Pharmacology Biochemistry and Behavior, Vol. 1, pp. 285–288. ANKHO International Inc., 1973. Printed in the U.S.A.

Amphetamine Reversal of Sexual Impairment Following Anterior Hypothalamic Lesions in Female Rats

JAMES G. HERNDON AND DARRYL B. NEILL[1]

Department of Psychology, Emory University, Atlanta, Georgia 30322

(Received 6 April 1973)

HERNDON, J. G. AND D. B. NEILL. *Amphetamine reversal of sexual impairment following anterior hypothalamic lesions in female rats.* PHARMAC. BIOCHEM. BEHAV. 1(3) 285–288, 1973. –Ovariectomized female rats were sexually receptive after systemic administration of estrogen and progesterone. Anterior hypothalamic lesions abolished their receptivity. Systemic administration of low doses of *d*-amphetamine restored their receptivity. The reversal may be related to an effect of amphetamine on brain catecholamines involved in female sexual behavior.

d-Amphetamine Anterior hypothalamus Female sexual behavior Lesion reversal Brain catecholamines

IT HAS been demonstrated that some behavioral disruptions following monoamine-depleting lesions can be ameliorated by exogenous replacement of the depleted amine [2,4]. Another method found effective in reversing some lesion-produced behavioral deficits is treatment with amphetamine, a drug which increases catecholamine release [8, 12, 16]. Systemic administrations of amphetamine have been found to reverse deficits in placing responses [9] and visual discrimination [3,5] following CNS damage.

Severe impairments of female sexual behavior have been observed in the rat following lesions of the anterior hypothalamus (AH) [6, 7, 15]. The present study is designed to determine whether these deficits in reproductive behavior might also be reversed by amphetamine treatment.

METHOD

Animals

Eighteen Sprague-Dawley derived female albino rats (Cherokee Breeding Co., Atlanta, Ga.) were maintained on a 12 hr on – 12 hr off light–dark cycle with free access to food and water.

Surgical Procedures

All animals were ovariectomized under ether anesthesia. One week later, they were given two subcutaneous injections of estradiol benzoate (EB), separated by 24 hr. Eighteen hr after the second EB injection, each animal received 0.5 mg progesterone. Six hr after the progesterone injection, the animals were given sexual experience by placing them in observation cages with sexually active males. All of the females were found to be receptive under these conditions.

Twelve animals were then anesthetized with sodium pentobarbital (45 mg/kg) and placed in a stereotaxic device. Bilateral lesions were made in the anterior hypothalamus by passing 2 ma anodal d.c. current for 20 sec (5 rats) or 10 sec (7 rats) between a stainless steel electrode and the stereotaxic frame. The electrode, which was insulated except for the cut tip, was aimed at stereotaxic co-ordinates A 6.8, H-2.0, L 1.0, according to the atlas of Pellegrino and Cushman [13].

The remaining six animals received sham operations instead of lesions. The sham procedure involved anesthesia, scalp incision and drilling of burr holes in the skull.

Histology

After the experiments, the lesioned animals were sacrificed with a lethal dose of pentobarbital and perfused through the heart with isotonic saline followed by 10% Formalin. The brains were removed and cut in 50 μ frozen sections, which were stained with cresyl violet. Typical small and large lesions are shown in Fig. 1.

[1]Address reprint request to Darryl B. Neill, Department of Psychology, Emory University, Atlanta, Georgia 30322. This research was supported by grants 1-RO3-MH-22395-01 from the National Institute of Mental Health and 8027 from the Emory University Research Committee to D. B. N. We thank Dr. Preston Perlman of the Schering Corp. for supplying estrogen and progesterone, and Smith, Kline and French for supplying *d*-amphetamine.

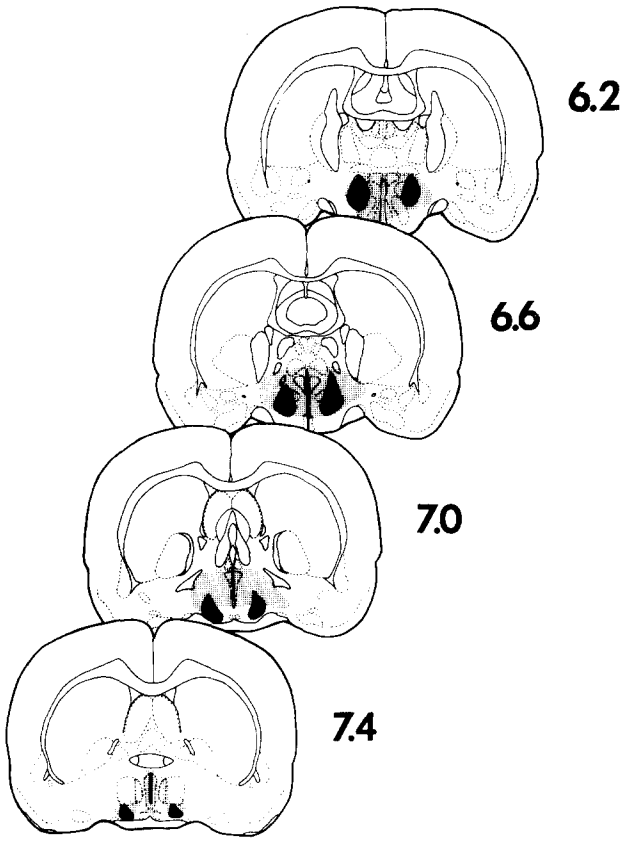

FIG. 1. Representative frontal sections through the anterior hypothalamus (9) showing a small lesion (solid black) and a large lesion (stipple).

Two lesioned animals were eliminated from the study on the basis of histology: one because of a large cortical tumor, and the other because of lateral misplacement of the lesions.

EXPERIMENT 1

The first experiment was designed to determine whether anterior hypothalamic lesions abolished sexual behavior in our own animals, and whether the behavior could then be restored by amphetamine treatment.

Procedure

At least one week was allowed for recovery from the stereotaxic surgery before tests for female sexual behavior were begun. Forty-eight and 24 hr prior to the behavioral test, 10 μg EB was subcutaneously administered. Eighteen hours after the second EB injection, 0.5 mg progesterone was given SC. Isotonic saline was administered IP 20 min prior to testing. Sexual receptivity was then assessed by placing the females into 30 x 30 x 45 cm observation cages with sexually vigorous Long-Evans male rats. All such tests were performed during the first four hours of the dark phase of the light–dark cycle. Females were scored for lordotic behavior on a four point scale: 0, no response; 1, back slightly flattened; 2, back flattened noticeably or slightly arched; 3, exaggerated arching of the back. Females received one score for each mount attempt by the male. A score of 0 or 1 on a particular mount was taken as an indication of nonreceptivity, while a score of 2 or 3 indicated a lordotic response for that mount. Tests were normally concluded after 10 mount attempts, although premature termination of a test session was occasionally required because of the extremely aggressive reaction shown by some of the lesioned females in response to a mount attempt. The receptivity quotient (RQ) for a given test session was defined as the percentage of mounts for which the female received a score of 2 or 3.

Following this initial test, the lesioned animals were injected with 1.0 mg/kg *d*-amphetamine sulfate and returned to their home cages. Twenty min later, each lesioned animal was again placed with an active male and again scored for receptivity as described above.

Results

Figure 2A indicates that lesioned animals were markedly less receptive after saline injection than were their sham operated counterparts (Mann-Whitney U, $p<0.002$). After amphetamine treatment, the receptivity of the lesioned group rose to a level not significantly different from that of controls ($p>0.15$). As shown in Table 1, seven of the lesioned animals showed increases in RQ's after amphetamine treatment, while the remaining three animals showed no receptivity after either saline or amphetamine treatment. The overall trend (Fig. 2A) is a significant increase (Mann-Whitney U, $p<0.05$).

TABLE 1

RECEPTIVITY QUOTIENTS FOR INDIVIDUAL ANIMALS WITHIN A SINGLE SESSION BEFORE AND AFTER ADMINISTRATION OF *d*-AMPHETAMINE

Animal No.	Saline	1.0 mg/kg *d*-amphetamine
Large Lesions		
1	0	100
2	0	0
3	0	80
4	0	0
5	0	30
Small Lesions		
6	0	0
7	50	100
8	0	60
11	0	90
12	0	70

EXPERIMENT 2

Although the results of Experiment 1 suggest that amphetamine facilitates the receptivity of AH lesioned animals, the possibility was considered that the facilitation was at least partly due to a warm-up effect, since amphetamine treatment followed an initial exposure to an active male. Experiment 2 was designed to examine the amphetamine effect in a situation which precluded confounding due to the possibility of warm-up, while simultaneously allowing for a blind testing procedure.

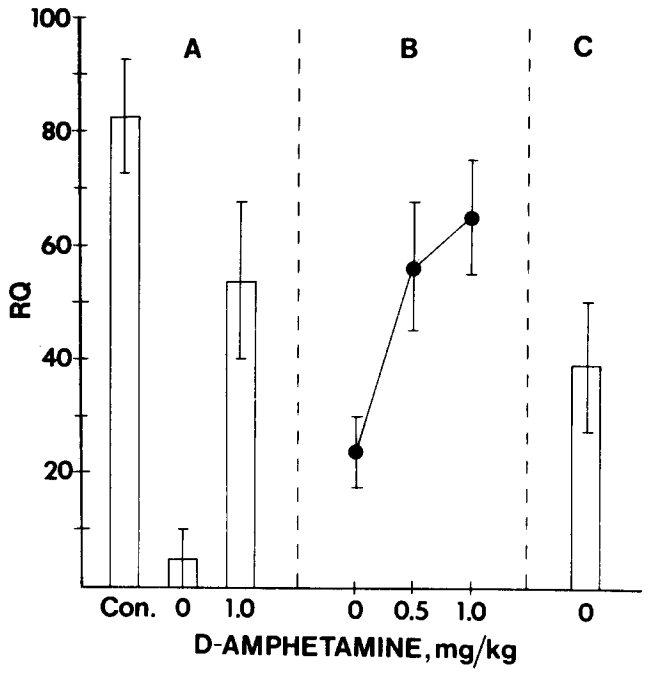

FIG. 2. Receptivity Quotients (with standard errors): (A) during a single session, (B) during a blind sequence of one dose per week, and (C) after the blind sequence under saline to evaluate recovery.

Procedure

The lesioned animals from Experiment 1 were tested for sexual receptivity on a weekly basis. Prior to each of these tests, the animals were treated with EB and progesterone as in Experiment 1. Twenty min before each test session, animals were given an IP injection of normal saline, 0.5 mg/kg *d*-amphetamine, or 1.0 mg/kg *d*-amphetamine. During the three week test sequence, every animal received each dose on one occasion. Dose order was varied between animals. Behavior was scored as described above, except that the testing was done by an experimenter who was unaware of the drug treatment of each animal.

Following this series of weekly tests, a final test of receptivity was conducted after injection of saline. The purpose of this final saline test was to assess any long-term effects on receptivity of the multiple drug treatments and behavioral tests.

Results

The results of the blind sequence of tests are plotted in Fig. 2B. The amphetamine facilitation of receptivity in this sequence is highly reliable ($F = 12.5$, $df = 2,18$, $p<0.001$).

The results of the final saline test are shown in Fig. 2C. This test revealed a higher mean receptivity than either the initial saline test ($p<0.05$) or the saline test during the blind sequence ($p<0.05$). However, the RQ scores for both 0.5 and 1.0 mg/kg amphetamine during the blind sequence were reliably higher than those of the final saline test ($p<0.05$).

DISCUSSION

These experiments demonstrate that amphetamine can reverse the sexual nonreceptivity of female rats bearing anterior hypothalamic lesions. Although some functional recovery occurred over the course of the experiment, the drug reversal is not dependent on functional recovery, since the effect can be seen in the first postoperative test. Furthermore, even in the final saline test, receptivity was lower than after either amphetamine dose ($p<0.05$).

Since pharmacological studies have suggested that the primary effect of amphetamine in the CNS is to facilitate catecholaminergic neurotransmission, the drug effect observed here might be related to catecholamine release. Although amphetamine may release serotonin as well as catecholamines [14], some evidence [10, 11, 18] indicates that serotonin has an inhibitory influence on female sexual behavior. Combined with the present results, these data suggest that catecholamine release is facilitatory with respect to female sexual behavior, while serotonin release is inhibitory. Histochemical mapping studies [17] have revealed the presence of both noradrenergic and serotonergic fibres in the anterior hypothalamus, providing anatomical support for this hypothesis.

While the present data suggest the possibility of an excitatory role for catecholamines in female sexual behavior, other investigators have suggested that catecholamines may inhibit receptivity. Ahlenius, *et al.* [1] have found that the facilitation of receptivity seen after administration of *p*-chlorophenylalanine or alpha-methyl tyrosine to estrogen-primed female rats closely follows the time course of the drug-produced depletion of norepinephrine and dopamine. Further research will hopefully resolve this apparent discrepancy.

Consideration must be given to the possibility that the amphetamine-reversal of sexually disruptive lesions is entirely unrelated to the normal occurrence of reproductive behavior in the unlesioned female. However, pilot data recently collected in our laboratory indicate that amphetamine treatment also facilitates receptivity in ovariectomized but unlesioned rats treated with suboptimal doses of estrogen.

Finally, it is possible that the animals with the large lesions were more difficult to reverse because the amine system in question had received too much damage. The better reversal seen in animals with small lesions may be related to the development of denervation supersensitivity. In this scheme, amphetamine would cause release of catecholamines from undamaged fibres and supersensitivity would potentiate the behavioral capabilities of the remaining neural system.

REFERENCES

1. Ahlenius, S., J. Engel, H. Eriksson and P. Sodersten. Importance of central catecholamines in the mediation of lordosis behavior in ovariectomized rats treated with estrogen and inhibitors of monoamine synthesis. *J. neur. Trans.* **33**: 247–255, 1972.
2. Berger, B. D., C. D. Wise and L. Stein. Norepinephrine: Reversal of anorexia in rats with lateral hypothalamic damage. *Science* **172**: 281–284, 1971.
3. Braun, J. J., P. M. Meyer and D. R. Meyer. Sparing of a brightness habit in rats following visual decortication. *J. comp. physiol. Psychol.* **61**: 79–82, 1966.

4. Harvey, J. A. and C. E. Lints. Lesions in the medial forebrain bundle: relationship between pain sensitivity and telencephalic content of serotonin. *J. comp. physiol. Psychol.* **74:** 28–36, 1971.
5. Jonason, K. R., S. M. Lauber, M. J. Robbins, P. M. Meyer and D. R. Meyer. Effects of amphetamine upon relearning pattern and black-white discriminations following neocortical lesions in rats. *J. comp. physiol. Psychol.* **73:** 47–55, 1970.
6. Kennedy, G. C. Hypothalamic control of the endocrine and behavioral changes associated with oestrus in the rat. *J. Physiol.* **172:** 383–392, 1964.
7. Law, T. and W. Meagher. Hypothalamic lesions and sexual behavior in the female rat. *Science* **128:** 1626–1627, 1958.
8. McKenzie, G. M. and J. C. Szerb. The effect of dihydroxyphenylalanine, pheniprazine and dextroamphetamine on the *in vivo* release of dopamine from the caudate nucleus. *J. Pharmac. exp. Ther.* **162:** 302–318, 1968.
9. Meyer, P. M., J. A. Horel and D. R. Meyer. Effects of *dl*-amphetamine upon placing responses in neodecorticate cats. *J. comp. physiol. Psychol.* **56:** 402–404, 1963.
10. Meyerson, B. Central nervous monoamines and hormone induced estrus behavior in the spayed rat. *Acta physiol. scand. Suppl.* **241:** 3–32, 1964.
11. Meyerson, B. and T. Lewander. Serotonin synthesis inhibition and estrus behavior in female rats. *Life Sci.* **9:** 661–671, 1970.
12. Moore, K. E. The role of exogenous norepinephrine in the toxicity of *d*-amphetamine in aggregated mice. *J. Pharmac. exp. Ther.* **144:** 45–51, 1964.
13. Pellegrino, L. J. and A. C. Cushman. *A Stereotaxic Atlas of the Rat Brain.* New York: Appleton-Century-Crofts, 1967.
14. Reid, W. D. Turnover rate of brain 5-hydroxytryptamine increased by *d*-amphetamine. *Br. J. Pharmac.* **40:** 483–491, 1970.
15. Singer, J. J. Hypothalamic control of male and female sexual behavior in female rats. *J. comp. physiol. Psychol.* **66:** 738–742, 1968.
16. Stein, L. and C. D. Wise. Release of norepinephrine from hypothalamus and amygdala by rewarding medial forebrain bundle stimulation and amphetamine. *J. comp. physiol. Psychol.* **67:** 189–198, 1969.
17. Ungerstedt, U. Stereotaxic mapping of the monoamine pathways in the rat brain. *Acta physiol. scand. Suppl.* **367:** 1–48, 1971.
18. Zelman, F. P., I. L. Ward, W. R. Crowley and D. L. Margules. Activation of lordotic responding in female rats by suppression of serotonergic activity. *Science* **179:** 1010–1011, 1973.