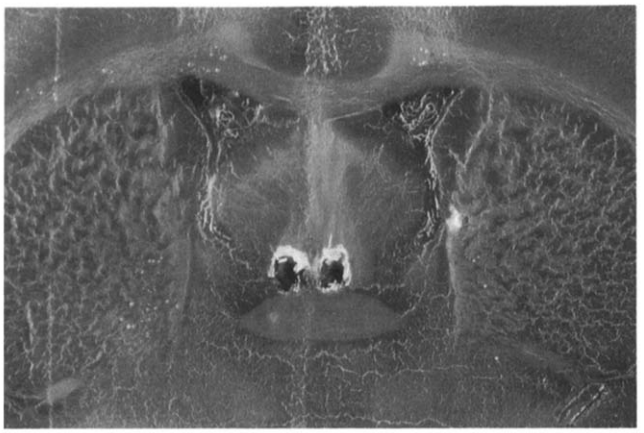

FIG. 1. Microphotograph showing typical extent of damage sustained to the fimbria fornix. Magnification × 20.

night cycle. All testing was conducted during their light cycle. Animals were divided into three groups. Two days after arrival, the first group (n = 12) received bilateral electrolytic lesions of the fimbria fornix (Fig. 1); animals were anesthetized with pentobarbital (50 mg/kg, IP) and an insulated stainless-steel electrode with a 0.5-mm uninsulated tip was placed, by means of a stereotaxic apparatus, at AP +8.7, L ±0.5, H +3.9 from the interaural line (14), through which an anodal current (6 mA, 7 s) was passed. Rats were handled daily during the 5 days after surgery (the first group) or for 1 week after arrival (the other groups). Next, to obtain behavioral sensitization to the locomotor-stimulant effects of MAP they were given a 0.5-mg/kg IP injection of *d*-methamphetamine HCl (MAP; Dainippon Pharmaceutical Co., Japan) every 4 days, up to a total of eight injections. At least 60 min prior to each injection, rats were placed individually into clear acrylic resin boxes (43 × 27 × 27 cm) equipped with MK-110 Animexes (Muromachi Co., Japan) for accommodation. The sensitivity of the Animex was adjusted so that only gross movements such as locomotion or rearing were detected, as described previously (27). The behavioral activity was recorded for 150 min following each injection.

After the eighth session was finished, the second group (n = 10) received bilateral electrolytic lesions of the fimbria fornix in the manner described above. The third group (n = 10) were given a sham operation, where the electrode tip was placed at coordinates AP +8.7, L ±0.5, H +4.9 but no current was delivered. One week after surgery, three more MAP injections and subsequent recordings of locomotor activity were carried out for these two groups. These treatments are summarized in Table 1.

Biochemical Determinations

Four days after the final MAP treatment in the behavioral study, half of each group of rats were given a challenge injection of MAP (0.5 mg/kg, IP) and the rest saline (1 ml/kg). They were decapitated 60 min later and brains removed in a cold box at 0°C and immediately frozen at −80°C. The nucleus accumbens was punched out from six 300-μm thick slices caudal to AP +11.7 with a 1.2-mm ID stainless steel tube. Histological verification of the lesion sites was made at this point. Each tissue was sonicated in 500 μl 0.4 N perchloric acid containing 30 ng isoproterenol as the internal standard. After 40 μl had been taken for protein assay, 50 μl chloroform was added to the homogenates. The mixture was vortexed for 10 s and centrifuged at 8,800×g for 15 min at 4°C. Fifty microliters of the supernatant was applied to a high-performance liquid chromatography column with electrochemical detection for the measurement of DA, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 3-methoxytyramine (3-MT). The mobile phase was 50 mM phosphate-citrate buffer containing 1 mM octasulfonic acid, 0.1 mM EDTA, and 12% methyl alcohol (pH 3.2). Analysis was performed at a flow rate of 1 ml/min at room temperature. Proteins were assayed according to the method of Lowry et al. (12).

Statistical Analysis

The data of the behavioral experiments were analyzed for each group by a one-way analysis of variance (ANOVA) with repeated measures on the number of injections, followed by Dunnett's multicomparison test on data of interest. The DA metabolism data were evaluated by Student's t-test.

RESULTS

Figure 2 shows the results of behavioral experiments. In the second and third groups of rats that received no surgery at the beginning of MAP treatments, locomotor activities evoked by the drug were increased according to the number of injections and reached a plateau around the fifth injection (the second group) or the seventh injection (the third group). There were significant effects of the number of injections in both groups [the second group, $F(7, 63) = 7.90$, $p < 0.001$; the third group, $F(7, 63) = 6.51$, $p < 0.001$], and significant changes were observed in the mean Animex counts following the fifth, sixth, seventh, and eighth injections (the second group) or the seventh and eighth injections (the third group) compared to those following each first injection.

The effects of "postsensitization" surgery were also shown in Fig. 2. In the second group of rats, which received bilateral electrolytic lesions of the fimbria fornix after the eighth administration of MAP, the level of response to the next three injections of MAP did not differ greatly from that following

TABLE 1

SUMMARY OF THE TREATMENTS IN THE THREE GROUPS OF RATS

Group	Lesion Before Drug Treatment	Drug Treatment	Lesion After Drug Treatment	Additional Drug Treatment
1st (n = 12)	Fimbria fornix	8 Times MAP	–	–
2nd (n = 10)	–	8 Times MAP	Fimbria fornix	3 Times MAP
3rd (n = 10)	–	8 Times MAP	Sham	3 Times MAP

MAP, methamphetamine

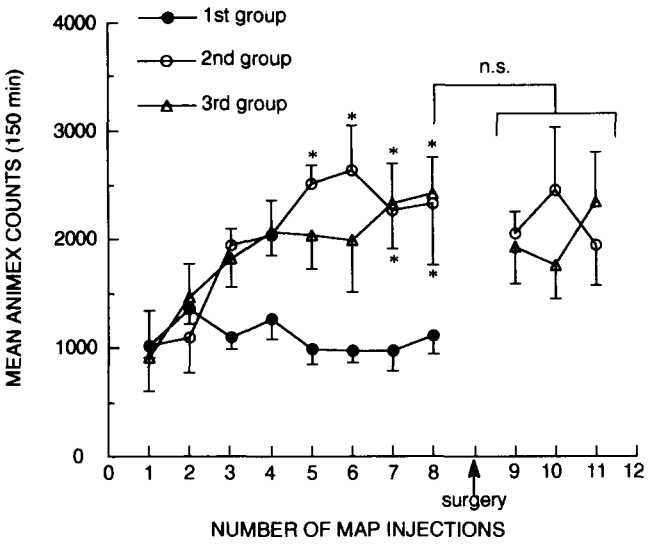

FIG. 2. Locomotor responses after repeated IP injections of 0.5 mg/kg methamphetamine (MAP) and the effects of "postsensitization" surgery on the subsequent responses to MAP. The details of treatment on each group are shown in Table 1. Data represent the mean ± SEM (n = 12 in the first and n = 10 in the other groups). Significant differences are at *$p < 0.05$ compared to the response to the first MAP injection of each group. n.s., not significant.

the eighth injection. The same was true of the third group, which received a sham operation after the eighth injection: In this group, the behavioral activities evoked by the 9th, 10th, and 11th injections were not significantly different from that evoked by the 8th injection.

In contrast, in the first group, which received the fimbria fornix lesion before the drug treatment, there were no significant changes in the mean Animex counts throughout the sessions, as previously reported; that is, the repeated injections of MAP did not produce any sensitization or tolerance in this group.

Although the data are not shown here, there was no effect of destruction of the fimbria fornix on basal locomotor activities such as a response to saline injection and a spontaneous activities during a dark period (27).

Biochemical analysis of DA and DA metabolites in the NAC after the challenge injections are summarized in Table 2. DA content after the MAP challenge was significantly increased compared to the saline treatment in the first group. Increases in DA after the MAP challenges were also seen in the second and third groups but were not significant partly because of greater dispersion. DOPAC and HVA did not alter after the MAP challenge in any of the three groups. The DOPAC/DA ratio, an index of the DA turnover rate, was significantly decreased in the first group after the challenge injection of MAP. In the second and third groups, the ratio did not change significantly although there was a tendency toward a decrease after the MAP challenge. The 3-MT level, which reflects DA release, rose significantly following the MAP challenge in the second and third groups but not significantly in the first group.

DISCUSSION

The present data demonstrate that the destruction of the fimbria fornix prevented the development of locomotor augmentation but the lesion could no longer reverse the previously acquired enhanced response to MAP. This implies that the fimbria fornix may convey information crucial for the initiation of sensitization but not play an important role in the expression of it. Karler et al. (8) reported that the NMDA subtype of excitatory amino acid receptor antagonists, ketamine and MK-801, could block the initiation of behavioral sensitization, but these compounds did not affect the expression of the phenomenon. Our anatomic manipulations may be correlated with their pharmacological findings, although they adopted stereotypy as an index of sensitization, because the fimbria fornix that we lesioned in the present study is shown to contain a glutamatergic pathway from the ventral subiculum of the HPC to the NAC (4,21). The distinction between "initiation" and "expression" is also reported in another form of neural plasticity, long-term potentiation. The induction of this electrophysiological phenomenon can be blocked by NMDA antagonists but its expression cannot (5,23). The precise mechanisms underlying these two contrasting processes in both phenomena are still unclear. In the case of behavioral sensitization, Kalivas and Stewart (7) emphasized in their recent review that increased somatodendritic DA release in the mesencephalon constitutes a common factor in the induction of it. Concerning the importance of stimulation of the mesencephalon, we speculated a possible involvement

TABLE 2

MEASUREMENTS OF DOPAMINE AND ITS METABOLITES IN THE NUCLEUS ACCUMBENS FOLLOWING SALINE (UPPER) OR METHAMPHETAMINE (0.5 mg/kg, IP, LOWER) CHALLENGE

Group	DA (ng/mg protein)	DOPAC (ng/mg protein)	DOPAC/DA	HVA (ng/mg protein)	3-MT* (% of saline)
1st	99.4 ± 3.54	25.2 ± 1.04	0.253 ± 0.008	7.39 ± 0.583	100 ± 3.18
	115 ± 0.967†	23.2 ± 0.680	0.201 ± 0.004†	7.35 ± 0.322	109 ± 6.58
2nd	89.6 ± 11.3	17.8 ± 1.24	0.199 ± 0.014	7.79 ± 0.813	100 ± 4.74
	103 ± 3.21	18.0 ± 0.640	0.178 ± 0.007	7.25 ± 0.488	121 ± 5.88‡
3rd	87.3 ± 3.21	19.3 ± 0.874	2.20 ± 0.010	7.00 ± 0.602	100 ± 4.21
	99.4 ± 3.73	19.5 ± 0.856	2.01 ± 0.009	6.57 ± 0.121	129 ± 3.56‡

Values are shown as the mean ± SEM (n = 6 in the 1st and n = 5 in the other groups).

*3-MT values for saline treatments are 2.34, 2.03, and 2.05 ng/mg protein in the first, second, and third groups, respectively.

†$p < 0.001$, ‡$p < 0.05$ as compared with saline challenge.

of the ventral tegmental area(VTA)-HPC-NAC connection, combining our results on the destruction of the hippocampo-accumbal pathway with the following fact: The ventral subiculum of the HPC appears to be the main target area for the hippocampal dopaminergic innervation from the VTA (20), and interestingly the same area in turn sends glutamatergic fibers to the NAC (21).

On the other hand, the expression of sensitization is shown to reflect augmented DA release at drug delivery in axon terminal fields. This enhanced response of DA release is proved with in vivo brain dialysis (9,16) and even at the tissue slice level (11). Thus, it may be that the induction of sensitization needs neural circuits originating in the VTA, but once it is established, properties indispensable for the expression would converge in the axon terminals. On the basis of this inference, our present results suggest that the hippocampo-accumbal pathway may play a role in the production of supposed functional changes at the presynaptic levels, that is, autoreceptor subsensitivity (25) and/or altered compartmentalization of the transmitter (26). However, to examine whether this is true or not, further studies are needed. In addition, it is also important to determine whether the lesion of the fimbria fornix might only block the development of conditioned activity based upon pairing of the environment with MAP rather than the development of sensitization itself (19).

The biochemical data show that in the saline treatment the DOPAC/DA ratio was lower and HVA was higher in the second group than in the other groups. These appear to be consistent with the report that, following hippocampal lesion, there was a decrease in DOPAC and DA utilization ([DOPAC + HVA]/DA) and a decrease in HVA at day 7, but these changes were recovered by day 28 (18). After MAP challenge, DOPAC and HVA changed little in any of the three groups, while DA had a tendency toward increase that is probably due to the monoamine oxidase (MAO) inhibitory effect of MAP (28). The change in DA was most prominent in the first group, and consequently DOPAC/DA, an index of DA utilization, was significantly decreased only in the first group. This result may be related to the behavioral observation that only the first group exhibited no sensitization. To corroborate this interpretation, we measured another DA metabolite, 3-MT, which reflects released DA and is assumed to be a more accurate parameter of the DA turnover rate (2). 3-MT is known to increase rapidly when rats are killed by decapitation (22); hence, care was taken to shorten the delay between decapitation and brain freezing, and make it the same in all groups in this study. 3-MT following MAP challenge was increased in all groups, but the change in the first group was small compared to the others. This seems to correspond to the behavioral outcome.

Finally, efforts to elucidate a functional attribute of the HPC to psychostimulant-induced sensitization seem to be intriguing, taking into account the recent clinical study showing that structural pathology of the HPC is observed in schizophrenics (6).

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REFERENCES

1. Alkon, D. L.; Amaral, D. G.; Bear, M. F.; Black, J.; Carew, T. J.; Cohen, N. J.; Disterhoft, J. F.; Eichenbaum, H.; Golski, S.; Gorman, L. K.; Lynch, G.; McNaughton, B. L.; Mishkin, M.; Moyer Jr, J. R.; Olds, J. L.; Olton, D. S.; Otto, T.; Squire, L. R.; Staubli, U.; Thompson, L. T.; Wible, C. Learning and memory. Brain Res. Rev. 16:193-220; 1991.
2. Brown, E. E.; Damsma, G.; Cumming, P.; Fibiger, H. C. Interstitial 3-methoxytyramine reflects striatal dopamine release: An in vivo microdialysis study. J. Neurochem. 57:701-707; 1991.
3. Criswell, H. E.; Mueller, R. A.; Breese, G. R. Long-term D1 dopamine receptor sensitization in neonatal 6-OHDA-lesioned rats is blocked by an NMDA antagonist. Brain Res. 512:284-290; 1990.
4. Groenewegen, H. J.; Vermeulen-Van der Zee, E.; Te Kortschot, A.; Witter, M. P. Organization of the projections from the subiculum to the ventral striatum in the rat. A study using anterograde transport of *Phasedus vulgaris* leucoagglutinin. Neuroscience 23: 103-120; 1987.
5. Harris, E. W.; Ganong, A. H.; Cotman, C. W. Long-term potentiation in the hippocampus involves activation of *N*-methyl-D-aspartate receptors. Brain Res. 323:132-137; 1984.
6. Jeste, D. V.; Lohr, J. B. Hippocampal pathogenic findings in schizophrenia. Arch. Gen. Psychiatry 46:1019-1024; 1989.
7. Kalivas, P. W.; Stewart, J. Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. Brain Res. Rev. 16:223-244; 1991.
8. Karler, R.; Chaudhry, I. A.; Calder, L. D.; Turkanis, S. A. Amphetamine behavioral sensitization and the excitatory amino acids. Brain Res. 537:76-82; 1990.
9. Kazahaya, Y.; Akimoto, K.; Otsuki, S. Subchronic methamphetamine treatment enhances methamphetamine- or cocaine-induced dopamine efflux in vivo. Biol. Psychiatry 25:903-912; 1989.
10. Kelley, A. E.; Domesick, V. B. The distribution of the projection from the hippocampal formation to the nucleus accumbens in the rat: An anterograde and retrograde horseradish peroxidase study. Neuroscience 7:2321-2335; 1982.
11. Kolta, M. G.; Shreve, P.; Uretsky, N. J. Effect of pretreatment with amphetamine on the interaction between amphetamine and dopamine neurons in the nucleus accumbens. Neuropharmacology 28:9-14; 1989.
12. Lowry, O. H.; Rosebrough, N. J.; Furr, A. C.; Randall, R. J. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265-275; 1951.
13. Mogenson, G. J.; Jones, D. L.; Yim, C. Y. From motivation to action: Functional interface between the limbic system and the motor system. Prog. Neurobiol. 14:69-97; 1980.
14. Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. 2nd ed. Sydney: Academic Press; 1986.
15. Robinson, T. E.; Becker, J. B. Enduring changes in brain and behavior produced by chronic amphetamine administration: A review and evaluation of animal models of amphetamine psychosis. Brain Res. Rev. 11:157-198; 1986.
16. Robinson, T. E.; Turson, P. A.; Bennett, J. A.; Bentgen, K. M. Persistent sensitization of dopamine neurotransmission in ventral striatum (nucleus accumbens) produced by prior experience with (+)-amphetamine: A microdialysis study in freely moving rats. Brain Res. 462:211-222; 1988.
17. Schiff, S. R. Conditioned dopaminergic activity. Biol. Psychiatry 17:135-145; 1982.
18. Springer, J. E.; Isaacson, R. L. Catecholamine alterations in basal ganglia after hippocampal lesions. Brain Res. 252:185-188; 1982.
19. Stewart, J. Conditioned stimulus control of the expression of sensitization of the behavioral activating effects of opiate and stimulant drugs. In: Gormezano, I.; Wasserman, E. A., eds. Learning and memory: Behavioral and biological substrates. Hillside, NJ: Erlbaum; 1991.

20. Verney, C.; Baulac, M.; Berge, B.; Alvarez, C.; Vigny, A.; Helle, K. B. Morphological evidence for a dopaminergic terminal field in the hippocampal formation of young and adult rat. Neuroscience 14:1039–1052; 1985.
21. Walaas, I.; Fonnum, G. The effects of surgical and chemical lesions on neurotransmitter candidates in the nucleus accumbens of the rat. Neuroscience 4:209–216; 1979.
22. Westerink, B. H. C.; Spaan, S. J. Estimation of the turnover of 3-methoxytyramine in the rat striatum by HPLC with electrochemical detection: Implications for the sequence in the cerebral metabolism of dopamine. J. Neurochem. 38:342–347; 1982.
23. Wigström, H.; Gustafsson, B. A possible correlate of the postsynaptic condition for long-term potentiation in the guinea pig hippocampus in vitro. Neurosci. Lett. 44:327–332; 1984.
24. Yang, C. R.; Mogenson, G. J. Hippocampal signal transmission to the pedunculopontine nucleus and its regulation by dopamine D2 receptors in the nucleus accumbens: An electrophysiological and behavioral study. Neuroscience 23:1041–1055; 1987.
25. Yi, S.-J.; Johnson, K. M. Chronic cocaine treatment impairs the regulation of synaptosomal ^{3}H-DA release by D2 autoreceptors. Pharmacol. Biochem. Behav. 36:457–461; 1990.
26. Yi, S.-J.; Johnson, K. M. Effects of acute and chronic administration of cocaine on striatal uptake, compartmentalization and release of [^{3}H]dopamine. Neuropharmacology 29:475–486; 1990.
27. Yoshikawa, T.; Shibuya, H.; Kaneno, S.; Toru, M. Blockade of behavioral sensitization to methamphetamine by lesion of hippocampo-accumbal pathway. Life Sci. 48:1325–1332; 1991.
28. Zetterström, T.; Sharp, T.; Ungerstedt, U. Further evaluation of the mechanism by which amphetamine reduces striatal dopamine metabolism: A brain dialysis study. Eur. J. Pharmacol. 132:1–9; 1986.