Pharmacology Biochemistry & Behavior, Vol. 20, pp. 707–713, 1984. © Ankho International Inc. Printed in the U.S.A. 0091-3057/84 $3.00 + .00

Six-Hydroxydopamine Induced Hyperactivity: Neither Sex Differences Nor Caffeine Stimulation Are Found

L. ERINOFF,[1] P. H. KELLY, M. BASURA AND S. R. SNODGRASS

Childrens Hospital of Los Angeles
Departments of Neurology and Physiology and Biophysics
University of Southern California

Received 21 February 1983

ERINOFF, L., P. H. KELLY, M. BASURA AND S. R. SNODGRASS. *Six-hydroxydopamine induced hyperactivity: Neither sex differences nor caffeine stimulation are found.* PHARMACOL BIOCHEM BEHAV **20**(5) 707–713, 1984.—We investigated possible sex differences in the development of locomotor activity in rats treated neonatally with desmethylimipramine (DMI) followed by intraventricular 6-hydroxydopamine (6-HDA). In addition, the locomotor response to the stimulant caffeine was investigated in the male rats after they had reached adulthood. Both male and female 6-HDA-treated rats exhibited increased activity relative to controls. No sex differences were seen in either the development or magnitude of this effect. Male rats were used to determine the dose effects function for caffeine (0.5, 5, 15, 30 mg/kg) on locomotor activity. Control rats exhibited increased locomotor activity whereas 6-HDA-treated rats showed no increases with any dose of caffeine. Large decreases in the dopamine content of the olfactory tubercle (−88%, −82%), nucleus accumbens (−96%, −95%), and striatum (−99%, −99%) were found in both male and female rats. Choline acetyltransferase and glutamic acid decarboxylase activities were unchanged.

Locomotor activity 6-Hydroxydopamine Dopamine Caffeine Choline acetyltransferase Glutamic acid decarboxylase Development Sex differences

INCREASED locomotor activity is seen in neonatal rat pups depleted of brain dopamine by intraventricular or intracisternal injection of 6-hydroxydopamine [7, 23, 25, 36, 39]. The duration of this hyperactivity seems to be a function of the degree of dopamine depletion [23] and environmental factors [26]. Most studies have used male rats or mixed groups of both sexes, although it has been reported that female pups do not become hyperactive after neonatal 6HDA treatment [3]. Since that study achieved relatively modest dopamine depletions and did not report the depletion data separately for males and females, the present report investigated possible sex differences in the development of locomotor activity following neonatal 6-HDA induced dopamine depletion.

Another aim of this study was to test some proposed mechanisms for the hyperactivity. One hypothesis [35] is that neonatal 6-HDA treatment causes greater destruction of dopaminergic neurons whose activity suppresses locomotor activity than of those whose activity increases locomotor activity. There is considerable evidence that the dopaminergic neurons whose activity stimulates locomotor activity are those of the mesolimbic dopamine system [5, 9, 18–20, 27, 28, 32] which innervates the nucleus accumbens and olfactory tubercle [43]. There is also evidence that dopamine released from nigrostriatal neurons inhibits locomotor activity since high doses of amphetamine cause less suppression of locomotor activity in rats with striatal 6-HDA lesions [18]. To investigate whether neonatal 6-HDA treatment produces greater destruction of nigrostriatal than of mesolimbic dopaminergic neurons, regional catecholamine concentrations were measured.

We also addressed the hypothesis that destruction of dopaminergic neurons in the early postnatal period leads to a failure of development or survival of non-catecholaminergic neurons which inhibit locomotor activity. Cholinergic neurons have been proposed as responsible for the suppression of locomotor activity seen with development [2], and injections of acetylcholine or gamma-aminobutyric acid (GABA) into the nucleus accumbens inhibit the locomotor activity provoked by systemic or centrally administered dopamine agonists [4, 24, 29, 30]. Therefore, we determined regional activities of choline acetyltransferase (ChAT) and L-glutamatic acid decarboxylase (GAD) to indicate possible effects of neonatal 6-HDA treatment on the development of

[1]Requests for reprints should be addressed to Dr. Lynda Erinoff, Neurology Research, Childrens Hospital of Los Angeles, P.O. Box 54700, Los Angeles, CA 90054.

cholinergic or GABAergic neurons. These biosynthetic enzymes are believed to be specific markers to cholinergic and GABAergic neurons respectively [21,40]. In previous reports, ChAT activity in whole brain was found to be unchanged by neonatal intracisternal 6-HDA treatment [37], but increased ChAT activity was demonstrated in the brainstem of rats selectively depleted of norepinephrine by neonatal subcutaneous injection of 6-hydroxydopa [16].

We also examined the effect of caffeine, a commonly used stimulant, on locomotor activity in male 6-HDA-treated and control animals. Administration of the stimulants, amphetamine and methylphenidate, has been shown to decrease the hyperactivity of 6-HDA treated developing rat pups [13, 34, 35], although this is not a universal finding [3, 6, 25, 41]. Many caffeine effects were formerly ascribed to inhibition of phosphodiesterase activity, however, they are now believed to be due to blockade of adenosine receptors [38]. Nonetheless, the locomotor stimulant action of caffeine has been shown to be antagonized by the dopamine antagonist pimozide [8,44] and by the catecholamine synthesis inhibitor, alpha-methyl-p-tyrosine [45].

METHOD

Sperm positive pregnant females (Hilltop Lab Animals, Scottdale, PA) were obtained at 14 days gestation and housed individually. They were provided with ad lib access to food and water and a diurnal lighting cycle (on 0600, off 1800). At 3 days of age pups were divided into 4 litters: male 6-HDA (14 pups), male vehicle (12 pups), female 6-HDA (12 pups), female vehicle (8 pups). Pups were injected with desmethylimipramine (DMI) 20 mg/kg SC (Merrell, Cincinnati, OH). One hour later pups were anesthetized with ether and injected with 100 μg/10 μl of 6-HDA base (6-HDA-HBr Sigma, St. Louis, MO) or vehicle (0.9% saline containing 0.1% ascorbic acid) into the right lateral ventricle. The procedure was repeated on day 6 except that the left lateral ventricle was injected.

The development of spontaneous locomotor activity was monitored every other day for one hour from day 10 to 58. The same 8 6-HDA and 7 vehicle male or female rats were tested each session with the male testing preceding (0800) the female (0900). Rats were placed in individual wire cages (40×24×18 cm high). The cages were equipped with two light-emitting diodes, 2.4 cm above the floor of the cage and 12 cm from each end, aimed at oppositely situated photodetectors, so that their infrared beams crossed the long axis of the cage. Beam interruptions per 10-minute period were automatically recorded by a modified (G and B Electronics, Royston, U.K.) AIM 65 (Rockwell, Anaheim, CA) microcomputer. For testing rats less than 18 days old a 1.3 cm thick wooden floor was placed over the wire mesh floor, and wooden inserts were used to decrease the size of the cage to 24×24×18 cm high, with one light beam crossing the center of the cage.

Pups were weaned at 22 days of age following activity testing. Supplementary feeding was begun soon after because the 6-HDA-treated pups failed to gain weight on dry chow. Gerber mixed cereal, Vivonex standard diet (Norwich-Eaton, Norwich, NY) and chocolate milk were mixed together and placed in petri dishes in the cages. This supplement was continued until rats showed signs of eating dry pellets (up to 3 weeks for male pups).

Drug testing of male rats, including some previously not tested (5 additional 6-HDA and 5 vehicle), began on day 72.

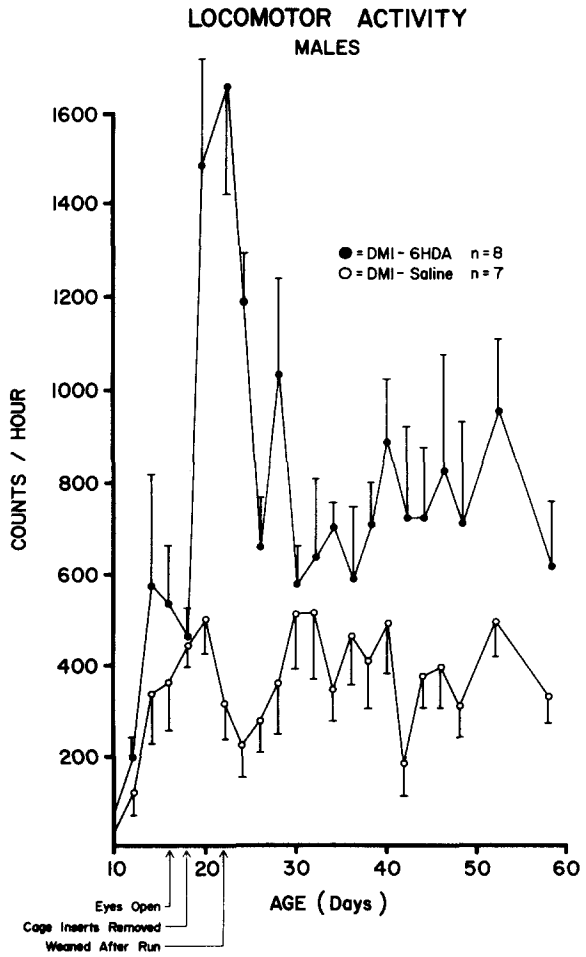

FIG. 1. The mean activity in counts per hour ±SEM for male rats treated with 6-HDA (100 μg intraventricularly) after DMI (20 mg/kg) or vehicle on days 3 and 6 is plotted against age in days. Significant differences were seen on days 20, 22, 24, 26, 28, 34, 38, 40, 42, 44, 48, and 52.

Caffeine (Sigma, St. Louis, MO) was dissolved in warm saline and injected in a volume of 1 ml/kg. Rats received saline or caffeine (5, 15, and 30 mg/kg) IP 20 minutes before activity testing. Injections followed a Latin square design; all doses were administered on each test day and after 4 test days each rat had received all the doses. All rats received a 0.5 mg/kg dose of caffeine on day 80.

The rats were killed by decapitation on day 111 and their brains dissected on an ice-cold glass plate [12,15]. The following regions were studied: olfactory tubercle, nucleus accumbens, caudate nucleus, hypothalamus, hippocampus, cerebral cortex, cerebellum and "rest of brain." Brain regions were wrapped in aluminum foil and immediately frozen in liquid nitrogen. These brain regions were homogenized in distilled water, and portions of the homogenates were immediately acidified with perchloric acid to give a final concentration of 0.2 N and assayed for catecholamines by high pressure liquid chromatography using electrochemical detection [14]. The remainder of the homogenates were stored at −80°C until assayed for choline acetyltransferase and L-glutamate decarboxylase [10].

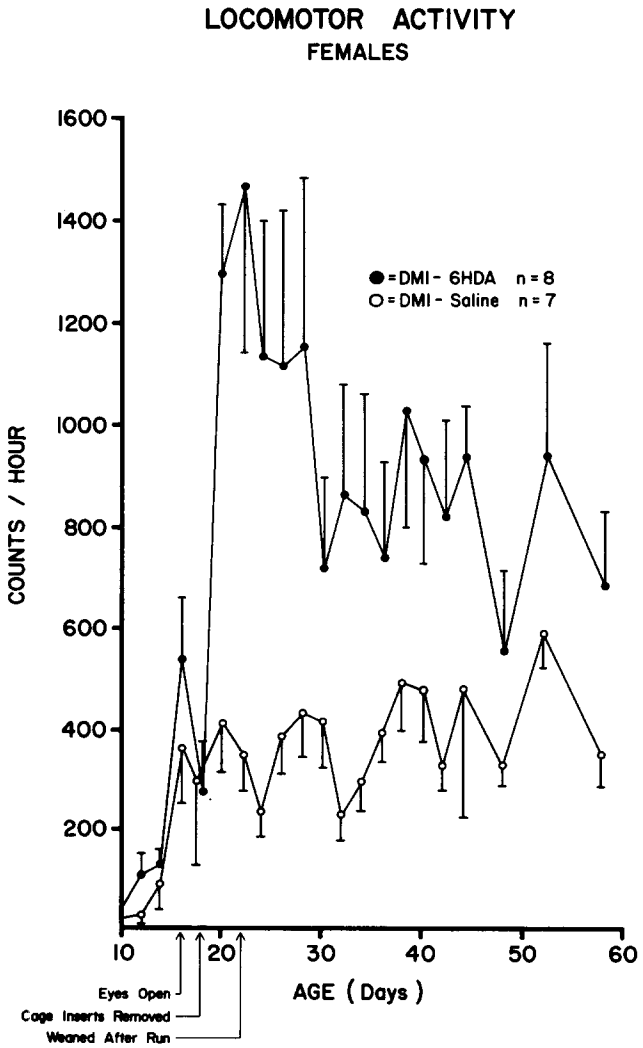

FIG. 2. The mean activity in counts per hour ±SEM for female rats treated with DMI-6HDA or vehicle is plotted against age in days. Significant differences were seen on days 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, and 52.

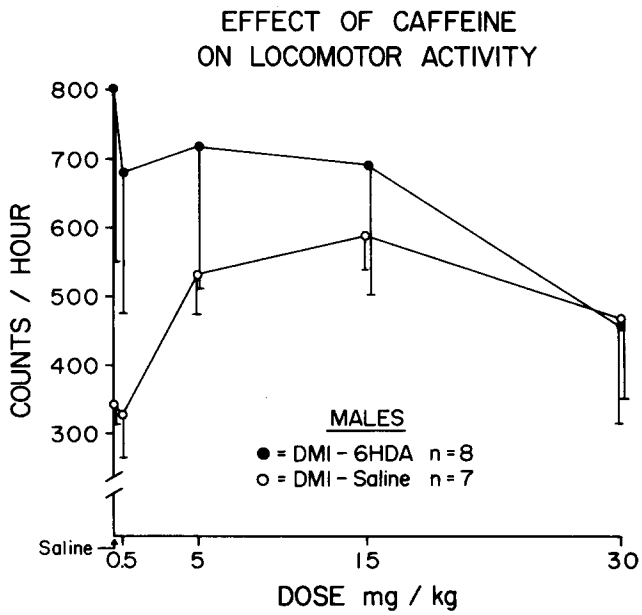

FIG. 3. Dose response function for caffeine determined in male rats. Drug testing began on day 72 and continued through day 80. A significant increase in locomotor activity was seen in vehicle control rats at 15 mg/kg.

RESULTS

The pattern of activity development was similar in male and female rat pups (Figs. 1 and 2). 6-HDA treatment led to sustained hyperactivity in both sexes (analysis of variance, three factor mixed design, repeated measures on one factor, males vs females, $F(1,26)=0.02$, not significant, control vs 6-HDA treated, $F(1,26)=22.06$, $p<0.001$, age, $F(18,468)=9.08$, $p<0.001$). Both male and female 6-HDA treated rats differed from controls on days 20, 22, 24, 26, 28, 34, 38, 40, 42, 44, and 52 ($p<0.05$, Duncan's Multiple-Range Test). At no time point was the activity of 6-HDA treated male rats different from that of females, nor were there differences between male and female controls. In both sexes the maximal differences between treated and control animals were seen during the 4th week of life. After the 6th week activity differences diminished in both male and female rats. Male rats tested on days 60 and 64 showed no significant differences between control and 6-HDA animals.

When the activity of control and treated rats was analyzed as two 30 minute periods instead of one hour, 6-HDA-treated rats had about equal activity in both the first 30 minutes and the second whereas control rats showed lower activity in the second half hour. For example, on day 26, 6-HDA-treated female rats had 598 counts in the first 30 minutes and 527 in the second 30 minutes. Female controls had 247 counts in the first 30 minutes and only 113 counts in the next. Male 6-HDA rats had 361 counts in the first 30 minutes and 297 in the second; control males had 197 counts in the first 30 minutes and 84 in the final 30 minutes.

Two-way analysis of variance of the caffeine dose response data (Fig. 3) for control and 6-HDA-treated rats indicated a significant effect of treatment group, $F(1,70)=6.47$, $p<0.05$, but no significant effect of dose or interaction between dose and treatment. The variability in the response of the 6-HDA rats was quite large and so obscured the effect of caffeine on control rats. In control animals the dose response function for caffeine was an inverted U with significant stimulation at 15 mg/kg ($p<0.01$, one-way ANOVA, $F(4,30)=4.32$, followed by Dunnett's t-test). Testing of a group of additional rats (5 treated, 5 control) without prior experience in the activity cages showed again that 6-HDA treated rats failed to respond to caffeine.

Figures 4 and 5 show the effect of 6-HDA on body weight in male and female rats. Three factor mixed design ANOVA showed significant effects of treatment, $F(1,26)=130.98$, $p<0.001$, the interaction between treatment and sex, $F(1,26)=5.15$, $p<0.05$ as well as significant effects of age, $F(14,364)=1,105$, $p<0.001$. There were slight differences in weight prior to weaning, but after weaning 6-HDA rats did not eat enough dry lab chow to gain weight so supplementary feeding was begun. The deficit in growth produced by 6-HDA was more pronounced in male rats than females, and the females began eating dry chow before the males. This sex

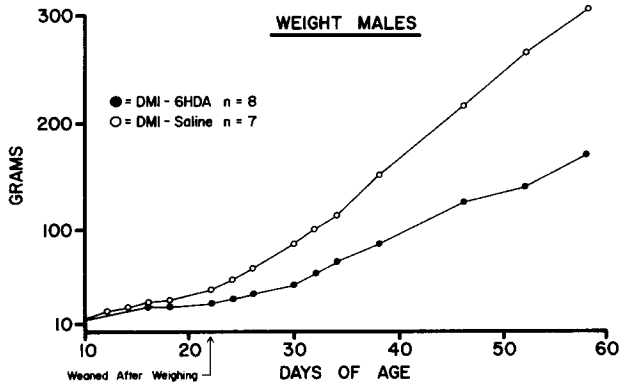

FIG. 4. Mean weight of male rats plotted against age in days (SEMs are not plotted because they do not extend beyond data points). Rats were weaned at 22 days.

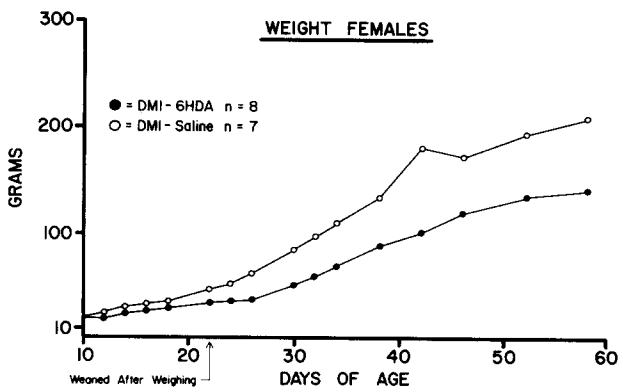

FIG. 5. Mean weight of female rats plotted against age in days (SEMs are not plotted because they do not extend beyond data points).

difference in the severity of the effect of 6-HDA on growth is consistent with that reported by Breese and Traylor [1], but contrasts with the data of Concannon and Schechter [3].

The data from the choline acetyltransferase and glutamic acid decarboxylase assays are summarized in Table 1. No significant differences were seen in any region. If the data from males and females are combined, a 21% increase in striatal GAD activity is seen in 6-HDA-treated rats ($p<0.05$). Table 2 summarizes the catecholamine data. 6-HDA treatment produced similar patterns of dopamine depletion in male and female rats. Striatal dopamine was most severely depleted (−99% in both males and females, $p<0.001$). In males there was a 88% decrease in olfactory tubercle and a 96% decrease in nucleus accumbens ($p<0.001$). In females these decreases were 82% and 95% respectively ($p<0.001$). There were no decreases in regional norepinephrine content. In fact, there was an increase in cerebellar norepinephrine concentration of 47% in males ($p<0.001$) and 30% in females ($p<0.02$). This was partially accounted for by a decrease in cerebellar weight of 24% in 6-HDA treated males ($p<0.001$) and 12% in 6-HDA treated females ($p<0.01$). There were also increases in the norepinephrine content of the olfactory tubercle of 45% in females ($p<0.01$) and 40% in males ($p<0.02$) although there was no change in the weight of this region in either males or females.

DISCUSSION

Our first aim was to determine if there is a sex difference in the development of locomotor activity after neonatal 6-HDA treatment. We found no sex difference; both male and female rats treated with intraventricular 6-HDA as neonates were hyperactive during development. Moreover, the data comparing the first 30 minutes in a session to the second indicates that both male and female 6-HDA-treated rats showed failure to habituate which has previously been reported after neonatal dopamine depletion [33,39]. It is interesting to note that while our female 6-HDA-treated rats did not show as great a growth retardation as the males, activity levels were comparable which does not support the hypothesis that the growth retardation produced by neonatal 6-HDA is sufficient to cause hyperactivity [22].

Our results differ from those previously reported by Concannon and Schechter [3] which may be accounted for by differences in the protocols of the two studies. We used litters grouped by sex and treatment while Concannon and Schechter used heterogeneous litter composition. Homogeneous litter composition enhances the syndrome produced by 6-HDA [26]. The present study used the intraventricular route of 6-HDA administration as opposed to intracisternal injection and produced greater dopamine depletion (approximately 90% depletion of telencephalic dopamine in our study versus 37.8% depletion in whole brain dopamine in their study). There may be a difference between males and females in the threshold dopamine depletion necessary to produce hyperactivity.

We also examined two hypotheses to account for the hyperactivity of neonatally treated 6-HDA rats. One hypothesis was that neonatal 6-HDA results in greater destruction of dopamine neurons whose effect is to inhibit locomotor activity (nigrostriatal) than of those whose effect is to stimulate activity (mesolimbic). While extensive dopamine depletion was produced in all telencephalic regions, striatal dopamine levels were reduced to a greater extent than those of nucleus accumbens or olfactory tubercle. However, the differences in dopamine depletion between striatal and mesolimbic regions were not disparate enough to prove our hypothesis.

The lack of change in choline acetyltransferase or glutamic acid decarboxylase activities suggests that early destruction of dopaminergic neurons does not cause the failure of development or survival of cholinergic or GABAergic neurons which inhibit locomotor activity. It is possible, of course, that neonatal 6-HDA treatment merely retards the development of cholinergic and/or GABAergic neurons. Differences in the marker enzymes might be found at the time of maximal difference between the locomotor activities of the treated and control groups. Schmidt and Bhatnagar [31] reported that catecholamine depletions measured in adult rats may not reflect the initial amount of damage produced by neonatal 6-HDA. For example, the increased cerebellar content of norepinephrine seen in our study may represent a recovery and hypertrophy of previously damaged neurons. We are unaware of other reports of increased norepinephrine content in olfactory tubercle following neonatal 6-HDA. The increased concentration of norepinephrine in this region may be due to either regenerative or collateral sprouting. Collateral sprouting has been reported

TABLE 1

EFFECT OF NEONATAL TREATMENT WITH DMI-6HDA ON REGIONAL MARKER ENZYMES

Group	Olfactory Tubercle	N. Accumbens	Striatum	Hypo-thalamus	Cortex	Hippo-campus	Cere-bellum	Rest
	Glutamic Acid Decarboxylase Activity (μmoles/100 mg protein/hr ± SEM)							
Male Control n=8	46.2±4.1	52.9±4.1	32.0±3.1	50.0±3.4	25.6±1.8	25.6±1.4	17.1±0.9	26.4±2.2
Male Treated n=8	47.1±3.4	52.8±3.9	38.8±2.7	51.8±4.6	27.4±1.7	28.0±1.5	18.9±1.6	30.5±1.8
Female Control n=6	51.0±6.3	56.6±4.7	31.3±1.6	50.7±3.7	24.8±1.6	24.5±1.1	16.8±0.5	26.5±2.3
Female Treated n=6	49.7±2.7	58.0±3.8	37.9±3.7	51.5±4.7	24.9±1.0	27.8±1.9	17.7±1.4	28.3±1.6
	Choline Acetyltransferase Activity (μmoles/100 mg protein/hr ± SEM)							
Male Control n=8	24.1±3.5	17.6±1.8	20.4±2.1	3.9±0.4	5.4±0.5	6.1±0.5	0.7±0.1	8.1±1.1
Male Treated n=8	24.1±1.7	18.6±1.4	22.1±1.6	4.5±0.5	6.1±0.4	5.9±0.5	0.8±0.1	7.5±0.6
Female Control n=7	23.8±2.2	18.8±1.2	21.0±1.4	4.4±0.4	5.6±0.5	6.1±0.4	0.7±0.1	7.9±0.4
Female Treated n=6	26.7±3.0	20.6±1.6	23.3±2.3	4.6±0.4	6.2±0.6	7.0±0.5	0.9±0.1	8.2±0.6

TABLE 2

EFFECT OF NEONATAL TREATMENT WITH DMI-6HDA ON REGIONAL CATECHOLAMINE CONTENT

Group	Olfactory Tubercle	N. Accumbens	Striatum	Hypo-thalamus	Cortex	Hippo-campus	Cere-bellum	Rest
	NE ng/g wet wt ± SEM							
Male Control n=7	109±12	337±37	95±11	1255±45	231±8	291±26	171±14	425±13
Male Treated n=8	153±12	295±19	105±14	1220±77	264±14	300±19	252±18	513±17
% Change	+40*						+47*	
Female Control n=7	141±7	376±44	98±14	1180±53	247±17	323±22	183±17	419±23
Female Treated n=8	205±14	401±49	126±7	1124±20	269±14	259±22	238±9	476±23
% Change	+45*						+30*	
	DA ng/g wet wt ±SEM)							
Male Control n=7	3268±383	6556±391	7370±436	315±6				
Male Treated n=8	396±100	274±74	77±10	253±23				
% Change	−88*	−96*	−99*	−20*				
Female Control n=7	3935±436	6417±472	6632±840	307±31				
Female Treated n=8	692±114	341±78	88±22	245±71				
% Change	−82*	−95*	−99*					

*Denotes $p<0.05$.

for dopaminergic terminals in the olfactory tubercle [11].

Caffeine produced an inverted U-shaped dose response function in control rats as reported by Thithapandha *et al.* [42]. However, no dose of caffeine was able to stimulate locomotor activity in 6-HDA-treated rats. Joyce and Koob [17] reported that adult rats with selective 6-HDA lesions of mesolimbic dopamine neurons showed unaltered caffeine stimulation of locomotor activity. The differences between their data and our own may be due to the different consequences of adult and neonatal 6-HDA treatment or the effects of a specific lesion of mesolimbic dopamine compared to the widespread destruction produced by intraventricular 6-HDA. We have seen (Erinoff and Snodgrass, unpublished observation) caffeine stimulation in neonatally treated 6-HDA rats whose dopamine depletions were less extensive (−83% in nucleus accumbens and −91% in striatum). Caffeine stimulation of locomotor activity is thought to be mediated by blockade of A_1 adenosine receptors [38]. Further studies are needed to determine if neonatal 6-HDA treatment alters the development of these receptors.

REFERENCES

1. Breese, G. R. and T. D. Traylor. Developmental characteristics of brain catecholamines and tyrosine hydroxylase in the rat: Effects of 6-hydroxydopamine. *Br J Pharmacol* **44:** 210–222, 1972.
2. Campbell, B. A., L. D. Lytle and H. C. Fibiger. Ontogeny of adrenergic arousal and cholinergic inhibitory mechanisms in the rat. *Science* **166:** 635–637, 1969.
3. Concannon, J. T. and M. D. Schechter. Hyperactivity in developing rats: Sex differences in 6-hydroxydopamine and amphetamine effects. *Pharmacol Biochem Behav* **14:** 5–10, 1981.
4. Costall, B., S.-C.G. Hui and R. J. Naylor. Hyperactivity induced by intra-accumbens dopamine: Actions and interactions of neuroleptic, cholinomimetic and cholinolytic agents. *Neuropharmacology* **18:** 661–665, 1980.
5. Costall, B. and R. J. Naylor. The behavioral effects of dopamine applied intracerebrally to areas of the mesolimbic system. *Eur J Pharmacol* **32:** 87–92, 1975.
6. Eastgate, S. M., J. J. Wright and J. S. Werry. Behavioral effects of methylphenidate in 6-hydroxydopamine-treated neonatal rats. *Psychopharmacology (Berlin)* **58:** 157–159, 1978.
7. Erinoff, L., R. C. MacPhail, A. Heller and L. S. Seiden. Age-dependent effects of 6-hydroxydopamine on locomotor activity in the rat. *Brain Res* **164:** 195–205, 1979.
8. Estler, C.-J. Influence of pimozide on the locomotor hyperactivity produced by caffeine. *J Pharm Pharmacol* **31:** 126–127, 1979.
9. Fink, J. S. and G. P. Smith. Relationships between selective denervation of dopamine terminal fields in the anterior forebrain and behavioral responses to amphetamine and apomorphine. *Brain Res* **201:** 107–127, 1980.
10. Friedle, N. M., P. H. Kelly and K. E. Moore. Regional atrophy and reductions in glutamate release and uptake after intrastriatal kainic acid. *Br J Pharmacol* **63:** 151–158, 1978.
11. Gilad, G. M. and D. J. Reis. Collateral sprouting in central mesolimbic dopamine neurons: Biochemical and immunocytochemical evidence of changes in the activity and distribution of tyrosine hydroxylase in terminal fields and in cell bodies of A10 neurons. *Brain Res* **160:** 17–36, 1977.
12. Glowinski, J. and L. L. Iversen. Regional studies of catecholamines in the cat brain. *J Neurochem* **13:** 655–669, 1966.
13. Heffner, T. G. and L. S. Seiden. Possible involvement of serotonergic neurons in the reduction of locomotor hyperactivity caused by amphetamine in neonatal rats depleted of brain dopamine. *Brain Res* **244:** 81–90, 1982.
14. Hegstrand, L. and B. Eichelman. Determination of rat brain tissue catecholamines using liquid chromatography with electrochemical detection. *J Chromatog* **222:** 107–111, 1981.
15. Horn, A. S., A. C. Cuello and R. J. Miller. Dopamine in the mesolimbic system of the rat brain: Endogenous levels and the effects of drugs on the uptake mechanism and stimulation of adenylate cyclase activity. *J Neurochem* **22:** 265–270, 1974.
16. Jaim-Etcheverry, G., G. Teitelman and L. M. Zieher. Choline acetyltransferase activity increases in the brain stem of rats treated at birth with 6-hydroxydopa. *Brain Res* **100:** 699–704, 1975.
17. Joyce, E. M. and G. F. Koob. Amphetamine-, scopolamine- and caffeine-induced locomotor activity following 6-hydroxydopamine lesions of the mesolimbic dopamine system. *Psychopharmacology (Berlin)* **73:** 311–313, 1981.
18. Kelly, P. H. and S. D. Iversen. Selective 6-OHDA induced destruction of mesolimbic dopamine neurons: Abolition of psychostimulant induced locomotor activity in rats. *Eur J Pharmacol* **40:** 45–56, 1976.
19. Kelly, P. H., P. W. Seviour and S. D. Iversen. Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum. *Brain Res* **94:** 507–522, 1975.
20. Koob, G. F., L. Stinus and M. Le Moal. Hyperactivity and hypoactivity produced by lesions to the mesolimbic dopamine system. *Behav Brain Res* **3:** 341–359, 1981.
21. Kuhar, M. J. The anatomy of cholinergic neurons. In: *Biology of Cholinergic Function,* edited by A. M. Goldberg and I. Hanin. New York: Raven Press, 1976, pp. 3–27.
22. Loch, R. K., L. S. Rafales, I. A. Michaelson and R. L. Bornschein. The role of undernutrition in animal models of hyperactivity. *Life Sci* **22:** 1963–1970, 1978.
23. Miller, F. E., T. G. Heffner, C. Kotake and L. S. Seiden. Magnitude and duration of hyperactivity following neonatal 6-hydroxydopamine is related to the extent of brain dopamine depletion. *Brain Res* **229:** 123–132, 1981.
24. Palfreyman, M. G., S. Huot, B. Lippert and P. J. Schecter. The effect of gamma-acetylinic GABA, an enzyme-activated irreversible inhibitor of GABA-transaminase, on dopamine pathways of the extrapyramidal and limbic systems. *Eur J Pharmacol* **50:** 325–336, 1978.
25. Pappas, B. A., J. V. Gallivan, T. Dugas, M. Saari and R. Ings. Intraventricular 6-hydroxydopamine in the newborn rat and locomotor responses to drugs in infancy: No support for the dopamine depletion model of minimal brain dysfunction. *Psychopharmacology (Berlin)* **70:** 44–46, 1980.
26. Pearson, D. E., M. H. Teicher, B. A. Shaywitz, D. J. Cohen, J. G. Young and G. M. Anderson. Environmental influences on body weight and behavior in developing rats after neonatal 6-hydroxydopamine. *Science* **209:** 715–717, 1980.
27. Pijnenburg, A. J. J. and J. M. Van Rossum. Stimulation of locomotor activity following injection of dopamine into the nucleus accumbens. *J Pharm Pharmacol* **25:** 1003–1005, 1973.
28. Pijnenburg, A. J. J., G. N. Woodruff and J. M. Van Rossum. Ergometrine induced locomotor activity following intracerebral injection into the nucleus accumbens. *Brain Res* **59:** 289–302, 1973.
29. Pycock, C. J. and R. W. Horton. Possible GABA-mediated control of dopamine-dependent behavioral effects from the nucleus accumbens of the rat. *Psychopharmacology (Berlin)* **49:** 173–178, 1976.
30. Scheel-Kruger, J., A. R. Cools and P. M. Van Wel. Muscimol a GABA-agonist injected into the nucleus accumbens increases apomorphine stereotypy and decreases the motility. *Life Sci* **21:** 1697–1702, 1977.

31. Schmidt, R. H. and R. K. Bhatnagar. Regional development of norepinephrine, dopamine beta hydroxylase and tyrosine hydroxylase in the rat brain subsequent to neonatal treatment with subcutaneous 6-hydroxydopamine. *Brain Res* **166:** 293–308, 1979.
32. Segal, D. S., P. H., Kelly, G. Koob and D. C. S. Roberts. Nonstriatal dopamine mechanisms in the response to repeated d-amphetamine administration. In: *Catecholamines: Basic and Clinical Frontiers*, edited by E. Usdin, I. J. Kopin and J. Barchas. New York: Pergamon Press, 1979, pp. 1672–1674.
33. Shaywitz, B. A., J. W. Gordon, J. H. Klopper and D. A. Zelterman. The effect of 6-hydroxydopamine on habituation of activity in the developing rat pup. *Pharmacol Biochem Behav* **6:** 391–396, 1977.
34. Shaywitz, B. A., J. H. Klopper and J. W. Gordon. Methylphenidate in 6-hydroxydopamine-treated developing rat pups. *Arch Neurol* **35:** 463–469, 1978.
35. Shaywitz, B. A., J. H. Klopper, R. D. Yager and J. W. Gordon. Paradoxical response to amphetamine in developing rats treated with 6-hydroxydopamine. *Nature* **261:** 153–155, 1976.
36. Shaywitz, B. A., R. D. Yager and J. H. Klopper. Selective brain dopamine depletion in developing rats: An experimental model of minimal brain dysfunction. *Science* **191:** 305–308, 1976.
37. Smith, R. D., B. R. Cooper and G. R. Breese. Growth and behavioral changes in developing rats treated intracisternally with 6-hydroxydopamine: Evidence for involvement of brain dopamine. *J Pharmacol Exp Ther* **185:** 609–619, 1973.
38. Snyder, S. H., J. J. Katims, Z. Annau, R. F. Bruns and J. W. Daly. Adenosine receptors and behavioral actions of methylxanthines. *Proc Natl Acad Sci USA* **78:** 3260–3264, 1981.
39. Stoof, J. C., H. Dijkstra and J. P. M. Hillegers. Changes in the behavioral response to a novel environment following lesioning of the central dopaminergic systems in rat pups. *Psychopharmacology (Berlin)* **57:** 163–166, 1978.
40. Storm-Mathisen, J. Localization of transmitter candidates in the brain: The hippocampal formation as a model. *Prog Neurobiol* **8:** 119–181, 1977.
41. Thieme, R. E., H. Dijkstra and J. C. Stoof. An evaluation of the young dopamine-lesioned rat as an animal model for minimal brain dysfunction (MBD). *Psychopharmacology (Berlin)* **67:** 165–169, 1980.
42. Thithapandha, A., H. M. Maling and J. R. Gillette. Effects of caffeine and theophylline on activity of rats in relation to brain xanthine concentrations. *Proc Soc Exp Biol Med* **139:** 582–586, 1972.
43. Ungerstedt, U. Stereotaxic mapping of the monoamine pathways in the rat brain. *Acta Physiol Scand* Suppl **367:** 1–48, 1971.
44. Waldeck, B. Sensitization by caffeine of central catecholamine receptors. *J Neural Trans* **34:** 61–72, 1973.
45. White, B. C., C. C. Simpson, J. E. Adams and D. Harleins, Jr. Monoamine synthesis and caffeine-induced locomotor activity. *Neuropharmacology* **17:** 511–513, 1978.