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6-OHDA-Treated Weanling Rats Show Normal Neuroleptic Sensitivity as Adults on LHSS

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SIDHU, K. S., J. R. STELLAR, D. GARITY, AND J. P. BRUNO. *6-OHDA-treated weanling rats show normal neuroleptic sensitivity as adults on LHSS.* PHARMACOL BIOCHEM BEHAV 44(4) 901-905, 1993. – Weanling rats receiving 6-hydroxydopamine (6-OHDA) ICV on postnatal days 15-20 and tested as adults have normal lateral hypothalamic self-stimulation locus of rise (LOR) reward thresholds but significantly lower operant motor/performance (MAX) capacity when compared to vehicle-treated controls using the rate-frequency method. These results are comparable to those previously seen in adult rats treated with 6-OHDA on postnatal day 3. In a second test, day 15-20 6-OHDA treated rats were tested as adults with pimozide (0.125-1.0 mg/kg) and showed LOR shifts ranging from 0.06-0.32 log Hz and MAX shifts of 83-47% of baseline. These results were not significantly different at any dose when compared to day 15-20 vehicle-treated rats. This second result contrasts with the pimozide subsensitivity previously reported in day 3 6-OHDA treated rats and suggests that DA depletions made later in neonatal life may involve different forms of recovery than those seen with earlier dopamine depletions.

Weanling rats Pimozide LHSS Reward Neonatal 6-OHDA

AS judged from adult lesion or receptor blockade experiments, dopamine (DA) appears to be a key neurotransmitter involved in the regulation of a variety of mammalian behaviors ranging from simple motor function to the more complex processes of reward or motivation. For example, disruption of DA function results in akinesia (23), catalepsy (23), somatosensory neglect (16), ingestive deficits and reduced or blocked reward effects of food (21), brain stimulation (10,11), and cocaine self-administration (27). Paradoxically, near-total DA depletions made early in development do not produce these striking behavioral dysfunctions either immediately or in subsequent adult life (3,22,25,26). Previous work in our laboratory with lateral hypothalamic self-stimulation (LHSS) has shown that rats sustaining severe DA lesions 3 days after birth (day 3) have similar LHSS reward thresholds to current-matched control rats but did show deficits in high-rate lever-pressing behavior (22). Further, these day 3 DA-depleted rats showed subsensitivity, not supersensitivity, to DA D_2 receptor blockade with systemic pimozide injections in both brain-stimulation reward and operant motor/performance measures of LHSS. The current study extends these observations to DA depletions sustained later in development where the neuroplasticity may be different (13).

As with previous work (22), LHSS reward and motor/performance function were assessed with the rate-frequency curve shift method, in which the rate of lever-press self-stimulation behavior is measured at a variety of stimulation pulse frequencies. According to published methods, (6,22), two statistics are derived from the rate-frequency curve. Reward threshold is the stimulation frequency required to sustain half the asymptotic maximal response rate and is termed the locus of rise (LOR). Numerous studies have supported the LOR as a reasonably specific measure of LHSS reward (6,8), in particular for shifts greater than 0.1 log Hz (10,22). Another statistic drawn from the rate-frequency curve is the maximum response rate (MAX), which is taken as a reflection of the animal's motor/performance capacity.

METHOD

Subjects

Subjects were male Sprague-Dawley rats born and raised in our colony in litters adjusted to contain 10 pups 2 days after birth. Standard Purina Laboratory Chow pellets and water were available ad lib. Animals were housed in plastic tubs in a reversed day-night cycle (lights on 1900-700 h). Pups remained with their litters until weaning at day 27, when they were group housed for 3-4 weeks, and then housed singly.

Brain DA Depletions

Fifteen or 20 days after birth, pups were given methoxyflurane anesthesia and received lateral ventricular injections of either 6-hydroxydopamine (6-OHDA, 150 μg, free base

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weight, in 10 μl) in a vehicle of 0.1% ascorbic acid in 0.9% NaCl or the vehicle alone. To administer the 6-OHDA, a small opening was made in the skull 0.5 mm anterior to bregma, and ±1.5 mm lateral to the saggital suture. A Hamilton syringe containing 6-OHDA or its vehicle was lowered to a depth of 3.5 mm and 5 μl (75 μg) was administered over 1 min into each lateral ventricle. The syringe was held in place for 30 s after each injection was complete. Thirty minutes prior to 6-OHDA or vehicle injection, pups were treated with desmethylimiprimine (DMI, 25 mg/kg, SC) to protect norepinephrine-containing terminals (2). The monoamine oxidase inhibitor pargyline (40 mg/kg, IP) was also administered along with DMI to potentiate the neurotoxic effects of 6-OHDA (2).

Electrode Implantation

Approximately 4–5 months after birth, vehicle- and 6-OHDA treated rats were anesthetized with Nembutal (55mg/kg, IP) and implanted with bipolar electrodes (Plastics One, Inc., Roanoke, VA) in the lateral hypothalamus. The bregma-based, level-skull coordinates were: AP −3.0, ML ±1.7 (from midsagittal sinus), DV −7.5 (from cortex). A ground wire from the electrode assembly was attached to skull screws, which, along with dental acrylic, anchored the entire assembly to the skull. Further details of the surgical procedures have been described previously (20).

Self-Stimulation Procedure

One week after electrode implantation, all rats were trained to bar-press in a standard operant chamber for a 1.0-s burst of 0.1 ms, square-wave, monophasic, constant-current pulses of brain stimulation delivered at a frequency of 100 Hz. Rats were first trained on a continuous reinforcement schedule and then switched to a VI schedule (3 s) for testing. During initial training, an optimal current was determined as one that yielded the highest rate of responding and lowest observable signs of aversiveness (e.g., retreat from lever, vocalization). A Basicon Co. microcontroller with Stimtek Co. stimulation interface was networked to an IBM PC to control all aspects of the self-stimulation experiment.

A rate-frequency curve was generated by varying the pulse frequency from trial to trial. Each trial began with the illumination of the houselights and delivery of a free stimulation burst. Trials lasted for 3 min, followed by a 10-s blackout of the houselights. Data from the first minute of responding of each frequency trial were discarded to allow the rat to adjust its response rate to the new frequency. Data from the last 2 min of responding were averaged to determine the response rate for that frequency. Response rate was collected on a VI 3-s schedule of reinforcement, with a 10-s houselight black-out period between the different frequencies. During the black-out period, responses did not deliver stimulation and subsequently were not recorded. Two rate-frequency curves were collected each day in 1 h of testing. Each curve included a warm-up period, which consisted of a high frequency (100 Hz) followed by a low frequency (1 Hz). The pulse frequency was then successively increased in 0.2 log unit steps in the range of 1.0–2.4 log Hz (10–251 Hz). Data from the rate-frequency curve was subjected to curve fitting according to the broken-line method (6) to yield LOR and MAX statistics. Rate-frequency curves were run until the LOR and MAX measures were stable day to day. Stability was judged to occur when LOR and MAX varied by no more than 0.1 log Hz or 20%, respectively, and when there was no upward or downward trend.

Experiments

First, average baseline LOR and MAX were determined over 10 test days for adult animals treated with 6-OHDA or its vehicle on either day 15 or 20. Then, in a second experiment all rats were given the DA D_2 receptor blocker (9) pimozide (Janssen Pharmaceutical Co.) and tested on the rate-frequency curve as described above. Pimozide was dissolved in a solution of warm dH_2O and tarturic acid (9 parts tarturic : 1 part pimozide) and administered (IP) 4 h before testing. Pimozide doses were 0.125, 0.25, 0.5, and 1.0 mg/kg and were given in random order to each rat. Between drug test days, each animal was allowed to reestablish its own baseline within 10% baseline LOR and MAX or subsequent pimozide testing was postponed. Rats were never given more than two drug injections in 1 week. All behavioral testing was completed within 2 months.

For both the DA-depleted and control groups, data were analyzed by finding the individual difference score for the LOR and MAX between the drug dose test day and the average baseline from no-drug test days. Pimozide dose-response curves were then constructed by averaging these individual difference scores within a group. LOR and MAX statistics were expressed as Log Hz and percent of baseline, respectively.

Neurochemical Analysis

Rats were sacrificed by decapitation and their brains were rapidly removed and frozen on powdered dry ice. Brains were sectioned and tissue punches (approx. 1 mm^3) were taken from the medial anterior striatum and nucleus accumbens. Levels of DA and its metabolite dihydroxyphenylacetic acid (DOPAC) were determined using high-performance liquid chromatography with electrochemical detection (HPLC-ED) according to previously described methods (26).

RESULTS

Behavioral results from the two groups of rats DA depleted at day 15 or 20 did not differ in any statistical comparison (Student's *t*-test) in either the baseline or at any doses of the pimozide challenge experiments ($p < 0.082$–0.896, df = 6, for LOR and $p < 0.342$–0.974, df = 6, for MAX) with an average p value <0.298, df = 6, for LOR and <0.711, df = 6, for MAX. Therefore, data were combined across these two age groups for all subsequent analysis.

HPLC-ED analysis (Table 1) revealed that animals treated with 6-OHDA on days 15–20 had near-total depletions of DA in caudate (97%) and severe depletions in accumbens (90%) DA. The extent and value of these DA depletions are consistent with previous reports (3,5,22).

As seen in Fig. 1, rats depleted of DA on days 15–20 had an average baseline LOR that was not significantly different from current-matched, vehicle-treated control rats in a two-tailed *t*-test ($p < 0.84$, df = 11). Rats depleted of DA as weanlings had a significantly lower MAX, however, when compared to current-matched, sham-lesioned controls (*t*-test, $p < 0.032$, df = 11).

Figure 2 shows that pimozide produced a dose-dependent increase in LOR and decrease in MAX that was comparable between the DA-depleted and control weanlings. An analysis

TABLE 1

POSTMORTEM TISSUE ANALYSIS FOR ANIMALS TREATED 15-20 DAYS POSTNATAL

	Caudate			Accumbens		
	DA	DOPAC	HVA	DA	DOPAC	HVA
6-OHDA	13.3 ± 9.3	32.22 ± 28.2	14.5 ± 10.5	35.4 ± 16.2	14.4 ± 7.5	6.4 ± 1.6
Vehicle	313.2 ± 51.4	173.8 ± 23.8	122.2 ± 23.9	396.1 ± 18.6	135.4 ± 13.2	97.0 ± 4.9

All values are expressed as pmol/mg protein. HVA, homovanillic acid.

of variance (ANOVA) on the LOR data revealed a significant effect of pimozide dose, $F(1, 4) = 336.5, p < 0.0001$, but no difference between DA-depleted and sham-depleted control groups, $F(1, 4) = 0.889, p < 0.366$, and an insignificant interaction pimozide × DA depletion, $F(1, 4) = 0.207, p < 0.933$. An ANOVA on the MAX data also revealed a significant effect of dose, $F(1, 4) = 13.94, p < 0.0001$, and no differences between DA-depleted and vehicle-treated control groups, $F(1, 4) = 0.579, p < 0.463$, but here the interaction was significant, $F(1, 4) = 3.044, p < 0.027$.

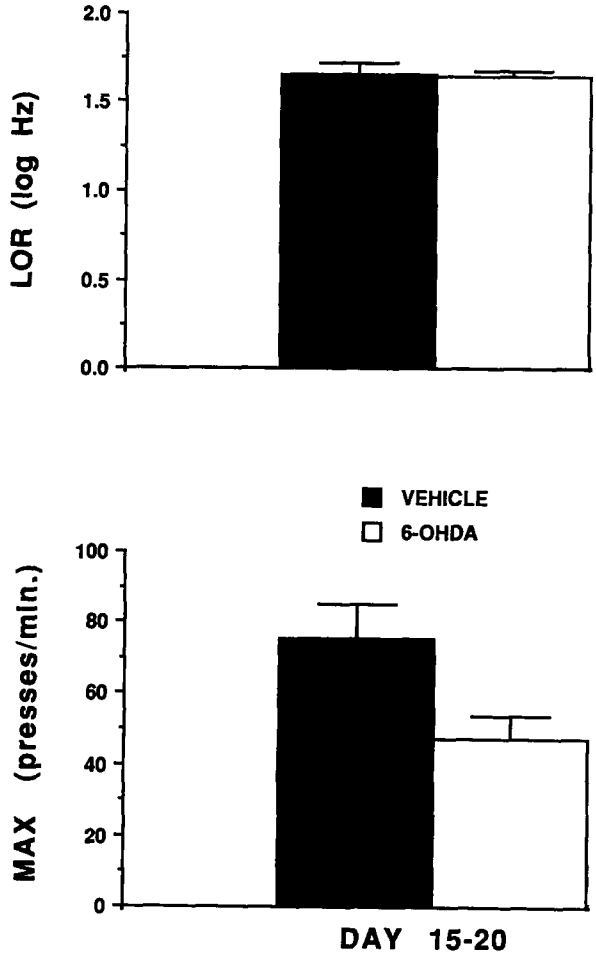

FIG. 1. Results of baseline self-stimulation testing for adults treated with 6-OHDA or vehicle on Days 15-20 for half-maximal reward threshold (LOR), and motor/performance (MAX). Error bars indicate 1 standard error of the mean.

DISCUSSION

Large DA depletions made on postnatal days 15-20 produce the same remarkable sparing of baseline adult LHSS reward function relative to normal rats as was previously reported in rats depleted of DA on day 3 (22). Also, a comparable deficit in normal baseline adult LHSS operant motor/performance capacity was seen relative to that seen in animals depleted of DA on day 3. However, when rats depleted of DA on day 15-20 were challenged as adults with the DA D_2 receptor blocker, pimozide, an important difference emerged from our previous work. Whereas the adult reward effect of LHSS in rats DA depleted on day 3 was previously shown to be less sensitive than vehicle-treated controls to pimozide [by as much as 0.2 log Hz (21)], rats DA depleted on days 15-20 appeared to be more normally sensitive to pimozide (less than 0.1 log Hz; Fig. 2).

Both the reactions of day 3 and days 15-20 DA-depleted rats to DA receptor blockade contrast with the supersensitivity seen in rats depleted of DA as adults (12). This supersensitivity to DA receptor blockade was recently extended to rats depleted of DA just after weaning on day 27 and tested as adults on LHSS (18) as well as to rats depleted as young juveniles on day 35 and tested as adults on ingestive behavior and akinesia (13). The lack of supersensitivity following day 3 DA depletions may be due to changes in receptor function within the DA system. For example, day 3 DA-depleted rats appear to be more sensitive to the sensorimotor effects of D_1 agonists than do adult DA-depleted rats (1), and if they are also more dependent upon D_1 receptors for LHSS this condition could yield the observation of pimozide (D_2) subsensitivity discussed above. Other studies on day 3 DA-depleted rats report that combining DA D_1 and D_2 receptor blocking drugs is in particular effective in inducing akinesia or ingestive deficits (13) or shifts in LHSS reward (18) when compared to DA D_1 or D_2 blocking drugs alone, a result that suggests the alteration in the normal cooperative coupling between DA receptors. Such a change could also make day 3 DA-depleted rats less sensitive to D_2 blockade with pimozide. The normal range LHSS sensitivity to pimozide for the days 15-20 DA-depleted rat seen in the current study might reflect a combination of two different mechanisms of sparing. According to this thinking, the subsensitivity seen after day 3 and supersensitivity seen after day 27 or adult DA depletions would cancel each other to leave a net effect of normal range pimozide sensitivity.

The previous discussion of potential postsynaptic mechanisms of behavioral sparing following early life DA depletions is based upon the notion that presynaptic DA release is occur-

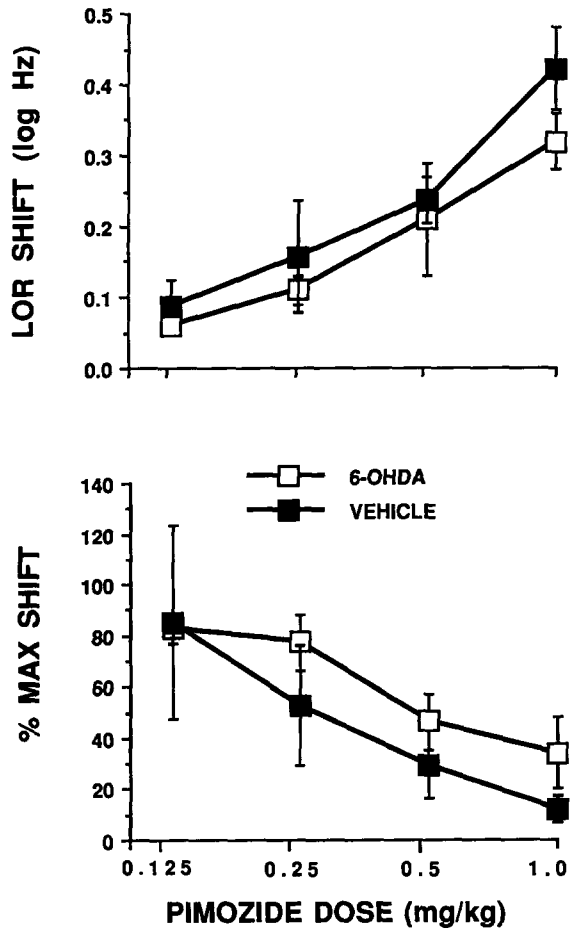

FIG. 2. Effects of Pimozide treatment on reward half-maximal thresholds (LOR) and motor/performance (MAX) in adults treated with 6-OHDA or vehicle on Days 15-20 and tested on a self-stimulation rate-frequency curve. Error bars indicate 1 standard error above the mean.

ring despite the fact that postmortum DA tissue levels are often less than 5% of normal. A recent study (7) using the microdialysis method shows that day 3 DA-depleted rats can release from caudate up to half the normal baseline amount of DA despite the low postmortem DA level determined by HPLC analysis. Adult lesioned rats also appear to have a greater than expected ability to release DA following substantia nigra lesions (17), so that a marked increase in presynaptic DA release in remaining cells appears to be a mechanism of recovery that is common to both neonatal and adult DA-depleted preparations. Another example of a common change following DA destruction is the hyperreactivity to accumbens injection of the enkephalin analog, *d*-ala-met-enkephalinamide, seen in LHSS with both neonatal (14) and adult (24) DA-depleted rats. On the other hand, only the neonatal DA-depleted rat and not the adult appear to develop a novel serotonin [5-hydroxytryptamine (5-HT)] projection to the rostral caudate (19). Unfortunately, and for simplicity in explanation, combined neonatal DA and 5-HT depletions do not result in severe behavioral deficits (4). Thus, one of the major challenges in studying the neonatal DA depletion preparation is to determine what mechanisms underlie the spared behaviors and how they differ from those mechanisms mediating recovery in adult DA-depleted animals.

The unusual response of the adult rat with severe neonatal DA depletion to DA agonists, their baseline hyperactivity in open-field tests (1), our observations of reduced high-rate operant motor function, and other incidental observations suggest that these animals are far from spared all behavioral deficits. Because adult rats with neonatal DA depletions have a roughly normal motivational appearance but decidedly abnormal limbic neurochemical mechanisms, such rats may well model minimal brain dysfunction syndromes or other behavioral syndromes that begin in infancy but that develop under age-, stress-, or drug-altered neurochemistry.

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