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Behavioral and Biochemical Effects of Neonatal Treatment of Rats with 6-Hydroxydopa[1]

JACK H. MCLEAN, RICHARD M. KOSTRZEWA AND JAMES G. MAY

Department of Psychology, University of New Orleans
New Orleans, LA 70122

and

Tulane University, School of Medicine, Department of Pharmacology

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MCLEAN, J. H., R. M. KOSTRZEWA AND J. G. MAY. *Behavioral and biochemical effects of neonatal treatment of rats with 6-hydroxydopa.* PHARMAC. BIOCHEM. BEHAV. 4(5) 601–607, 1976. – Rats receiving injection of either 6-hydroxydopa (60 μg/g) or saline on Days 1, 3, and 5 of life were studied in adulthood on a number of behavioral tasks before being sacrificed at 8 or 12 months for NE assay. The treated rats exhibited impaired passive avoidance, less shock-induced aggression, and more locomotor open-field activity than the control rats. There were no differences between the groups in male copulatory behavior, food and water intake, or thermoregulation. In comparison to the saline rats, 6-hydroxydopa rats showed elevated levels of endogenous NE in lower brainstem regions, e.g., midbrain and pons-medulla, as well as cerebellum. Hypothalamic NE level was not affected. Significant depletions of NE were obtained in the hippocampus and neocortex.

6-Hydroxydopa Norepinephrine Avoidance Hippocampus Shock-induced aggression

SINCE the presence of catecholamine (CA)-containing neurons in the central nervous sytem was first described [1, 11, 12, 16], several investigators have presented detailed mappings of central monoaminergic nuclei, fiber tracts, and terminals [21,45]. A number of behaviors has been postulated as likely mediated by these catecholamine-containing neurons [23].

Evidence which implicates the central catecholaminergic system in the control of behavior stems primarily from research with reserpine and 6-hydroxydopamine (6-OHDA). Reserpine was first reported to effect a depletion of norepinephrine (NE), dopamine (DA), and serotonin, as well as disrupt a number of behaviors [6,7]. Subsequent research reported 6-OHDA to be more specific than reserpine in that NE and DA were depleted with little effect on serotonin [3,18]. However, 6-OHDA does not cross the blood-brain barrier and must be injected intracranially to have a central effect. Central injections of 6-OHDA have been shown to lower brain CA levels without having an effect on peripheral structures. Although not replicated reliably from study to study, among behaviors that have resulted from central injections of 6-OHDA are hypophagia [5,14], reduced activity [15], increased emotionality and/or aggression [13,33], impaired acquisition and performance in a double T-maze [20], and reduced rates of intracranial self-stimulation [2,4].

A precursor to 6-OHDA, 6-hydroxydopa (6-OHDOPA), has recently produced findings which complement and extend those from reserpine and 6-OHDA. Systemic injections of 6-OHDOPA were found to be effective in crossing the blood-brain barrier and causing marked reduction in whole brain NE [21]. Further, this effect of 6-OHDOPA was selective in that it destroyed noradrenergic terminals without affecting other types of neurons [21, 38, 39]. Histochemical observation of a number of brain areas revealed regional variation, with significant NE depletion in the hippocampus, cortex, and cerebellum [21, 37, 40].

The behavioral effects of 6-OHDOPA have been investigated in four studies, all using intraventricular administration, and all reporting approximately similar results. The first study reported significant increases in shock-induced fighting that appeared on the fourth day after the injection [43]. The second report of behavioral effects of 6-OHDOPA found reductions in food intake and activity, with both effects lasting for 5 days before returning to preinjection levels [37]. Thermoregulation was unaltered. The treated animals also exhibited significantly longer emergence latencies and higher emotionality ratings than controls. These effects persisted for up to 70 days, a time course which paralleled NE depletion in the telencephalon and hindbrain.

Another study found aphagia, reduced activity, and

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hypothermia in the first few hours after injections, but behavioral differences between experimental and control animals had essentially disappeared after 24 hours [25]. The fourth and most recent report found 6-OHDOPA to effect reductions in food intake, water intake, and locomotor activity for the first few post injection days before returning to control levels [36]. Emotionality ratings of treated animals were significantly higher than controls throughout the 14 day testing period. In summary, common findings from intraventricular 6-OHDOPA injections are aphagia, adipsia, reduced motor activity, and increased emotionality and aggression.

The purpose of the present research was to determine the effects of 6-OHDOPA on the developing nervous system, by administering the agent systemically during the first week of life and following the pattern of CA alteration from a few weeks up to a year later. Behavioral testing began in adulthood and was designed to assess 6-OHDOPA effects on a number of motivational variables, both appetitive and aversive: food and water intake, copulation, approach-avoidance behavior in a straight runway, open field activity, pain-elicited aggression, and thermoregulation.

METHOD

Animals

Sprague-Dawley male albino rats were used in all studies. Animals were treated from the day of birth and at 48-hr intervals with either 6-OHDOPA (60 μg/g IP) or the diluent, saline (0.9%)-ascorbic acid (0.1%). Rats received a total of 3 treatments.

All rats were weaned at about 25 days of age and were maintained during the suckling period on a 10 hr–14 hr light-dark cycle at a colony room temperature of 22°C. After weaning, animals were housed individually in wire mesh cages and were not handled again until adulthood when behavioral testing began. After the weaning stage animals were maintained on a reversed light–dark cycle, on at 2200 hr, off at 800 hr. Water and standard Purina lab chow were available ad lib except during approach-avoidance training when access to food was limited.

Catecholamine Analysis

All animals used in the behavioral studies were sacrificed by decapitation at 8 months or 1 year, postnatal age. Brain (minus, the olfactory bulbs, pituitary gland and pineal gland) was rapidly removed and dissected into several regions [17]. Regions of the CNS taken for study were the pons-medulla, midbrain, hypothalamus, neocortex, hippocampus, striatum, cerebellum, and cervical-thoracic spinal cord. Peripheral tissues taken for assay of CA were the cardiac atria and ventricles.

Tissues taken for CA analysis were rapidly frozen over dry ice and stored at −50°C until the time of assay. Each brain region was analyzed for CA content by the hydroxyindole fluorometric method of Hogans [32]. Tissue specimens were homogenized in acidified butanol and the CA was subsequently extracted into phosphate buffer (0.1M, pH 6.5). Iodine oxidation was used to form the fluorescent trihydroxyindole from NE or the dihydroxyindole from DA. Recovery of CA was routinely 85 to 95%. Values reported are uncorrected for recovery.

Behavioral Tests

Food and water consumption. Food intake, corrected for spillage, was monitored daily in 10 treated and 10 control rats for a 10 day interval beginning at day 80 after birth. Subsequently, water intake was measured for the following 10-day interval.

Copulation. Treated and control rats were tested with estrous females during three 30-min sessions, separated by one week intervals. The female lures, previously ovariectomized under ether anesthesia, were brought into heat by IM injections of 6.6 μg estradiol benozate and 0.5 mg progesterone at 48 hr and 6 hr, respectively, prior to a test session. Male copulatory behavior was scored by trained observers using an Esterline-Angus event recorder. Responses recorded were mounts without intromission, mounts with intromission and ejaculations. Mount and intromission latencies were transcribed from the event records at a later date.

Approach-avoidance behavior. For this portion of the experiment, the animals were maintained on reduced food intake in order to approximate 85% ad lib weight. After initial familiarization in a straight runway (stem 132 cm, start and goal boxes each 21 cm), each rat was run for 5 trials a day for 5 days (approach phase). On each trial in which the rat successfully entered the goal box, he found a 45 mg Noyes pellet in the food dish. On the 6th day when the rat attempted to eat the food pellet, he completed an electric circuit between the metal food dish and a metal plate on the floor and received a 2.5 mA shock. The rats were then run for food reward for an additional 5 days, 5 trials a day (avoidance phase). Dependent measures taken were start box latency, total running time (upper limit of 1 min), and distance travelled. The last measure was recorded since it was anticipated that most of the rats would not enter the goal box during the avoiance phase.

Pain-elicited aggression. The rats within each group were randomly paired and tested for pain-elicited aggression during three 5-min sessions. The shock (2.0 mA, scrambled) was delivered through the grid floor of a 30 × 32 × 30 cm Plexiglas chamber. The frequency of the shocks was random, with a mean of 15 per min. The number of fight bouts in each session was recorded.

Open field activity. General activity was measured at 4 periods during the course of the experiment: Test 1, 100 110 days; Test 2, 130 160 days; Test 3, 290 295 days; and Test 4, 345–365 days of age. Each rat was placed in a circular open field (110 cm dia.) which was marked off into approximately equal area segments. The number of segments entered in 5 min was recorded.

Thermoregulation. The ability to regulate body temperature was compared in 6 treated and 6 control rats at 1 year of age. Animals were placed in individual metal cages at 4°C for 4 hr. Rectal temperature (T_R) was measured with a thermistor after 30 min and thereafter at hourly intervals.

Statistical Analyses

All behavioral measures were analyzed using Analysis of Variance (ANOVA). One factor was always between groups (treated vs control). Where appropriate, additional factors were included for repeated measures. When there occurred an unequal number of rats between groups, subjects were randomly voided to give an equal-N analysis. Student's *t*-test was used to evaluate differences between CA levels in

tissues of control and 6-OHDOPA-treated groups at each time period.

Drugs

Norepinephrine (free base) and dopamine hydrochloride, used in the biogenic amine assays, as well as dl-6-hydroxydopa were obtained from Regis Chemical Co., Chicago, Il. Estradiol benzoate and progesterone used in the copulation tests were obtained from Nutritional Biochemical Corp., Cleveland, Ohio. Other reagents and chemicals used in analytical procedures were A. C. S. grade or spectro-grade.

RESULTS

Catecholamine Analysis

When 6-OHDOPA-treated rats were sacrificed at 8 or 12 months of age, marked alterations in NE content were found in various regions of the CNS (Figs. 1 and 2). In the pons-medulla, which contains the major portion of noradrenergic perikarya, NE was elevated by 60 to 65% at both time periods. Similarly, in the midbrain, NE was elevated to the same degree as in the pons-medulla at each age. The changes in NE content in the cerebellum were similar to those found in other brainstem regions. At 8 months of age NE was increased by 20%, while at 12 months NE was elevated more than 35%. However, in the hypothalamus, NE remained unchanged. Therefore, neonatal 6-OHDOPA treatment of rats failed to reduce NE levels in the brainstem and cerebellum, but rather, produced a permanent elevation in NE content in these more caudal structures.

In marked contrast to these findings was the permanent reduction in NE levels found in the spinal cord and telencephalic regions. In the neocortex NE was reduced by 47 and 72% at 8 and 12 months, respectively. Such a decrease would likely be accompanied by a similar reduction in the number of noradrenergic terminals in the region. In the hippocampus NE content was reduced to an even greater degree, by approximately 80% at both the 8 and 12 month periods. In the cervical-thoracic spinal cord NE was decreased by 62 and 70% below control levels at 8 and 12 months, respectively. Thus, the effects of neonatal 6-OHDOPA on noradrenergic neurons in the telencephalic regions and spinal cord were qualitatively similar, as reflected by changes in NE content. At neither age level did the striatal DA content of treated rats differ from that of controls. Thus, the CNS effects of 6-OHDOPA were due primarily to alterations in noradrenergic neurons.

Despite the widespread effects of 6-OHDOPA on noradrenergic neurons in the CNS, sympathetic terminals in the peripheral nervous system apparently were unaltered in the groups of animals studied (Fig. 3). In both cardiac atria and ventricles, NE levels were unaltered at 8 and 12 months of age. Therefore, it appears that the permanent chemical lesioning effects of 6-OHDOPA are localized to noradrenergic neurons in the CNS.

Behavioral Tests

Food and water consumption. No significant differences were found between the control and 6-OHDOPA rats for either food or water consumption (all F's< 1.00, *df* = 1, 18).

Copulation. None of the measures of male copulatory behavior approached significance, with most of the ANOVA's yielding F values of 1.00 or less.

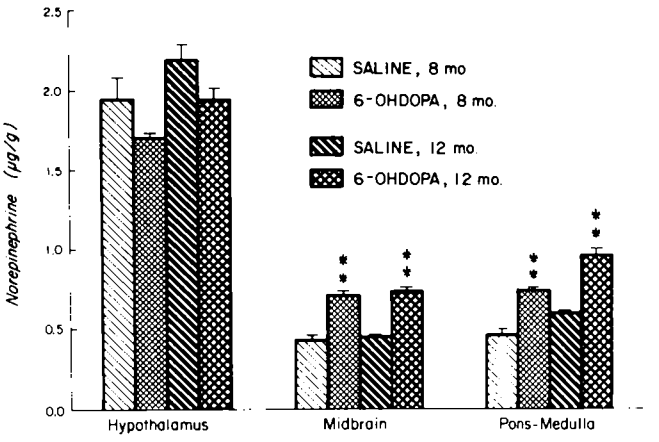

FIG. 1. NE content of neocortex, hippocampus, cerebellum, and spinal cord of rats treated with 6-OHDOPA (60 μg/g IP) or saline on Days 1, 3, and 5 of life and sacrificed at 8 (diagonal lines) or 12 months (crosshatched). Each column represents the mean ± S.E.M. of 3–6 animals at 8 mo and 6 -8 animals at 12 months. *$p<0.05$; **$p<0.005$.

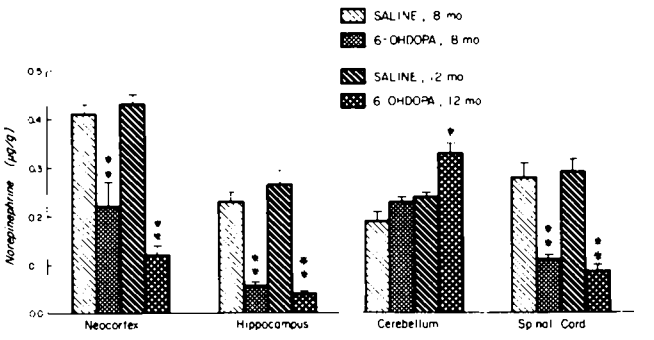

FIG. 2. NE content of hypothalamus, midbrain, and pons-medulla. Legend as in Fig. 1.

Approach-avoidance behavior. Treated and control animals did not differ during the approach phase of runway training (all F's< 1.00). However, during the avoidance phase there was a non-significant trend for the 6-OHDOPA-treated rats to leave the start box sooner, $F(1,26) = 1.82$, $p<0.18$, and approach the goal box more closely than the saline rats, $F(1,26) = 3.53$, $p<0.07$. The mean start-box emergence speed (1/sec) was 0.123 for the control and 0.168 for the the 6-OHDOPA group. The mean distance travelled during the avoidance phase is shown in Fig. 4 where avoidance is undergoing extinction in both groups, but the treated animals traversed a greater distance than the controls on all 5 tests.

Pain-elicited aggression. The mean number of fight bouts exhibited by both groups during the 3 sessions is shown in Fig. 5. Control rats were significantly more aggressive than the treated rats on the first test, $F(1,14) = 6.87$, $p<0.025$. In the remaining 2 sessions, however, the number of fight bouts exhibited by the saline animals decreased to approximately the same level as that of the 6-OHDOPA rats.

Open-field activity. The mean number of sections entered during the 4 open-field tests is shown in Fig. 6. Although there were no differences between groups in the

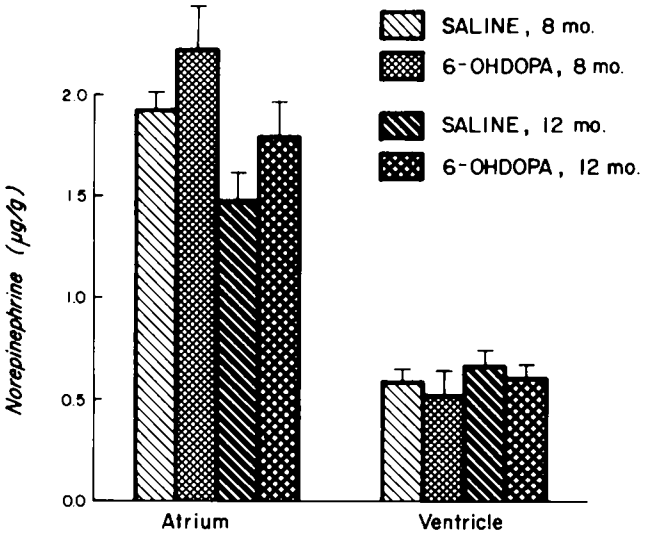

FIG. 3. NE content of cardiac atria and ventricles. Legend as in Fig. 1.

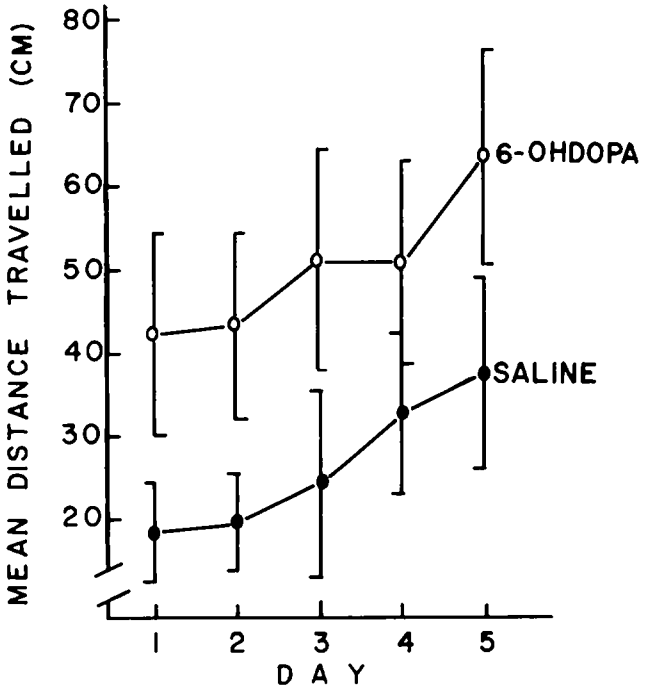

FIG. 4. Mean (± SEM) distance travelled in the Approach-Avoidance task on the 5 days after receiving electric shock in the goal box (avoidance phase). (N = 14 per group).

first 2 tests, the treated rats were significantly more active than the controls on Test 3 ($p<0.025$) and Test 4 ($p<0.01$). More specifically, both groups exhibited a decrease in activity, but the decrease was much larger in the saline group.

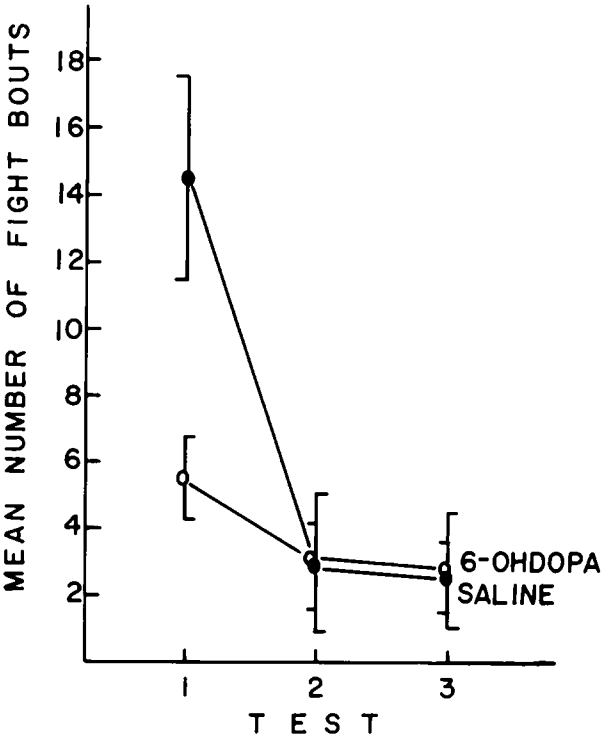

FIG. 5. Mean (± SEM) number of fight bouts exhibited by each group during three 5-min pain elicited aggression sessions. (N = 8 per group).

Thermoregulation. There were no differences between the 6-OHDOPA and control animals in ability to maintain body temperature at any of the intervals measured ($p<0.5$).

DISCUSSION

The resultant alterations in CA levels in the brain can be explained by the permanence of neonatal 6-OHDOPA treatment on noradrenergic neurons. The alterations observed in NE levels in the neocortex and hippocampus at 8 and 12 months are identical to the changes observed during the initial 8 week period after birth [26] when noradrenergic neurons are in the most rapid phase of growth and development [8, 9, 10, 35]. Such a reduction in NE content apparently would reflect a similar decrease in the number of binding sites for NE in the neurons, as would result when the terminals had degenerated [44,46]. Separate studies have already demonstrated a decrease in the in vitro uptake velocity of H^3-NE by synaptosomes isolated from the neocortex at intervals up to 1 year, thereby demonstrating quantitatively an apparent diminution in the number of noradrenergic terminal endings in the telencephalic regions [26]. In all brainstem regions NE levels were either elevated or unchanged in the treated group when compared to the control at 8 or 12 months. Such alterations are similar to those changes seen in the initial 8 week postnatal developmental period. There are three likely explanations for these brainstem NE increases: (1) an

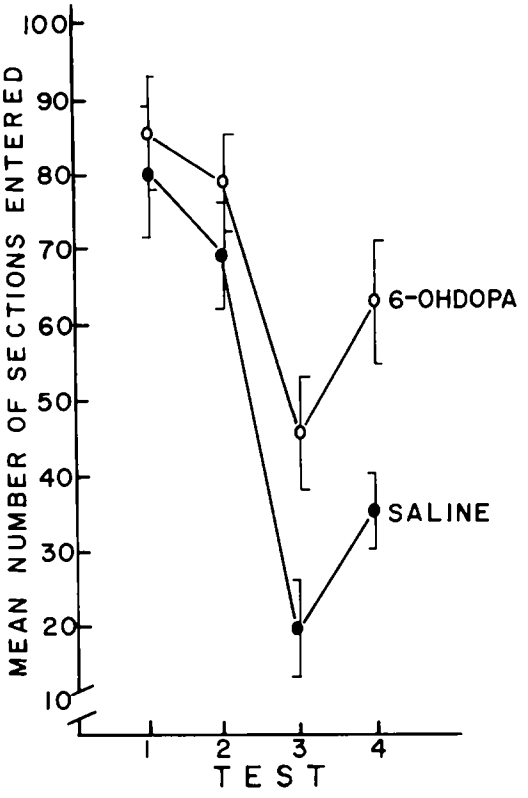

FIG. 6. Mean (± SEM) number of sections entered by each group during four 5-min open field activity tests. (N = 10 per group).

increase in the number of noradrenergic terminal endings, (2) intra-axonal accumulation of NE (preterminal build-up), and (3) an increase in the concentration of NE in the nerve terminals. The nature of the change in NE in brainstem regions following 6-OHDOPA is under study, but a combination of such factors has been found in the pons-medulla following 6-OHDA treatment of neonates [22]. Because of the failure of 6-OHDOPA to alter DA content of the striatum at any time period, it appears that any behavioral changes would reflect an alteration of noradrenergic neurons. In addition, the behavioral alterations are likely due primarily to central effects of 6-OHDOPA, since NE levels are unchanged at 8 and 12 months in the atria and cardiac ventricles. Numerous other studies have shown that noradrenergic terminals in the cardiac ventricle are the most susceptible to damage by 6-OHDA/6-OHDOPA when compared to all other peripheral end organs [30]. Therefore, lack of destruction in the heart would suggest a normal component of noradrenergic terminals in other peripheral end organs. Thus, the general prevailing feature in the experimental group is a decrease in the number of noradrenergic terminals in the neocortex and hippocampus.

The present biochemical findings are in agreement with other neonatal studies employing 6-OHDOPA [27, 38, 47]. All reports, including the present one, show that NE levels are greatly reduced in telencephalic regions [27,47] or that uptake of ^{3}H-NE by slices of synaptosomes from this region is similarly reduced in 6-OHDOPA animals [26,39]. In brainstem regions NE levels are elevated in 6-OHDOPA-treated rats [27,47]. Prior studies with neonatal 6-OHDA indicate the same type alterations in brainstem NE levels [30]. Such a change has been associated with a regenerative sprouting of noradrenergic neurons in these regions after 6-OHDA [38], but not after 6-OHDOPA [26]. Because sympathetic noradrenergic neurons are not permanently altered by 6-OHDOPA, this agent appears to offer some advantages over use of 6-OHDA in neonates. The latter agent, given either IV, IP, or SC, produces a profound impairment of sympathetic neuronal growth and development [30], and therefore, complicates behavioral testing. Even when given intracisternally to neonates, 6-OHDA produces long-term reductions in NE content of various peripheral organs, presumably because of the decrease in growth rate of the animal [31]. Thus, neonatal 6-OHDOPA is similar to but not identical with neonatal 6-OHDA treatment.

In contrast to the pharmacological findings, the behavioral results are not in accord with existing literature. From previous research with 6-OHDA and 6-OHDOPA one would likely have predicted hypophagia [5, 14, 25, 36, 37], reduced activity [25, 36, 37], increased emotionality and/or aggression [36, 37, 43], and enhanced acquisition of avoidance [42]. For each of these predictions the present study either did not confirm, or produced results contrary to the prediction. When compared to the saline treatment the neonatal 6-OHDOPA treatment resulted in no change in food or water intake, increased open field activity, reduced aggression, and decreased avoidance. One might effectively characterize the experimental animals as being minimally affected by noxious stimuli. In fact, behavioral differences began to emerge only after the animals were exposed to foot-shock. This first occurred in the approach-avoidance experiment, when the 6-OHDOPA rats were more likely to approach the goal box where shock had been delivered than were the controls. The other measure that involved footshock was the pain-elicited aggression task, and here, too, the experimental rats were less affected by the shock and consequently exhibited fewer fight-bouts. The differential reaction to noxious stimulation is likely reflected in the activity scores as well. Both the tasks that involved footshock were administered between the second and third activity measures. Since the only open field differences derive from decreases in activity by the control group on Tests 3 and 4, the differences are likely due to the prior exposure to aversive stimulation.

This lack of behavioral reaction to aversive stimulation is similar to that observed in animals with lesions in the hippocampus, an area where marked NE depletion was obtained in the present study as a result of neonatal 6-OHDOPA. For example, rats with bilateral posterior hippocampal lesions are more likely to return to a goal box where they have been previously shocked [24]. This impaired retention of a passive avoidance task is qualitatively similar to the findings in the avoidance phase of the present study.

Although the data suggest that the 6-OHDOPA animals were affected less by aversive stimuli than the saline animals, the reason for the obtained differences is not entirely clear. One obvious possible explanation involves the motivational or arousal system: perhaps neonatal 6-OHDOPA renders an organism less emotional. He would therefore display only minimal fear to noxious stimulation. A second possibility involves the sensory systems. If 6-OHDOPA depresses pain sensitivity then footshock would elicit less of a reaction. A third, alternate, explanation involves the memory system: perhaps the experimental treatment interferes with long-term memory. Here the

depressed reactivity would result because the treated animal fails to maintain the association between footshock and various aspects of the experiment (i.e., handling, goal box entry, etc.).

The impaired memory hypothesis is consistent with the approach-avoidance and open-field activity results, but fails to explain why the experimental animals exhibited fewer fight bouts in the shock-induced aggression tests. The motivational and sensory hypotheses, however, can handle all three findings. Both a fearless as well as a painless animal would be more likely than a control to approach a goal box where he had been previously shocked, to fight less with another rat when shocked, and be more active in an open field. Although the sensory explanation is more elementary and perhaps more parsimonious than the motivational, the present data do not favor one hypothesis over the other.

Systemic neonatal administration of 6-OHDOPA offers a highly selective research tool for the investigation of NE effects in the central nervous system. Behavioral alterations would likely be attributed to central NE depletion as there were no peripheral effects at the age of testing nor was dopamine level affected. The anatomical pattern of NE depletion in the present study (*e.g.*, hippocampus and neocortex) suggests that the neurotoxic actions of 6-OHDOPA were primarily in terminal regions of the dorsal noradrenergic bundle (DNB). However, other studies measuring the uptake of H^3-NE by hypothalamic synaptasomes indicate that noradrenergic terminals are permanently destroyed in this region as well, in spite of a lack of change in endogenous NE [26].

In summary, the results of the present study indicate that neonatal 6-OHDOPA treatment, with the schedule employed, produces the greatest degree of destruction in the hippocampus and neocortex. The behavioral studies show that 6-OHDOPA rats demonstrated less pain-elicited aggression, had greater activity in the open field and tended to be less fearful of aversive stimuli in the approach-avoidance task. These behavioral effects are distinctly different from those obtained with animals treated neonatally [34,42] or in adulthood [5, 13, 14, 15, 33] with 6-OHDA, as well as those treated with 6-OHDOPA [25, 36, 37, 43] in adulthood.

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