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Male rat copulation following 6-OHDA lesions of the medial preoptic area: resistance to repeated administration and rapid behavioral recovery

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Dopamine (DA) in the medial preoptic area (MPOA) has been shown to facilitate male rat sexual behavior. However, injections of the catecholamine (CA) neurotoxin 6-OHDA into the MPOA did not impair copulation in tests 3 days after injection. In the present study, three weekly (serial) injections produced no copulatory deficits compared to animals that received a single injection or to preinjection copulatory behavior scores. However, blocking CA synthesis, which did not impair control rats, produced deficits in both single and serial lesion animals, with significantly fewer serial than single lesion animals initiating copulation. Biochemical analysis of tissue punches showed no difference in MPOA concentrations of dopamine, norepinephrine, epinephrine, or the dopamine metabolite DOPAC between the two groups. Additional animals were tested at earlier intervals after 6-OHDA injections into the MPOA. Tests conducted 30 min after an MPOA injection of 6-OHDA revealed that all measures of copulation were impaired, relative to scores 24 h later. However, these scores were not significantly different from animals tested 30 min after a vehicle injection. A final group, tested 4 h after injection, showed impairment of all measures of copulation compared to vehicle injections and to tests 24 h later. Furthermore, in the tests 24 h later, 6-OHDA animals were not different from vehicle animals. Results from all experiments show that 6-OHDA injections into the MPOA impair copulation for at least 4 h, but that behavioral recovery is complete 24 h later. However, deficits can be reinstated by inhibiting DA synthesis, suggesting that increased synthesis in undamaged terminals contributed to behavioral recovery.

INTRODUCTION

Extensive electrolytic lesions of the medial preoptic area (MPOA), or lesions of fibers innervating this structure, severely impair or completely abolish male copulatory behavior[2,6]. The effects of small lesions of the MPOA in rats are more variable than those of large lesions. Arendash and Gorski[2] reported that discrete lesions of the dorsal MPOA induced deficits, but that similar discrete lesions in other subregions of the MPOA were ineffective. In addition, recovery from the copulatory deficits produced by discrete lesions of the MPOA, has been reported[6].

We have shown that DA stimulation of the MPOA, using the DA agonist apomorphine, enhances rate of copulation in male rats[10]. Furthermore, MPOA injections of the DA antagonist *cis*-flupenthixol impaired those measures of copulation that were enhanced by apomorphine[19].

In a previous study, Bitran and colleagues[4] reported that selective lesions of DA fibers innervating the MPOA, using 6-OHDA microinjections, did not impair copulatory behavior in tests 3 days later. However, a dose of the CA synthesis inhibitor α-methyl-*p*-tyrosine methyl ester (AMPT) that did not impair intact animals, did reduce copulatory efficiency in 6-OHDA injected animals. Furthermore, Bitran and colleagues[4] found that although 6-OHDA decreased DA concentration in the MPOA by 23%, there was an increase in the DOPAC/DA ratio, suggesting an increase in DA metabolism in these animals. It was hypothesized that the lack of any deificits 3 days after 6-OHDA treatment was due to insufficient depletion and/or metabolic compensation in remaining terminals.

The present experiments were designed to test whether multiple treatments of 6-OHDA would induce greater behavioral deficits than a single treatment, and whether deficits would be apparent at an earlier time.

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Furthermore, if rapid mechanisms of recovery in previous studies had masked early 6-OHDA induced deficits, we wished to determine the time course for this recovery to normal levels of copulatory behavior.

MATERIALS AND METHODS

Animals

Adult male Long–Evans rats (300–400 g) purchased from Blue Spruce Farms (Altamount, NY), were housed singly in large plastic cages (40 × 20 × 20 cm) and maintained under a reversed light cycle (11.00–21.00 h lights off/21.00–11.00 h lights on), with food and water available ad lib. Only males that had copulated to at least one ejaculation on a screening test were selected for experimental testing. Ovariectomized stimulus females of the same strain (200–250 g) were brought into behavioral estrus with a subcutaneous injection of estradiol benzoate (20 μg) 48 h prior to behavioral testing. Only females displaying receptive behavior with non-experimental males were used for testing.

Surgery and cannulae

Male rats selected for experiments were anesthetized with ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (4 mg/kg) i.m., and received unilateral stainless steel guide cannulae ending 1 mm above the MPOA (AP: 2.4, ML: 0.2, DV: –7.3, incisor bar: +5 mm)[20]. Pilot animals were injected with dye to assure a 2-μl injection from this unilateral placement would adequately diffuse throughout the MPOA. Cannulae were constructed from thin-walled 23-gauge stainless steel tubing and were held in place by dental cement that was anchored by four machine screws secured in the skull. An obturator was inserted into the cannula to prevent entry of debris. Construction of cannulae and obturators are described in detail elsewhere[10].

Drugs

Desmethylimipramine hydrochloride (DMI, Sigma; 25 mg/kg s.c.), was used to inhibit uptake of 6-OHDA into noradrenergic (NA) terminals. It was dissolved in sterile distilled water and injected 30 min preceding 6-OHDA microinjection. Alpha-methyl-*p*-tyrosine methyl ester (AMPT, Sigma; 100 mg/kg i.p.) was dissolved in isotonic saline and injected 8 h and again 2 h prior to behavioral testing.

6-Hydroxydopamine hydrochloride (6-OHDA, Sigma; 8 μg/2μl) was dissolved in ice-cold isotonic saline with 0.2% ascorbic acid to prevent auto-oxidation. Once dissolved, 6-OHDA was kept on ice for the duration of intracranial injections. Intracranial injections were accomplished using a Harvard microinfusion pump.

Behavioral testing

All behavioral tests were conducted in the male's home cage between 14.00 and 17.00 h. Each test lasted for 30 min after the first intromission, or 30 min after the introduction of the female if no intromission occurred. The following measures were recorded: mount latency, latency from the introduction of the female to the first copulative behavior (mount or intromission); intromission latency, latency from the introduction of the female to the first intromission; ejaculation latency, latency from the first intromission to the first ejaculation; postejaculatory interval, latency from the first ejaculation to the first intromission of the second ejaculatory series. The numbers of mounts and intromissions per ejaculatory series and the number of ejaculations per test were also recorded.

Histology

Following the completion of experiments not requiring biochemical analysis, animals were anesthetized using ether anesthesia and killed by decapitation. Coronal sections (40 μm) were collected, stained with cresyl violet, and examined using a projection magnifier for accuracy of placements. Animals with cannula placements ending more than 0.5 mm from the intended implantation site were excluded from statistical analysis.

Biochemical analysis

Animals were killed by decapitation. Brains were quickly removed and frozen on dry ice. Four bilateral micropunches of tissue 300 μm thick and 1 mm in diameter were taken from the MPOA using Pellegrino et al.[20] atlas of the rat brain for visualization of the area. Micropunch techniques are described elsewhere[18].

Tissue punches were homogenized in 100 μl Tris-HCl buffer (50 mM, pH 7.4) and 0.1 M perchloric acid. The homogenate was centrifuged at 1,600 × *g* for 2 min at 4°C. CA analysis was determined by injecting 20 μl of the filtrate onto a high performance liquid chromatography column (Alltech, C-18, 5 μ), using electrochemical detection. The mobile phase consisted of potassium phosphate buffer (0.07 M, pH 4.0), EDTA (0.5 mM), acetonitrile (8%), methanol (10%) and sodium ocytyl sulphate (100 mg/l). The medium was pumped over the column at a flow rate of 1 ml/min. The working electrode (glassy carbon, Bioanalytic Systems) was set at 0.65 V with a sensitivity of 2 nA/V. The amounts of CA were based upon the peak heights of the sample compared to the peak heights of known standards. Peak heights were measured using a recording integrator.

Concentrations of norepinephrine (NE), epinephrine (Epi), dopamine (DA), dihydroxyphenylacetic acid (DOPAC) were measured. Protein determinations were made using a modified Lowry procedure[15].

Statistics

For single vs. serial injected animals, Student's *t*-test was used for comparison of the two groups. A two-way ANOVA was used to compare animals' scores prior to 6-OHDA injection, with their scores after 6-OHDA injection. A two-way ANOVA was also used to compare animals scores following AMPT and vehicle injection.

For 30 min vs. 24 h groups, a 3-way mixed ANOVA, one between, two within, was used to show no interactive effect of counterbalancing rats that had received an injection of 6-OHDA the previous week. A two-way within subjects ANOVA was then used to compare the following tests: 30 min following 6-OHDA, 30 min following vehicle, 24 h following 6-OHDA, and 24 h following vehicle. Duncan's tests were used for posthoc evaluation. Scores for 4 h vs. 28 h groups were analyzed in a like manner.

Cochran's *Q*-test was used to analyze changes in numbers of animals initiating behaviors. Chi square tests were used to determine changes in percent animals initiating behaviors.

RESULTS

Experiment 1. Behavioral and biochemical assessment of single vs. serial 6-OHDA injections

Ten rats received a single MPOA injection of 6-OHDA, followed by vehicle injections of the same volume 7 and 14 days later. Ten rats received 6-OHDA injections on days 1, 7, and 14. All animals were treated with DMI prior to all 6-OHDA and vehicle injections. Animals were tested 3 days after each intracranial injection.

Following the completion of behavioral testing in Experiments 1 and 2, biochemical analysis was performed to determine concentrations of DA, DOPAC, NE and Epi in the MPOA of single and serial 6-OHDA injected animals.

No significant differences in copulatory behavior were found between animals receiving a single injection of

TABLE I

Comparison of MPOA catecholamine concentrations in animals receiving single and serial injections of 6-OHDA

Concentrations are expressed as ng/mg protein ± S.E.M.

Neurochemical	*Single*	*Serial*
DA	2.699 ± 0.286	2.643 ± 0.293
DOPAC	1.112 ± 0.156	1.227 ± 0.154
NE	23.542 ± 1.843	21.811 ± 2.234
EPI	6.659 ± 0.818	5.874 ± 0.624

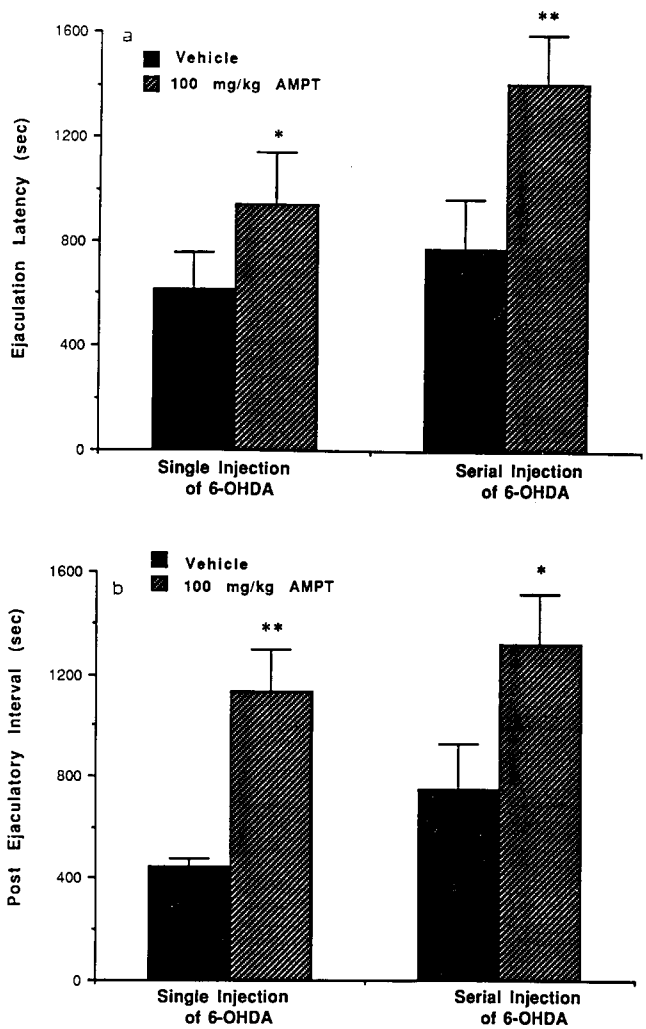

Fig. 1. a: mean ejaculation latency (± S.E.M.) following intraperitoneal injection of AMPT and vehicle in animals that received either a single MPOA injection of 6-OHDA or three weekly (serial) injections of 6-OHDA. $*P < 0.05$, $**P < 0.005$. b: mean post-ejaculatory interval (± S.E.M.) following intraperitoneal injection of AMPT and vehicle in animals that received either a single MPOA injection of 6-OHDA or three weekly (serial) injections of 6-OHDA. $*P < 0.02$, $**P < 0.005$.

6-OHDA and animals receiving three weekly injections of 6-OHDA. In addition, no significant differences were found between baseline copulation scores before 6-OHDA injections, and copulation scores following these injections. Biochemical analysis revealed no significant differences between single and serial lesion groups in concentrations of DA, DOPAC, NE and Epi (Table I).

Experiment 2. Response to AMPT in single and serial injected rats

In order to test whether increased synthesis may have masked potential behavioral deficits, the catecholamine synthesis inhibitor AMPT was administered before copulatory behavior testing.

The twenty male rats used in Experiment 1 were used in the present study. One week following the completion of Experiment 1, 5 rats from the single lesion group and 5 rats from the serial lesion group were treated with AMPT prior to copulatory behavior testing. The remaining five animals from each group received similar injections of vehicle only. The following week injections of AMPT and vehicle were counterbalanced so that each animal was used as its own control.

An additional group of 10 rats that had received no 6-OHDA was tested separately to verify that the dose of AMPT chosen would not affect copulation in intact animals. The control group was tested using the same counterbalanced procedure as 6-OHDA-injected groups.

AMPT impaired copulation in both groups of 6-OHDA-treated animals when compared to their scores following a vehicle injection. Rate of copulation was

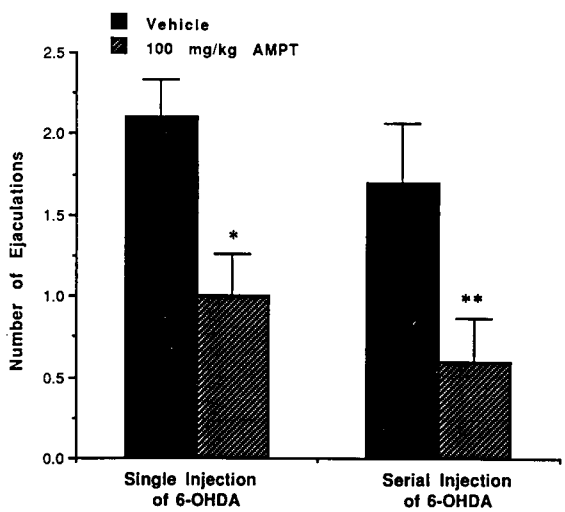

Fig. 2. Mean number of ejaculations (± S.E.M.), following intraperitoneal injection of AMPT and vehicle in animals that received either a single MPOA injection of 6-OHDA or three weekly (serial) injections of 6-OHDA. $*P < 0.01$, $**P < 0.001$.

TABLE II

(A) Mean latencies (s ± S.E.M.) for animals 30 min and 24 h after MPOA injections of 6-OHDA or vehicle

	Mount	*Intromission*	*Ejaculation*	*Postejaculatory*
30 min				
Veh	709.5 ± 173.2	1003.5 ± 185.1	1254.4 ± 135.7	1172.2 ± 144.4
6-OHDA	1032.2 ± 183.1	1417.5 ± 154.5	1517.2 ± 121.4	1495.7 ± 123.9
24 h				
Veh	580.0 ± 161.1*	718.1 ± 167.9*	1046.8 ± 147.9*	944.5 ± 144.8*
6-OHDA	549.4 ± 130.9*	874.9 ± 159.8*	1100.4 ± 131.4*	957.3 ± 131.7*

(B) Number of animals exhibiting behaviors 30 min and 24 h after MPOA injections of 6-OHDA or vehicle

30 min			
Veh	14/20	10/20	10/20
6-OHDA	10/20	6/20	5/20
24 h			
Veh	16/20	15/20	13/20
6-OHDA	19/20**	16/20**	14/20*

Significance levels are shown for comparison to 30-min 6-OHDA group. *$P < 0.05$, **$P < 0.01$.

slowed by AMPT, as indicated by an increase in ejaculation latency (single lesion $F_{1,9} = 6.3$, $P < 0.05$; serial lesion $F_{1,9} = 16.6$, $P < 0.005$, Fig. 1a), and a lengthening of the postejaculatory period (single lesion $F_{1,9} = 17.7$, $P < 0.005$; serial lesion $F_{1,9} = 8.64$, $P < 0.02$, Fig. 1b). The number of ejaculations per test was also decreased (single lesion $F_{1,9} = 12.2$, $P < 0.01$; serial lesion $F_{1,9} = 37.5$, $P < 0.001$; Fig. 2).

In addition, the total number of intromissions was decreased by AMPT (single lesion $F_{1,9} = 5.2$, $P < 0.05$; serial lesion $F_{1,9} = 26.0$, $P < 0.001$). This decrease was primarily due to a reduction in the number of animals achieving one or more intromissions to 40% of vehicle controls (4/10 vs. 10/10) in serial lesion animals, and to 80% of vehicle controls (8/10 vs. 10/10) in single lesion animals (single lesion, $\chi^2_1 = 4.5$, $P < 0.05$; serial lesion, $\chi^2_1 = 8.17$, $P < 0.005$; Fig. 3). Significantly fewer serial lesion animals than single lesion animals intromitted after AMPT injections ($\chi^2_1 = 13.33$, $P < 0.001$). No significant deficits in copulatory behavior were found in control rats after administration of AMPT when compared to vehicle injection.

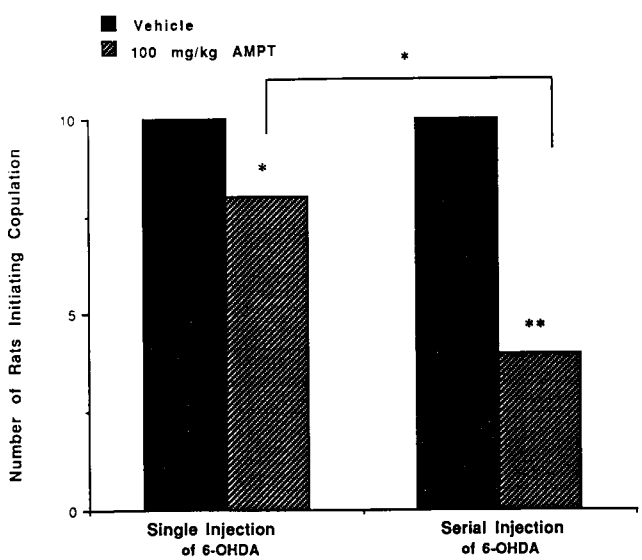

Fig. 3. Number of rats from single 6-OHDA injected group ($n = 10$) and serial 6-OHDA injected group ($n = 10$) that initiated copulation following intraperitoneal injection of AMPT and vehicle. *$P < 0.05$, **$P < 0.005$.

Experiment 3. Effects of 6-OHDA on copulatory behavior 30 min and 24 h after MPOA injection

In order to test whether behavioral deficits would be observed before mechanisms of recovery could compensate for neuronal loss, animals were tested 30 min and 24 h after an intracranial injection of 6-OHDA. Ten rats received a single MPOA injection of 6-OHDA; 10 additional rats received vehicle injections of the same volume. Since 6-OHDA lesions of the MPOA were previously shown to have no lasting behavioral effects[4] (and Experiment 1), injections of 6-OHDA and vehicle were counterbalanced the following week, so that each animal was used as its own control. All animals were treated with DMI to protect NE terminals.

There was no interaction resulting from order of vehicle or 6-OHDA on weeks one and two, using a three-way mixed ANOVA (one between two within). Tests 30 min after 6-OHDA showed deficits in all behaviors compared to scores 24 h after both 6-OHDA and vehicle in-

TABLE III

Mean latencies (s ± S.E.M.) for animals 4 h and 28 h after MPOA injections of 6-OHDA or vehicle

	Mount	*Intromission*	*Ejaculation*	*Postejaculatory*
4 h				
Veh	551.2 ± 177.2**	983.9 ± 185.3*	1054.7 ± 169.9*	1064.8 ± 150.9*
6-OHDA	1236.7 ± 192.3	1476.9 ± 172.8	1570.1 ± 129.1	1547.7 ± 135.0
28 h				
Veh	849.7 ± 191.9	1097.7 ± 187.8	1145.6 ± 164.7*	1099.7 ± 151.2*
6-OHDA	321.7 ± 117.6**	615.6 ± 178.2**	1009.5 ± 160.3*	935.4 ± 141.4**

Significance levels are shown for comparison to 4-h 6-OHDA group.
*$P < 0.05$, **$P < 0.01$.

jections.

For measures affected, two-way within subjects ANOVA scores are shown followed by the level of significance for Duncans posthoc comparison with deficits 30 min following 6-OHDA (Table IIA): increased mount latency $F_{3,57} = 2.25$, compared with their scores 24 h after either vehicle or 6-OHDA ($P < 0.05$); increased intromission latency $F_{3,57} = 3.32$ ($P < 0.05$) compared with their scores 24 h after either vehicle or 6-OHDA ($P < 0.05$); increased ejaculation latency $F_{3,57} = 2.73$ compared with their scores 24 h after either vehicle or 6-OHDA ($P < 0.05$); increased postejaculatory interval $F_{3,57} = 3.56$ ($P < 0.05$) compared with their scores 24 h after either vehicle or 6-OHDA ($P < 0.05$); and decreased ejaculation frequency $F_{3,57} = 2.64$ compared with their scores 24 h after either vehicle or 6-OHDA ($P < 0.05$, Fig. 4).

The number of animals initiating copulation was also decreased at 30 min compared to 24 h after 6-OHDA (Table IIB). This decrease in number of animals copulating was reflected in a decrease in animals achieving mounts ($Q = 12.51$, $P < 0.01$), intromissions ($Q = 12.33$, $P < 0.01$), and ejaculations ($Q = 9.48$, $P < 0.05$). No other significant differences were found.

Experiment 4. Effects of 6-OHDA on copulatory behavior 4 h and 28 h after MPOA injection

Animals in the previous experiment were impaired at 30 min following 6-OHDA, compared with their scores 24 h after either 6-OHDA or vehicle. However, scores 30 min after 6-OHDA were not significantly different from scores 30 min after vehicle. Since DMI and/or the injection of a cold substance may have contributed to a slight impairment in both groups at 30 min, an additional experiment tested behavior at a somewhat longer interval after injection.

Ten rats received a single injection of 6-OHDA, 10 additional rats received vehicle control injections of the same volume. Injections of 6-OHDA and vehicle were counterbalanced the following week, so that each animal was used as its own control. All animals were treated

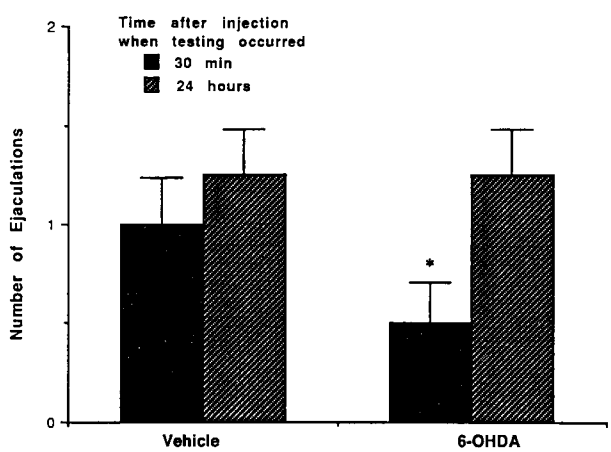

Fig. 4. Mean number of ejaculations (± S.E.M.) during tests conducted 30 min and 24 h after an MPOA injection of 6-OHDA or vehicle. *$P < 0.05$.

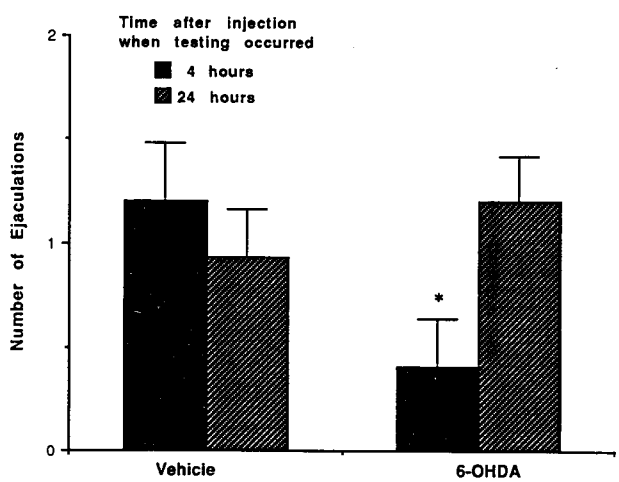

Fig. 5. Mean number of ejaculations (± S.E.M.) during tests conducted 4 h and 28 h after an MPOA injection of 6-OHDA or vehicle. *$P < 0.05$.

with DMI prior to 6-OHDA injection. Behavioral testing was conducted 4 h after intracranial injections were completed. A second behavioral test was conducted 24 h after the first test.

As in Experiment 3, there was no interaction resulting from order of vehicle or 6-OHDA on weeks 1 and 2. Ejaculation latency and postejaculatory interval were lengthened in animals tested 4 h after 6-OHDA compared to animals tested 4 h after vehicle and to animals tested 28 h after either vehicle or 6-OHDA. Mount and intromission latency were lengthened in animals tested 4 h after 6-OHDA compared to vehicle animals tested 4 h after vehicle and animals tested 28 h after 6-OHDA. A two-way within subjects ANOVA revealed the following interactive effects: mount latency $F_{1,42} = 14.99$, $P < 0.001$, intromission latency $F_{1,42} = 9.21$, $P < 0.005$, ejaculation latency, $F_{1,42} = 5.36$, $P < 0.05$, postejaculatory interval, $F_{1,42} = 4.73$, $P < 0.05$, and ejaculation frequency $F_{1,42} = 6.27$, $P < 0.02$.

Duncan's posthoc tests showed the following deficits in animals tested 4 h after 6-OHDA injection: increased mount latency compared to vehicle at 4 h and 6-OHDA at 28 h ($P < 0.01$), increased intromission latency compared to vehicle at 4 h ($P < 0.05$) and 6-OHDA at 28 h ($P < 0.01$), increased ejaculation latency compared to each of the other three groups ($P < 0.05$), increased postejaculatory interval compared to vehicle at 4 h and 28 h ($P < 0.05$) and 6-OHDA at 28 h ($P < 0.01$) (Table III) and decreased ejaculation frequency compared to vehicle at 4 h and 6-OHDA at 28 h ($P < 0.05$) (Fig. 5).

DISCUSSION

Three MPOA injections of 6-OHDA, given on successive weeks, produced no greater copulatory deficits than a single injection. However, AMPT significantly impaired behavior in both single and serial lesion groups, with significantly fewer serial lesion than single lesion animals initiating copulation after this CA synthesis challenge. Biochemical analysis revealed no significant differences between single and serial lesion groups in concentrations of NE, Epi, DA or DOPAC.

There was significant impairment in all measures of copulatory behavior 30 min after an MPOA injection of 6-OHDA, compared to scores 24 h after vehicle or 6-OHDA injections. However, no significant differences were found between 6-OHDA and vehicle scores 30 min after injection, possibly because of a slight impairment of vehicle animals due to DMI or the cold injection.

In a separate group of animals tested 4 h after an MPOA injection of 6-OHDA, there were significant decreases in all measures of copulatory behavior compared to scores 4 h following a vehicle injection.

Bitran and colleagues[4] observed no behavioral deficits in copulatory behavior in animals tested as early as 3 days following 6-OHDA injections into the MPOA. Furthermore, DA concentration was decreased by only 23%. Although this decrease was statistically significant, it was not sufficient to induce behavioral deficits. In addition, the DOPAC/DA ratio was significantly elevated in those animals that received 6-OHDA, compared to vehicle-injected animals. The ratio of DOPAC/DA is used as a measure of DA metabolism[1,7,8,17]. Thus, it appeared that surviving neurons had increased their rate of metabolism to compensate for lost terminals. A dose of the CA synthesis inhibitor AMPT (100 mg/kg) that did not produce copulatory deficits in control animals did impair copulation in animals sustaining 6-OHDA lesions[4]. Britan and colleagues[4] suggested the relative resistance of MPOA terminals to 6-OHDA, as well as mechanisms of recovery (i.e. increased DA metabolism, receptor supersensitivity), had prevented expression of copulatory behavior deficits.

The present experiments tested whether multiple intracranial injections of 6-OHDA into the MPOA would induce greater behavioral and/or biochemical changes than a single injection, and whether behavioral effects could be observed at an earlier time. Willis and Smith[27] found that repeated injections of 6-OHDA were more effective in decreasing hypothalamic DA concentrations than was a single 6-OHDA injection. In the present study, multiple injections produced no greater behavioral deficits or neurotransmitter depletion than a single lesion, suggesting that MPOA neurons are relatively resistant to the neurotoxic effects of 6-OHDA. This resistance may result in part from the high rate of turnover of MPOA DA neurons[14]. Since neurotoxicity depends on intracellular accumulation of a threshold concentration[11,12], rapid release of 6-OHDA back into extracellular fluid may protect the terminal. Furthermore, if metabolic rate is increased in remaining terminals following 6-OHDA[4] repeated lesions should further increase metabolism and therefore, resistance.

A second factor contributing to the resistance of DA neurons to multiple injections of 6-OHDA is the cells' inability to accumulate a threshold amount of this neurotoxin due to damage of the reuptake system caused by the initial injection. Thoenen and Tranzer[22] have suggested than an initial dose of 6-OHDA that does not produce maximal damage may prevent uptake of additional doses by causing temporary damage to reuptake systems.

A third possible contribution to the resistance of MPOA DA neurons is the distribution of DA neurons relative to noradrenergic (NA) neurons in the same region. The vast majority of CA fibers innervating the

MPOA are NA[14]. The use of DMI to protect NA fibers from the effects of 6-OHDA may cause an extracellular accumulation of NE by blocking reuptake of this transmitter[9]. High concentration of extracellular NE may then compete with 6-OHDA for reuptake at DA releasing terminals[9,21]. These factors would be further accentuated by a greater NA/DA ratio following destruction of a portion of DA terminals by an initial injection of 6-OHDA.

In summary, it appears that resistance of MPOA DA neurons to neurotoxic effects may be the result of high metabolic activity, impairment of reuptake mechanisms and/or protection by surrounding NA fibers. It thus appears that destruction of a greater number of DA neurons in the MPOA would be extremely difficult.

Experiment 3 showed that 6-OHDA did produce deficits 30 min after administration when compared to behavioral measures prior to 6-OHDA injection and 24 h following the injection. However these deficits were not statistically significant compared with tests 30 min following vehicle injections. A factor that may have minimized the apparent effectiveness of 6-OHDA, compared to vehicle at 30 min, was the slight decrease in copulatory behavior of vehicle animals. Although not statistically significant, this decrease may have led to a 'floor effect' so that the additional impairment by 6-OHDA was not sufficient to result in a statistically significant difference. This decrease may have resulted from the DMI pretreatment or the infusion of a cold solution into the MPOA.

In Experiment 4, tests 4 h after injection did reveal significant impairment by 6-OHDA compared to both a vehicle injection at 4 h and to 6-OHDA-injected animals retested 24 h later. The 4-h interval was chosen because degenerative effects resemble neural axotomy at this time[11] and because hypothalamic CA levels showed a greater reduction at 4 h than at 30 min following intraventricular administration of 6-OHDA[3]. In addition, the effects of DMI and of cold intracranial injections were expected to have diminished 4 h after injection.

The behavioral deficits observed 30 min and 4 h after 6-OHDA suggest that initial neurodegeneration can produce behavioral deficits before mechanisms of recovery can compensate. Furthermore, recovery to baseline levels of copulatory behavior occurred in both of these lesion groups within 24 h of the first test session.

The rapid time course of recovery seen in the present study is in agreement with at least two reports of submaximal depletion of DA following 6-OHDA. Marshall[16] found that rats sustaining a mean depletion of 52% showed deficits in somatosensory localization to touch one day after DA depletion of the ventralis tegmenti of the forebrain DA pathway. This deficit was significantly reduced when subjects were retested 24 h later. Of even greater relevance to the present study, Kitchen[13] reported that 6-OHDA compared to vehicle decreased circulating luteinizing hormone (LH) 2, 3 and 4 h after injection into the preoptic area and medial forebrain bundle. However, when tested 24 h after injection, 6-OHDA animals showed no significant difference in LH levels compared to control animals.

Metabolic increase has been shown to occur within 24 h of denervation[26]. Although one study has shown behavioral supersensitivity normally associated with postsynaptic changes 24 h after 6-OHDA administration[23], it is unlikely that a rapid increase in receptor number or sensitivity is responsible for recovery in the present experiments. Previous studies have shown that for supersensitivity to occur, DA depletion must exceed 70% of controls[5,24,28]. It is unlikely the level of DA depletion in the present studies reached 70%, based on the 23% depletion found in a previous study using the same lesion protocol[4].

The present results also lend additional support to the contention that DA input at the MPOA is essential for expression of normal levels of male copulatory behavior. Previous studies have shown that MPOA stimulation using the DA agonist apomorphine increased both rate and efficiency of male copulatory behavior[10]. Likewise, blockade of DA receptors in the MPOA using the DA antagonist *cis*-flupenthixol decreased the rate and efficiency of copulation[19], the number of ex-copula penile reflexes and motivation to be with a receptive female[25].

In summary, 6-OHDA injected into the MPOA produced an initial decrease in copulatory efficiency of male rats. Furthermore, deficits seen in the first 4 h after injection were completely reversed within 24 h. The primary mechanism of recovery is likely an increase in metabolism at remaining terminals, since increased receptor density is associated with a greater amount of DA depletion and since inhibition of CA synthesis with AMPT impaired copulation after behavioral recovery in Experiment 2.

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REFERENCES

1 Altar, C.A., Marien, M.R. and Marshall, J.F., Time course of adaptations in dopamine biosynthesis, metabolism, and release following nigrostriatal lesions: implications for behavioral recovery from brain jury, *J. Neurochem.*, 48 (1987) 390–399.

2 Arendash, G.W. and Gorski, R.A., Effects of discrete lesions of the sexually dimorphic nucleus of the preoptic area or other medial preoptic regions on the sexual behavior of male rats, *Brain Res. Bull.*, 10 (1983) 147–154.
3 Bell, L., Uretsky, N. and Iversen, L.L., Time course of the effects of 6-hydroxydopamine on catecholamine containing neurones in rat hypothalamus and striatum, *Br. J. Pharmacol.*, 40 (1970) 790–799.
4 Bitran, D., Hull, E.M., Holmes, G.M. and Lookingland, K.J., Regulation of male rat copulatory behavior by preoptic incertohypothalamic dopamine neurons, *Brain Res. Bull.*, 20 (1988) 323–331.
5 Creese, I. and Snyder, S.H., Nigrostriatal lesions enhance striatal 3H-apomorphine and 3H-spiroperidol binding, *Eur. J. Pharmacol.*, 56 (1979) 277–281.
6 Ginton, A. and Merari, A., Long range effects of MPOA lesions on mating behavior in the male rat, *Brain Res.*, 120 (1977) 158–163.
7 Hefti, F., Enz, A. and Melamed, E., Partial lesions of the nigrostriatal pathway in the rat — acceleration of transmitter synthesis and release of surviving dopaminergic neurons, *Neuropharmacology*, 24 (1985) 19–23.
8 Hefti, F., Melamed, E. and Wurtman, R.J., Partial lesions of the dopaminergic nigrostriatal system in rat brain: biochemical characterization, *Brain Res.*, 195 (1980) 123–137.
9 Herve, D., Studler, J.M., Blanc, G., Glowinski, J. and Tassin, J.P., Partial protection by desmethylimipramine of the mesocortical dopamine neurones from the neurotoxic effect of 6-hydroxydopamine injected in ventral mesencephalic tegmentum. The role of noradrenergic innervation, *Brain Res.*, 383 (1986) 47–53.
10 Hull, E.M., Bitran, D., Pehek, E.A., Warner, R.K., Band, L.C. and Holmes, G.M., Dopaminergic control of male sex behavior in rats: effects of an intracerebrally-infused agonist, *Brain Res.*, 370 (1986) 73–81.
11 Jonsson, G., Studies on the mechanisms of 6-hydroxydopamine cytotoxicity, *Med. Biol.*, 54 (1976) 406–420.
12 Jonsson, G. and Sachs, Ch., Uptake and accumulation of 3H-6-hydroxydopamine in adrenergic nerves, *Eur. J. Pharmacol.*, 16 (1971) 55–62.
13 Kitchen, J.H., Effects of intracerebral injections of 6-hydroxydopamine on LH and FSH release in male rats, *Neuroendocrinology*, 15 (1974) 240–244.
14 Lookingland, K.J. and Moore, K.E., Dopamine receptor-mediated regulation of incertohypothalamic dopaminergic neurons in the male rat, *Brain Res.*, 304 (1984) 329–338.
15 Markwell, M.K., Hass, S.M., Tolbert, N.E. and Bieber, L.L., Protein determinations in membrane and lipoprotein samples: manual and automated procedures, *Meth. Enzymol.*, 72 (1981) 296–303.
16 Marshall, J.F., Somatosensory inattention after dopamine-depleting intracerebral 6-OHDA injections: spontaneous recovery and pharmacological control, *Brain Res.*, 177 (1979) 311–324.
17 Mitchell, J.B. and Stewart, J., Effects of castration, steroid replacement, and sexual experience on mesolimbic dopamine and sexual behaviors in the male rat, *Brain Res.*, 491 (1989) 116–127.
18 Palkovits, M., Isolated removal of hypothalamic or other brain nuclei of the rat, *Brain Res.*, 59 (1973) 449–450.
19 Pehek, E.A., Warner, R.K., Bazzett, T., Bitran, D., Band, L.C., Eaton, R.C. and Hull, E.M., Microinjection of *cis*-flupenthixol, a dopamine antagonist, into the medial preoptic area impairs sexual behavior of male rats, *Brain Res.*, 443 (1988) 70–76.
20 Pellegrino, L.J., Pellegrino, A.S. and Cushman, A.J., *A Stereotaxic Atlas of the Rat Brain*, 2nd edn., Plenum, New York, 1979.
21 Robinson, T.E. and Whishaw, I.Q., Normalization of extracellular dopamine in striatum following recovery from a partial unilateral 6-OHDA lesion of the substantia nigra: a microdialysis study in freely moving rats, *Brain Res.*, 450 (1988) 209–224.
22 Thoenen, H. and Tranzer, J.P., Chemical sympathectomy by selective destruction of adrenergic nerve endings with 6-hydroxydopamine, *Naunyn-Schmiedeberg's Arch. Pharmacol. Exp. Pathol.*, 261 (1968) 271–288.
23 Ungerstedt, U., Striatal dopamine release after amphetamine or nerve degeneration revealed by rotational behaviour, *Acta Physiol. Scand.*, Suppl. 367 (1971) 49–68.
24 Waddington, J.L., Relationship between functional supersensitivity and extent of denervation of dopamine receptors, *Physiol. Behav.*, 26 (1981) 627–629.
25 Warner, R.K., Thompson, J.T., Markowski, V.P., Loucks, J.A., Bazzett, T., Eaton, R.C. and Hull, E.M., Microinjection of the dopamine antagonist *cis*-flupenthixol into the MPOA impairs copulation, penile reflexes and sexual motivation in male rats, *Brain Res.*, 540 (1991) 177–182.
26 Westerink, B.H.C., Van der Heyden, J.A.M. and Korf, J., Enhanced dopamine metabolism after small lesions in the midbrain of the rat, *Life Sci.*, 22 (1978) 749–756.
27 Willis, G.L. and Smith, G.C., Effects of intrahypothalamic multistage versus single injections of 6-hydroxydopamine, *Brain Res.*, 245 (1982) 345–352.
28 Zigmond, M.J. and Stricker, E.M., Supersensitivity after intraventricular 6-hydroxydopamine: relation to dopamine depletion, *Experientia*, 36 (1980) 436–438.