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Effects of Striatal or Accumbens Lesions on the Amphetamine-Induced Abolition of Latent Inhibition

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KONSTANDI, M. AND E. KAFETZOPOULOS. *Effects of striatal or accumbens lesions on the amphetamine-induced abolition of latent inhibition.* PHARMACOL BIOCHEM BEHAV **44**(4) 751–754, 1993.—In this study, we tested the effects of nucleus accumbens or corpus striatum lesions on the abolition of latent inhibition induced by *d*-amphetamine. In the latent inhibition paradigm, animals learn to ignore a repeatedly presented nonreinforced stimulus. In this paradigm, the repeated nonreinforced preexposure to a stimulus retards subsequent conditioning to that stimulus. Pharmacological manipulations that enhance the dopaminergic function (e.g., *d*-amphetamine) abolish this ability to ignore an irrelevant stimulus. Previous studies have revealed a major role of the nucleus accumbens in the *d*-amphetamine-induced abolition of latent inhibition because intraaccumbens injections of the drug mimic its systemic effects. The results of this study, however, revealed a significant increase in the disruption of latent inhibition by *d*-amphetamine between corpus striatum-lesioned and sham-operated rats, but a marginal difference between nucleus accumbens lesioned and sham-operated rats, which had been preexposed to the stimulus. These findings indicate that the corpus striatum plays also a major role in the disruption of latent inhibition by *d*-amphetamine. It seems, therefore, that the nucleus accumbens and corpus striatum may represent a functionally common system regarding the expression of latent inhibition, although different experimental manipulation can favor the one structure over the other, reflecting probably their complex function.

Latent inhibition *d*-Amphetamine Corpus striatum Nucleus accumbens

WHEN *d*-amphetamine (AMPH) is administered in low or moderate doses to rats, it increases locomotor activity and stereotyped behaviour (3), as well as some other conditioned behaviours (2). It has been suggested that locomotor activity induced by AMPH is mediated by an increase in dopamine (DA) neurotransmission in the nucleus accumbens septi (NAS) because intraaccumbens injection of AMPH mimics its systemic stimulant effects, while 6-dihydroxydopamine (6-OHDA) lesions within the NAS abolish the locomotor response to this drug (4,7,10). However, electrolytic (8) or cytotoxic lesions of the NAS (6) did not abolish the locomotor response to AMPH, indicating that other DA-rich structures, such as the corpus striatum, may be involved in the AMPH-induced hyperactivity (1). The purpose of the present study, therefore, was to test the effects of cytotoxic lesions of the NAS and corpus striatum on AMPH-induced changes in conditioned behaviour using the latent inhibition (LI) paradigm, which also appears to be sensitive to both systemic and direct intraaccumbens administration of AMPH.

In the latent inhibition paradigm, the animal is exposed to two opposite environmental contingencies, the preexposure and conditioning stages. In the former, the target stimulus is consistently followed by nonreinforcement, and in the latter the same stimulus is followed by reinforcement. The central point is that during preexposure the animal must learn that the stimulus signals are followed by no event of consequence and are, therefore, irrelevant. This acquired stimulus irrelevance must control the animal's behaviour in conditioning if the LI effect is to be obtained. Indeed, in normal animals earlier learning of irrelevance impairs subsequent contingency learning in the conditioning stage. In other words, animals are under the control of their previous learning of irrelevance rather than the new changed environmental contingency (15). Solomon and Weiner demonstrated that AMPH-treated animals were retarded in their ability to develop LI (12,14,17). Solomon and Staton (13) obtained a similar effect after injection of AMPH into the NAS, indicating that the AMPH-induced disruption of this conditioned behaviour may be mediated by this structure. To examine AMPH effects on LI, Solomon and Staton (13) used a conditioned emotional response (CER) procedure in rats licking for water. In brief, the CER procedure consists of three stages: a) *preexposure,* in which the conditioned stimulus (tone) was repeatedly presented without being followed by reinforcement; b) *acquisi-*

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tion, in which the preexposed stimulus was paired with negative reinforcement (shock); and c) *test,* in which latent inhibition is indexed by the animal's suppression of licking during tone presentation. In the present study, in light of data from Weiner et al. (15), showing that AMPH did not disrupt LI unless both preexposure and conditioning stages were conducted under the influence of the drug, AMPH was administered prior to preexposure, the conditioning session, and the test session. AMPH at a dose of 1.5 mg/kg has been reported to be an optimal dose for the disruption of LI (17) and is the dose used in our study to test the effects of the drug on LI after striatal or accumbens lesions.

METHOD

Subjects

Naive male Wistar rats bred in this laboratory, weighing 170–220 g at the time of operation, were used. They were housed in a light- (12 L : 12 D cycle) and temperature-controlled room.

Surgery

Rats were anesthetized with sodium pentobarbital (40 mg/kg, IP) and placed in a David Kopf (Kopf, Tujunga, CA) stereotaxic frame. The skull of rats was orientated according to the Paxinos and Watson atlas (9). For dorsal striatal lesions (n = 22), 7 μg ibotenic acid (Sigma Chemical Co., St. Louis, MO) in 1 μl phosphate buffer (pH 7.4) were injected bilaterally at the following coordinates: AP, 0.2 mm; L, 2.5 mm; and V, 4.5 mm. Anteroposterior coordinates were taken from bregma, lateral from midline and ventral from skull surface. For nucleus accumbens lesions (n = 21) 7 μg ibotenic acid in 1 μl phosphate buffer were injected bilaterally at the following coordinates: AP, 1.7 mm; L, 1.5 mm; and V, 7.0 mm. All stereotaxic injections were made with a 5-μl Hamilton syringe (Hamilton Co., Reno, NV) over a period of 3 min and the injection cannula was left in place for 5 min following each injection to attenuate leakage of ibotenic acid up the cannula track. A third group (n = 24) was sham operated, lowering only a guide cannula at the dorsal striatum.

Apparatus

The CER apparatus consisted of a Plexiglas cage with a grid floor. A drinking bottle could be inserted into the cage through a 2-cm diameter hole that was 10 cm above the grid floor and 5 cm from the right side of the cage. Licks were detected by an electronic drinkometer. The shock grid was made from stainless steel bars 0.25 cm in diameter set at 1.5-cm intervals. Scrambled shock was supplied by a Master Shocker (Lafayette Instrument Co., Model 82404/5-55) set at 1 mA. An electronic counter (Lafayette 5804) was used for equipment programming and data recording, controlled by a microcomputer.

Procedure

After the surgery, rats were kept for 30 days with food and water available ad lib. They were divided in groups of five to six animals each for tests on different conditions. All tests were performed between 10 p.m. and 3 a.m. in a sound-attenuated room. One day before baseline began, animals were weighed and placed on a 23-h water deprivation schedule that continued throughout the experiment.

Baseline. On each of the five baseline days, rats were individually placed into the experimental chamber and allowed to make licks for 15 min. Rats were then returned to their home cage and allowed access to water for 30 min.

Preexposure (PRE). On day 6, with the bottle removed, each animal was placed in the experimental chamber. PRE animals received 45 3-s tone presentations with an interval of 50 s. Nonpreexposed (NONPRE) animals were confined to the chamber for the identical period of time but did not receive the tone.

Conditioning. On day 7, with the bottle removed, each animal was given two tone–shock pairings. Tone parameters were identical to those used in preexposure. The 1-mA, 1-s shock immediately followed tone termination. The first tone–shock pairing was given 5 min after the start of the conditioning session. Five minutes later, the second pairing was administered. After the second pairing, animals were left in the experimental chamber for an additional 5 min.

Test. On day 8, each animal was placed in the chamber and allowed to drink from the bottle. When the subject completed 90 licks, the tone was presented. The tone continued until 10 additional licks were completed. If the subject failed to complete the last 10 licks within 300 s, the session was terminated and a score of 300 was recorded. The time between licks 80–90 and 90–100 were recorded. The amount of suppression of licking was indexed using a suppression ratio, $A/A + B$, where A is the time to complete licks 80–90 (pre-CS period) and B is the time to complete licks 90–100 (CS period). A suppression ratio close to zero indicates almost complete suppression (no LI) and a ratio of 0.50 indicates no change in response time from the preCS to the CS period (LI). The appropriate drug, either 1.5 mg/kg AMPH sulphate (Sigma) dissolved in 1 ml isotonic saline or an equivalent volume of the vehicle alone, was administered prior to each stage (preexposure, conditioning, and test).

Statistics

A two × two × three analysis of variance (ANOVA) with main factors condition (preexposure vs. nonpreexposure), drug (saline vs. AMPH), and lesion (sham vs. accumbens vs. striatum) was performed on the mean suppression ratio. A second two × three ANOVA with condition and lesion as factors was performed on the AMPH data. Subsequent Student's t-tests were conducted between all comparable groups.

Histology

At the end of the experiments, a routine histological analysis was performed to verify the site and extent of the lesions. Rats were sacrificed using an overdose of pentothal, and after intracardial perfusion with formalin their brains were removed and fixed. Coronal sections, cut at 30 μ in a cryostat, were stained with toluidine blue. The extent of the lesions was verified under microscopic examination and transferred visually on the corresponding plates of the Paxinos and Watson atlas (8).

RESULTS

Ibotenic acid injections into the dorsal striatum or nucleus accumbens selectively destroyed the cell bodies of the neurons of these structures without affecting more distant areas. A profound loss of neuronal perikarya with glial response and elargation of the ventricles was found after the lesions, with a proliferation of nonneuronal elements within the limits of the lesioned area (Fig. 1).

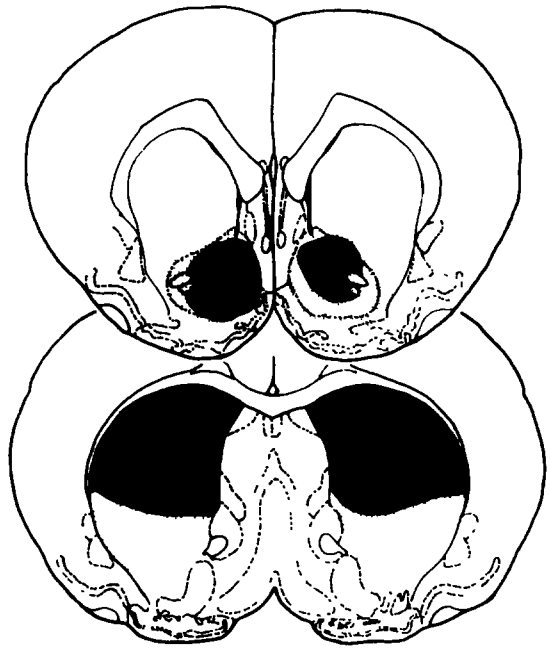

FIG. 1. Reconstruction of the ibotenic acid lesions of the nucleus accumbens (upper diagram) and the dorsal striatum (lower diagram) transferred visually on the corresponding plates of the Paxinos and Watson (8) atlas. The black area represents the smaller region and the speckled area the largest region, the limits of the remaining lesions lying roughly between them.

The results of the lesion effects on latent inhibition in the two × two × three ANOVA are summarized in Fig. 2, which shows that nonpreexposed (NONPRE) animals had a poorer suppression ratio, which was evident in all groups. This is supported by a significant main effect of condition $F(1, 66) = 47.5, p < 0.001$. AMPH administration increased suppression in sham-operated rats in both PRE ($t = 3.53, p < 0.001$) and NONPRE groups ($t = 2.74, p < 0.025$), but in striatal- and NAS-lesioned rats the AMPH effect was restricted to NONPRE animals ($t = 3.7, p < 0.005$, and $t = 2.24, p < 0.025$), but in striatal- and NAS-lesioned rats the AMPH effect was restricted to NONPRE animals ($t = 3.7, p < 0.005$, and $t = 2.24, p < 0.05$, respectively).

The two × three ANOVA on AMPH data revealed a significant condition effect, $F(1, 33) = 55.5, p < 0.001$, as well as a significant lesion effect, $F(2, 33) = 5.86, p < 0.008$. These results were confirmed by subsequent t-test analyses, which revealed a significant difference between the suppression ratio of sham-operated and striatum-lesioned PRE rats ($t = 4.52, p < 0.005$) but a marginal difference between sham- and NAS-lesioned PRE rats ($t = 2.0, 0.05 < p < 0.1$).

DISCUSSION

Robinson and Becker's model of behavioral sensitization focused on amphetamine-induced motor effects, that is, sensitization of locomotion and stereotypy (11). Weiner et al. (16) extended the model to attentional processes, demonstrating that the behavioral sensitization paradigm produces in addition to motor effects a cognitive analog of the schizophrenic syndrome, namely, an inability to ignore irrelevant stimuli.

As for the brain mechanisms underlying these AMPH-induced attentional deficits, the most convincing clue up to

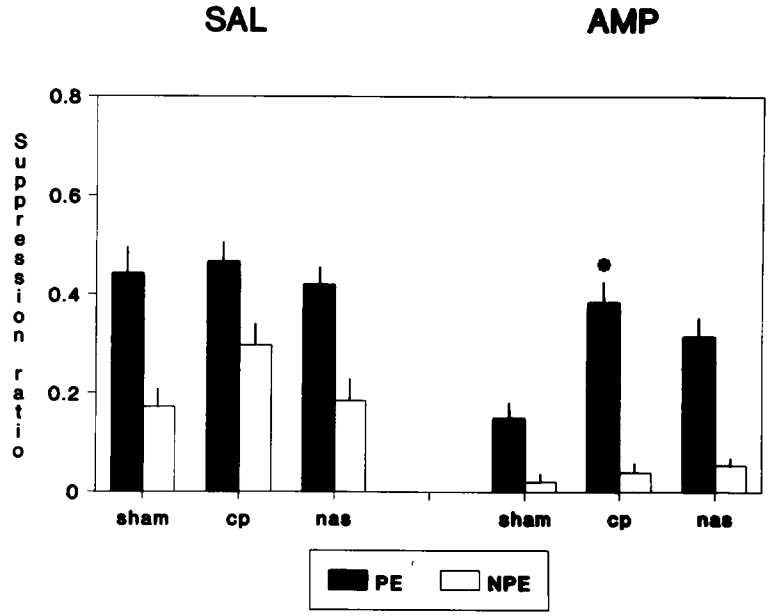

FIG. 2. Mean suppression ratio of the preexposed (PE) and nonpreexposed (NPE) groups of sham-operated control rats (sham) or rats with ibotenic acid lesions of the corpus striatum (cp) or the nucleus accumbens septi (nas) (mean ± SEM) after saline (SAL) or d-amphetamine (AMP) treatment. *Significant difference ($p < 0.005$) between sham-operated and corpus striatum-lesioned rats in the AMPH-treated group.

now has been provided by Solomon and Staton (13), who demonstrated a dissociation between the mesolimbic and striatal dopamine systems in the mediation of LI. They reported that microinjections of AMPH into the nucleus accumbens, but not the caudate putamen (CP), abolished LI. The results of our study, however, revealed only a marginal difference between the suppression ratio of sham- and NAS-lesioned rats but a significant difference between sham- and CP-lesioned PRE rats, indicating that under our experimental setting LI disruption seems to be mediated mainly by the striatal output system.

This disagreement between our and Solomon's and Staton's findings may be related to the different methods used. Our finding, however, that CP lesioning produces an inhibition in the AMPH-induced disruption of LI that is more profound than that of NAS lesioning could strengthen the hypothesis that the AMPH effect could be indepted also in striatal mechanisms. This is in agreement with Fink's and Smith's suggestion about a mass action between DA terminal fields in the anterior forebrain (5). Fink and Smith observed that less extensive DA denervations in both structures failed to abolish the locomotor response to AMPH and suggested that locomotion after a low dose of the drug in the intact rat requires simultaneous release of DA in most, if not all, of the terminal fields in the anterior forebrain. One would predict, therefore, that after a necessary minimum area of denervation was achieved the decrease in AMPH-induced locomotion would be a function of the extent of denervation rather than of a specific site of denervation.

The mechanisms underlying this AMPH-induced interruption of LI cannot be elucidated solely on the basis of our results. It is known that neurotoxic lesions of the DA-rich structures (e.g., NAS) produce different behavioural effects than 6-OHDA-induced denervation of the same structure. It was found that 6-OHDA lesion of the NAS abolished the hyperactive response to AMPH, while electrolytic or kainic acid lesion did not (6,8). We could suggest, therefore, that these discrepancies between the behavioural effects of direct AMPH injections, 6-OHDA lesions, or neurotoxic lesions are not due only to methodological differences but may reflect a complex substrate, as well as a complicated function of the underlying structures.

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