Pharmacology Biochemistry & Behavior, Vol. 14, pp. 5–10. Printed in the U.S.A.

Hyperactivity in Developing Rats: Sex Differences in 6-Hydroxydopamine and Amphetamine Effects

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Received 17 December 1979

CONCANNON, J. T. AND M. D. SCHECHTER. *Hyperactivity in developing rats: Sex differences in 6-hydroxydopamine and amphetamine effects.* PHARMAC. BIOCHEM. BEHAV. **14**(1) 5–10, 1981.—Possible sex-related differences in the effects of 6-hydroxydopamine (6-OHDA) and d-amphetamine on activity were investigated in rats administered intracisternal injections of 6-OHDA or its vehicle at 5 days of age. Administration of the dopamine neurotoxin resulted in a significant depletion of whole brain dopamine to 62.2% of controls as indicated when the brains of animals sacrificed at 35–36 days of age were analyzed by high pressure liquid chromatography. In normal developing rat pups, activity for rats of both sexes increased from low levels at 12 days of age to fairly high levels at 21–24 days of age. Thereafter, activity levels in control male pups decreased to levels typical for adults, whereas female rats displayed little decrease from their peak level of activity. Hyperactivity was detected in 6-OHDA treated male rats at 24 and 27 days of age, after the decline in activity to adult levels in control (vehicle-treated) pups. Females treated with 6-OHDA, on the other hand, were hypoactive at 27 days of age relative to vehicle-injected female controls. Furthermore, d-amphetamine injections increased the activity of 6-OHDA treated males to a lesser extent than it did in their vehicle-injected controls, at 24 and 27 days of age, while just the opposite was true for female 6-OHDA treated rats administered d-amphetamine. This altered responsiveness to d-amphetamine associated with the 6-OHDA treatments was not related to body weight, which was virtually unaffected by the 6-OHDA treatment. Thus, no conclusive evidence was found for the ability of d-amphetamine to decrease 6-OHDA-induced hyperactivity. However, the results implicate the role of sex of subjects in modulating the effects of 6-OHDA and d-amphetamine on activity in developing rats and generally support clinical findings which suggest that hyperactivity may be largely confined to male, pre-adolescent organisms.

6-Hydroxydopamine Dopamine d-Amphetamine Sex-differences Activity Developing rats

THE childhood hyperkinetic syndrome represents numerous behavioral problems as cardinal features, including overactivity, short attention span, impulsivity, and impaired learning ability [13]. In order to investigate possible underlying neurophysiological substrates of hyperkinesis, various animal models have been proposed which attempt to produce at least some of these cardinal features, most often overactivity, by neurochemical [14] or surgical [20] brain area lesioning, chronic neuroleptic treatment [1] or neonatal exposure to lead [17, 18, 19]. Perhaps the most accurate animal model of hyperkinesis has been proposed by Shaywitz and his associates [11, 12, 14–17] and involves the neonatal administration of 6-hydroxydopamine (6-OHDA), which produces preferential central dopamine depletion when it is administered after desmethylimipramine (DMI). This model appears to replicate the human hyperkinetic syndrome in that it: (a) produces many of the cardinal features of the hyperkinetic syndrome; (b) bears some relationship, in terms of onset, duration, and offset of overactivity, to the pathogenesis of hyperkinesis in humans; and, perhaps most importantly, (c) the hyperactivity produced responds well to psychostimulant medication [15,16].

Since Shaywitz and his colleagues first reported this model [14] a number of other laboratories have attempted to replicate their findings with various degrees of success. For example, Stoof *et al.* [21] and Erinoff *et al.* [7] have shown that either intracisternal or intraventricular administration of 6-OHDA produces overactivity in rat pups from approximately 15 to 30 days of age. However, in contrast to Shaywitz's work, neither laboratory was able to detect an offset period of hyperactivity in which 6-OHDA-treated rats return to the level of activity observed in vehicle-injected controls. Furthermore, Eastgate *et al.* [6] were unable to produce overactivity by neonatal 6-OHDA administration, although they did detect less of an activity increase in 6-OHDA rats that were administered methylphenidate.

Difficulty in replicating Shaywitz's findings may stem from two important variables: (a) within-litter vs. between-litter assignment of animals to 6-OHDA and vehicle groups [6,11], and (b) sex differences of the animals within a treatment group. For example, Shaywitz and associates [11] have found that raising 6-OHDA treated rats along with vehicle-treated littermates tended to suppress hyperactivity relative to 6-OHDA treated rats raised in different litters from vehicle-treated pups (i.e., the homogenous litter condition: [11]). Hence, heterogenous litter composition may attenuate evidence for production of 6-OHDA-induced hyperactivity. Perhaps more importantly, no study has examined possible

sex-related differences in overactivity and psychostimulant responsiveness in 6-OHDA *vs.* vehicle-injected animals. For example, Erinoff *et al.* [7] used male rats exclusively, whereas Shaywitz *et al.* [11, 12, 14–17] and Stoof *et al.* [21] never analyzed for sex differences and Eastgate *et al.* [6] did not report the sex of their rat pups. Failure to examine such sex-differences appears to be an oversight in experimental design in light of the report that the human hyperkinetic syndrome is diagnosed more frequently in pre-pubescent males than in same-age females [10]. Hence, a sex-related variable may play a critical role in the inability to model Shaywitz' criteria of hyperkinesis and it may provide some starting point for further elucidation of the source of higher incidence of hyperkinesis in the male population. Accordingly, the intent of the present experiment was to use Shaywitz' procedures to examine 6-OHDA-induced overactivity and responsiveness to d-amphetamine in an experimental design in which sex was investigated as a contributing or modulating factor in this animal model of hyperkinesis.

METHOD

Animals

Sprague-Dawley-derived (Charles River) rats, born and raised in the Department colony, served as subjects. The parents were paired in single plastic breeding cages and the male was removed as soon as it was physically apparent that the female was pregnant. Within 2 days after birth, litters were culled to 8 rat pups with an equal number of males and females. On occasion, a litter with less than 8 pups was fortified by addition of animals culled from other litters born on the same day. A total of 10 litters were used in this experiment. Throughout all phases of breeding and behavioral observation, animals were housed under controlled temperature and a 12-hr light/12-hr dark cycle. Food and water were provided ad lib.

Procedure

At 5 days of age, rats were toe-clipped for identification and were randomly assigned to one of two treatment groups: (a) desmethylimipramine (DMI) and intracisternal 6-OHDA, or (b) DMI and intracisternal vehicle injection. Activity was determined at 12, 15, 18, 21, 24, and 27 days of age, between the hours of 1300 and 1600, using the time-sampling technique described in detail by Shaywitz *et al.* [14]. The present procedure differed, however, in that an equal number of male and female rat pups were explicitly assigned to each treatment group. Due to the anticipation of deaths resulting from 6-OHDA injections, the ratio of 6-OHDA to vehicle-injected animals was approximately 3:2.

Starting at 12 days of age, and at 3 day intervals thereafter, rat pups were removed from the mother and administered an intraperitoneal injection of d-amphetamine or an equal volume of physiological saline. Assignment to treatment was random with the assigned animal always receiving the same drug treatment. Thirty minutes after injection, pups were again removed from the mother and individually placed in 46×24×14.5 cm clear plastic cages for behavioral observation. Each cage was scanned every min for 1 hr, thus generating 60 measures for each animal on each observation day. This activity was determined by one of two observers who showed 92.3% agreement on an activity categorization detailed in Shaywitz *et al.* [14]. All observations were recorded "blind" in that the observer had no systematic information concerning either the brain treatment at 5 days of age (6-OHDA vs. vehicle) or the substance administered prior to the activity session (d-amphetamine vs. saline).

Drugs and Dosage Rationale

6-OHDA HBr (Aldrich Chemical Co., Milwaukee, WI), 100 μg, calculated as free base, was dissolved immediately before administration in a 25 μl solution of 0.9% saline with 0.4 mg l-ascorbic acid/ml added to retard oxidation. The vehicle-treated littermate control group received an injection of an equivalent volume of the 6-OHDA vehicle. All 6-OHDA or vehicle injections were preceded 60 min earlier by an intraperitoneal injection of 20 mg/kg desmethylimipramine HCl (as base) dissolved in 0.9% saline and administered in a volume of 1.0 μl/g body weight. This DMI-6-OHDA treatment regimen has been reported to produce selective depletion of central dopamine levels with little or no effect on central norepinephrine levels [14].

d-Amphetamine sulfate (Sigma Chemical Co., St. Louis, MO) was dissolved in physiological saline and administered intraperitoneally at a dose of 0.5 mg/kg (as base) in a volume of 0.005 ml/g; control animals within the same litters received an equivalent volume of the saline vehicle. This dose of d-amphetamine has been reported to be effective in altering activity in both 6-OHDA and normal rat pups between the ages of 12 and 30 days [15], i.e., it produces overactivity in normal pups and "paradoxical calming" in 6-OHDA treated pups. Only the 0.5 mg/kg dose was used since the reduction in activity in 6-OHDA-treated rat pups was apparently not increased at higher doses (e.g., 1.0 and 5.0 mg/kg [15]).

Biochemical Determinations

All animals were sacrificed by decapitation at 35–36 days of age. Brains were immediately removed, weighed and frozen at −70 °C for later analysis. The frozen whole brains were then cut into approximately 4–6 parts and homogenized in 0.05 μ $HClO_4$ with a Brinkmann (Westbury, NY) polytron homogenizer for 20–30 sec at setting 6. The tubes were then centrifuged at 15,000 G for 15 min. Two ml (1/5 of the volume) was added to a conical reaction vial containing 100 mg of acid washed alumina, 1 ml Tris buffer pH=8.6 (3.0 M), and 100 μl dihydroxybenzylamine (DBA), the internal standard (1 ng/μl). The alumina was shaken and washed as described in Felice *et al.* [8]. The eluate was injected, using a 1.0 ml syringe, into a Varian Model 5020 high pressure liquid chromatograph equipped with 100 μl loop injection and Micropak C-18 reverse phase column and model LC-2A electrochemical detector with glassy carbon electrode set at 720 MV *vs.* AgCl electrode. Mobile phase consisted of 3 parts citric acid (0.1 M) to 2 parts Na_2HPO_4 (0.1 M) with 0.3 mM in sodium octyl phosphate and 10 ml acetonitrile.

Data were analyzed using a Varian CDS 111L data system based on the response of dopamine and norepinephrine standards *vs.* the internal standard DBA in tissue samples.

Statistical Methods

Measurements of each category of activity were calculated as percentage of occurrence of the 60-min observation period (i.e. number of times active÷60×100). For brevity of reporting, and for direct comparison to the reports of Shaywitz, only the category of "Total Activity" was analyzed by using a 2(Sex) × 4(Treatment) × 6(Age) mixed

TABLE 1

EFFECT OF SEX AND BRAIN INJECTIONS ON BODY WEIGHT

	Body Weights (Mean ± S.E.M.) Age (Days)							
Treatment Group	5	12	15	18	21	24	27	35–36
Female/Vehicle	11.50 ± 0.34	23.11 ± 0.81	29.64 ± 0.79	34.64 ± 1.14	42.39 ± 1.50	53.64 ± 1.82	67.61 ± 1.82	106.75 ± 3.18
Female/6OHDA	11.30 ± 0.24	21.50 ± 0.80	27.61 ± 0.71	33.11 ± 0.89	40.36 ± 1.36	52.05 ± 1.82	65.94 ± 2.30	105.30 ± 3.46
Male/Vehicle	12.20 ± 0.31	23.33 ± 1.12	30.00 ± 1.31	37.20 ± 1.40	46.33 ± 2.12	60.17 ± 2.74	76.37 ± 3.25	126.50 ± 5.55
Male/6OHDA	12.08 ± 0.29	24.21 ± 0.93	30.42 ± 1.18	37.82 ± 1.15	46.74 ± 1.74	59.74 ± 1.98	75.00 ± 2.66	122.34 ± 5.35

unweighted means analysis of variance (ANOVA), with Sex and Treatment as between-group factors and Age representing the repeated measurement. Subsequent to this analysis, all between-group comparisons were made utilizing Duncan's Multiple Range Test using the appropriate within-group error term. Similar results were obtained using completely between-group two-way ANOVAs at each level of the repeated measure (i.e., Age). Throughout the experiment $p<0.05$ was considered to be statistically significant.

RESULTS

Mortality

A total of 14 of the 80 brain-injected animals died within 3 or 4 days from the time of injection. Twelve of these 14 animals (85.7%) were 6-OHDA-treated. The lethal effect of 6-OHDA, however, was equally distributed between males (7 of 8 deaths) and females (5 of 6 deaths).

Weight Loss

Table 1 presents the body weight data for male and female 6-OHDA and vehicle-injected animals from 5 days of age until sacrifice (35–36 days of age). These data clearly show that, although there were consistent sex-related differences in body weight (males outweighing females) which increased as the animals matured, there were no reliable differences in body weight attributable to the 6-OHDA *vs.* vehicle brain injections. In fact, the maximum difference between 6-OHDA and vehicle-treated rats at any age was less than 6%.

Development of Activity in 6-OHDA and Vehicle-Injected Rats

Few, if any, reports have examined the course of development of activity in 6-OHDA *vs.* vehicle-injected rat pups as a function of sex in the age range examined. Depicted in Fig. 1, therefore, is the percentage of "Total Activity" [14] as a function of age and type of brain injection for males (top panel) and females (lower panel) administered saline injections prior to behavioral observations. (The baseline data in absolute values are presented in Table 2.) These results are similar from 12 days of age until 21 days of age, with a nearly linear increase in activity from very low levels (about 20%) to very high levels (approximately 80%). Thereafter, the typical decline in activity to adult levels in male vehicle-injected rats [5,14] is observed, whereas similarly treated female rats show no significant decline in activity from 21 to 27 days of age. In fact, the female saline-injected group shows higher activity than the male saline-injected group at 24 and 27 days of age. Sex-dependent differences in activity, with pubescent females being more active than males, have previously been reported for somewhat older animals [2,3].

TABLE 2

MEAN ABSOLUTE TOTAL ACTIVITY SCORES FOR ALL TREATMENT GROUPS

Treatment Group*	Age (days) 12	15	18	21	24	27
M/V/S	13.2 (7)	31.8	45.0	46.8	30.0	29.4
M/V/A	48.6 (8)	52.8	56.4	54.0	51.0	49.8
M/6/S	18.6 (10)	28.8	50.4	46.8	45.0	45.6
M/6/A	42.0 (9)	56.4	55.8	53.4	52.2	52.2
F/V/S	10.2 (7)	28.8	37.8	46.2	45.0	46.8
F/V/A	49.8 (7)	55.8	57.6	53.4	54.0	55.2
F/6/S	13.2 (9)	28.2	40.2	46.8	36.6	33.0
F/6/A	45.6 (9)	51.0	52.2	54.6	54.6	54.6

*M=male, F=female; V=vehicle, 6=6-OHDA; S=saline, A=amphetamine.

( )Indicates sample size for each group.

Furthermore, there are sex-related differences in activity in 6-OHDA-treated rats which emerge at 24 and 27 days of age. At those times, male rats are more active than their vehicle-injected controls. The pattern of results is quite different for the females, since 6-OHDA did not significantly alter activity at 24 days of age, but it depressed activity at 27 days of age. These differences in activity within a sex were not related to differences in body weight (Table 1).

Responsiveness to d-Amphetamine in 6-OHDA and Vehicle-Injected Rat Pups

The results shown in Fig. 1 indicate that, in general, the dose of amphetamine used increased activity in all treatment groups. It appears that the male 6-OHDA-treated animals are less sensitive than their vehicle-treated controls to the activity-increasing effects of amphetamine at 24 and 27 days of age, although this finding may be complicated by "ceiling" effects in the 6-OHDA/amphetamine groups. Similarly, ceil-

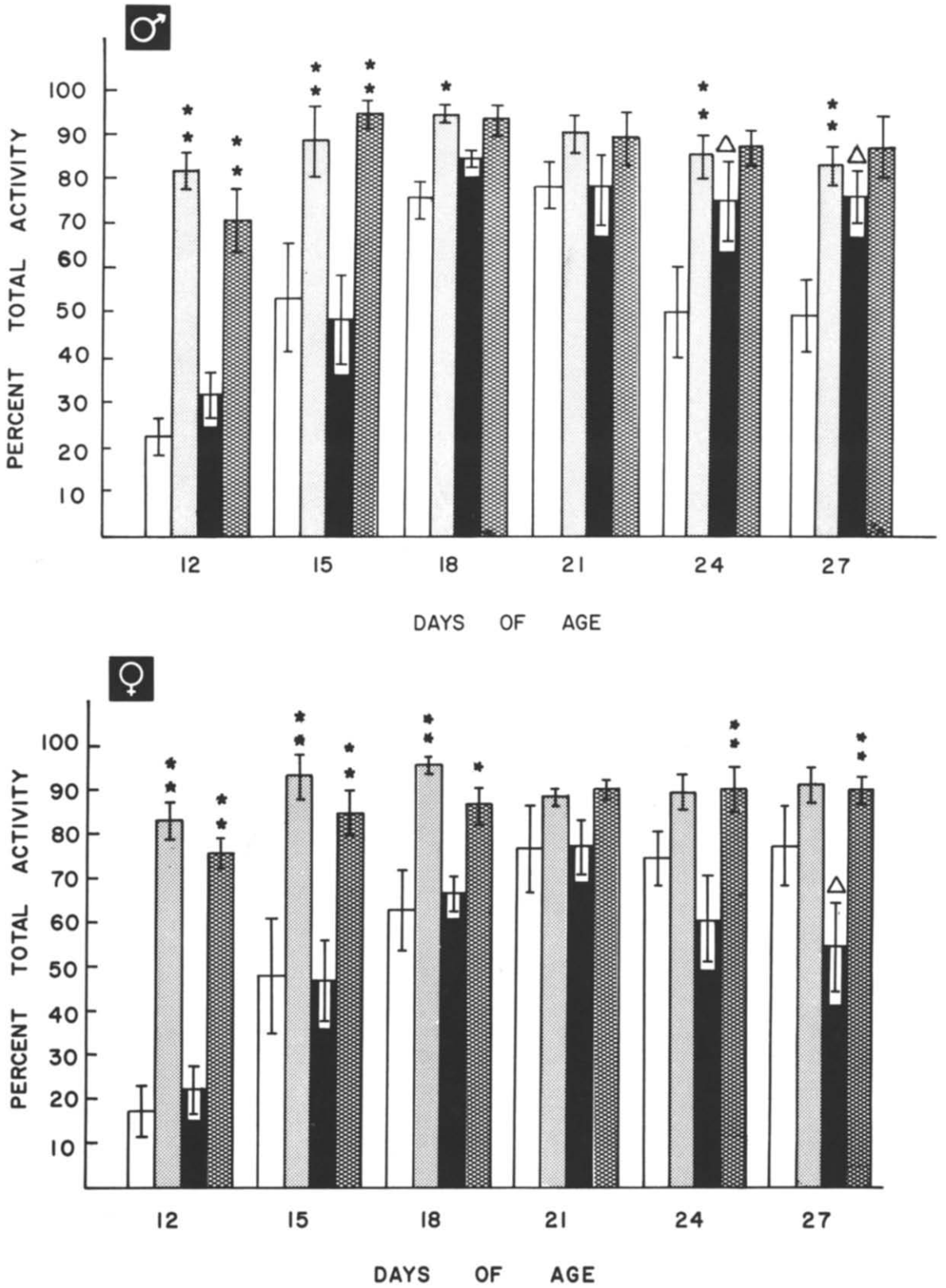

FIG. 1. Mean (±S.E.M.) percent total activity for male (upper panel) and female (lower panel) rat pups during development. Ordinate: activity represented as a percent of total observations during 60-min period. Abscissa: postnatal age in days. Open column, vehicle-saline; stippled column, vehicle-amphetamine; solid column, 6-OHDA-saline; hashed column, 6-OHDA-amphetamine. *Differs from saline animals within same treatment, $p<0.05$; **$p<0.001$. $^{\Delta}$6-OHDA-saline group differs from vehicle-saline group, $p<0.01$ using Duncan's Multiple Range Test.

ing effects in the vehicle-treated female amphetamine groups may explain the fact that female 6-OHDA-treated groups seem to be more sensitive to the activity-increasing effects of amphetamine, relative to either their vehicle-injected control group or the male 6-OHDA treated group. Hence, there is no unequivocal evidence for an altered response to amphetamine due to the 6-OHDA treatment [6] and certainly no indication of a "paradoxical" calming effect of amphetamine in 6-OHDA treated pups [15] of either sex.

Dopamine Concentrations

Analysis of whole brain dopamine concentrations in the 6-OHDA-treated rats sacrificed on day 35 or 36 showed a mean concentration (±S.E.M.) of 428.8±38.1 ng/g tissue and in vehicle-treated rats a mean dopamine concentration of 689.7±41.2 ng/g tissue. This represents a 37.8% depletion and a significant difference between group means. Norepinephrine levels were also examined in a small random subsample of the animals. Whole-brain concentrations of NE revealed an 11% depletion in NE which was not statistically significant.

DISCUSSION

Despite the large number of studies examining the ontogeny of activity in normal [5] and 6-OHDA treated rats [6, 7, 14–17], no studies have reported sex differences in 6-OHDA-induced overactivity. The present findings suggest that an important sex-related variable, other than body weight differences, may be responsible for the reported inability to detect 6-OHDA-induced hyperactivity [6]. More importantly, these data suggest that 6-OHDA-induced overactivity may be confined largely to males, mirroring the prevalence in the human population [10]. Hence, in addition to neurotoxin, stimulant administration, and age, the sex of the animal may be an important determinant of whether Shaywitz' methods will produce results meeting the sound criteria of an animal model of hyperkinesis, i.e., whether (a) the cardinal feature of hyperactivity is produced, (b) the time course of pathogenesis in humans is paralleled, and (c) the model is favorably responsive to stimulant medication.

These results provide only limited support for the contention of Shaywitz *et al.* [15,16] that neonatal 6-OHDA administration results in hyperactivity and a paradoxical calming response to psychostimulants in developing rat pups, i.e., although we report 6-OHDA-induced hyperactivity at 24 and 27 days of age, it was exclusively confined to the males (Fig. 1). Furthermore, the most striking effect of 6-OHDA on male rats' activity came after, rather than before, the decline in their normal activity [7]. Whether this issue represents a fair point of comparison is debatable, since Shaywitz *et al.* sometimes [16], but not always [14], detected hyperactivity when normal activity declined to adult levels. Detection of hypoactivity in 6-OHDA-treated females represents another point of contention, not previously reported, which cannot be explained simply by ceiling effects in female-vehicle-saline animals at 27 days of age. That is, ceiling effects would only explain why female-6OHDA-saline animals were not hyperactive, but not why they were hypoactive. The underlying basis of this hypoactivity remains to be discovered. In addition, we were unable to clearly detect either a paradoxical calming effect of amphetamine or an altered response to amphetamine [6,15] as a result of the 6-OHDA treatment, although the dose was unquestionably effective in increasing activity in vehicle-treated male pups at ages when hyperactivity was detected. These results, along with those reported by other laboratories [6,22] suggest that the "paradoxical calming" effects of psychostimulants, occurring after 6-OHDA-induced central dopamine (DA) denervation and subsequent denervation supersensitivity [15,16], may represent a fairly weak phenomenon in developing rats.

At present, it remains unclear exactly why we were unable to precisely reproduce Shaywitz' profile of hyperactive behavior in developing rat pups. First, like Shaywitz, we utilized littermate controls for 6-OHDA treated animals in order to obviate the possibility of systematic experimenter-produced bias and the potential confound of differential maternal care of pups receiving 6-OHDA *vs.* vehicle treatments [12]. Second, although we obtained only a 38% depletion in whole brain DA, which is somewhat less than that found by Shaywitz [11,14], the depletion, nonetheless, is quite substantial statistically. More substantial brain DA depletions might prove useful in producing both hyperactivity [14] and a "paradoxical" response to amphetamine. Third, we utilized the same dose of d-amphetamine that provides marked overactivity in normal rat pups and calming of overactivity in 6-OHDA-treated rat pups [15]. Dose-response functions were not determined, however, since the paradoxical response to d-amphetamine appears to be equal across a dose range from 0.5 to 5.0 mg/kg [15]. Nonetheless, dose-response data may help to resolve some apparent inconsistencies, particularly related to the issue of DA supersensitivity, in the different investigative reports. At present, we are examining dose-response relationships in 6-OHDA and vehicle-treated pups administered several doses of d-amphetamine in order to test more fully DA supersensitivity [15]. Fourth, we detected only slight decreases in body weight gain associated with the 6-OHDA treatment, differences similar to those reported by Erinoff *et al.* [7], but rather less substantial than those typically reported by Shaywitz and others [4, 11, 17]. A possible source of these discrepancies may be that our most severely DA-depleted animals were those that had died, thereby altering our estimates of both body weight gain and DA depletion. Furthermore, differences in body weight gain between 6-OHDA and control animals depend on whether animals are raised in heterogeneous or homogeneous litters [11], with vehicle-treated heterogeneous litters consistently outweighing all other comparison groups. While we did not explicitly examine the issue of this type of litter composition, we found no differences in body weight gain attributable to 6-OHDA in our heterogeneously-raised litters. Such differences in body weight gain may have prevailed had we found a more substantial reduction of brain DA [11]. More substantial DA depletions may, however, produce a dilemma, particularly if DA depletion is directly related to body weight loss, since stunted body weight growth (in the absence of other treatments) has been implicated in both the production of hyperactivity and the "paradoxical calming" response to d-amphetamine [9].

Given that the above problems can be resolved, further attention should be drawn to the possibility that 6-OHDA induced hyperactivity is sex-related. Although some studies employing neonatal 6-OHDA administration have reported using an approximately equal number of male and female rats [14, 15, 21], no other study has investigated the differential effects of 6-OHDA upon the activity of males and females. Examination of possible sex-related differences are important for a number of reasons, e.g., 6-OHDA has been shown to lead to more profound retardation of body weight

growth in male than in female rat pups [4]. Growth retardation may be a contributory factor in both overactivity and the "paradoxical" response to amphetamine in 6-OHDA treated pups [9], particularly if a proportionately higher number of males are used. On the other hand, if weight loss *per se* is not related to either overactivity [11,17] or to the paradoxical calming effect of stimulants, then males and females may still differ in their responsiveness to 6-OHDA and/or amphetamine due to sex-related differences in neurotransmitter enzymes in the central and peripheral nervous systems [23,24] or to differential responsiveness of the sexes to a constant amount of drug in the brain.

The present findings indicate that male rats are more likely to become hyperactive after neonatal 6-OHDA treatment than are females and this observation may take the basic Shaywitz design one step closer to paralleling the human situation in which males are more frequently diagnosed as hyperkinetic [10]. Pending replication, then, sex-related differences in 6-OHDA-induced hyperactivity and amphetamine responsiveness may provide a reasonable starting point for the investigation of new therapeutic regimens designed to control one or more of the cardinal features of the hyperkinetic syndrome.

ACKNOWLEDGEMENTS

The authors would like to thank Ray Christopher and Eric Cornish for their technical assistance and Patricia McGinley and Denise Lovano for the brain assays. Supported by Public Health Service grant MH-33636.

REFERENCES

1. Ahlenius, S., J. Engel, E. Hard, K. Larsson, P. Lundborg and P. Sinnerstedt. Open field behavior and gross motor development in offspring of nursing rat mothers given penfluridol. *Pharmac. Biochem. Behav.* **6**: 343–347, 1977.
2. Beatty, W. B. and R. G. Fessler. Ontogeny of sex differences in open-field behavior and sensitivity to electric shock in the rat. *Physiol. Behav.* **16**: 413–417, 1976.
3. Blizard, D. A., H. R. Lippman and J. J. Chen. Sex differences in open-field behavior of the rat: The inductive and activational role of gonadal hormones. *Physiol. Behav.* **14**: 601–608, 1975.
4. Breese, G. R. and T. D. Traylor. Developmental characteristics of brain catecholamines and tyrosine hydroxylase in the rat: Effects of 6-hydroxydopamine. *Br. J. Pharmac.* **44**: 210–222, 1972.
5. 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.
6. Eastgate, S. M., J. J. Wright and J. S. Werry. Behavioral effects of methylphenidate in 6-hydroxydopamine-treated neonatal rats. *Psychopharmacology* **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. Felice, F. J., J. D. Felice and P. T. Kissinger. Determination of catecholamines in rat brain parts by reverse-phase ion-pair liquid chromotography. *J. Neurochem.* **31**: 1461–1465, 1978.
9. 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.
10. Ney, P. G. Four types of hyperkinesis. *Can. Psychiatr. J.* **19**: 543–550, 1974.
11. Pearson, D. E., M. H. Teicher, B. A. Shaywitz, P. 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.
12. Piccirillo, M., D. J. Cohen, B. A. Shaywitz, J. E. Alpert and D. Marinelli. Maternal care received by rat pups treated with 6-hydroxydopamine. *Physiol. Behav.* **22**: 69–75, 1979.
13. Rosenthal, R. H. and T. W. Allen. An examination of attention, arousal, and learning dysfunctions of hyperkinetic children. *Psychol. Bull.* **85**: 689–715, 1978.
14. 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.
15. 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.
16. Shaywitz, B. A., J. H. Klopper and J. W. Gordon. Methylphenidate in 6-hydroxydopamine-treated developing rat pups: Effects on activity and maze performance. *Archs Neurol.* **35**: 463–469, 1978.
17. Shaywitz, B. A. and D. A. Pearson. Effects of phenobarbital on activity and learning in 6-hydroxydopamine treated rat pups. *Pharmac. Biochem. Behav.* **9**: 173–179, 1978.
18. Silbergeld, E. K. and A. M. Goldberg. Lead-induced behavioral dysfunction: An animal model of hyperactivity. *Expl Neurol.* **42**: 146–157, 1974.
19. Sobotka, T. J. and M. P. Cook. Postnatal lead acetate exposure in rats: Possible relationship to minimal brain dysfunction. *Am. J. ment. Defic.* **79**: 5–9, 1974.
20. Stinus, L., O. Gaffori, H. Simon and M. LeMoal. Small doses of apomorphine and chronic administration of *d*-amphetamine reduce locomotor hyperactivity produced by radiofrequency lesions of dopaminergic A10 neurons area. *Biol. Psychiat.* **12**: 719–732, 1977.
21. 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* **57**: 163–166, 1978.
22. 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* **67**: 165–169, 1980.
23. Vaccari, A., S. Brotman, J. Cimino and P. S. Timaris. Sex differentiation of neurotransmitter enzymes in central and peripheral nervous system. *Brain Res.* **132**: 176–185, 1977.
24. Wilson, W. E. and A. K. Agrawal. Brain regional levels of neurotransmitter amines as neurochemical correlates of sex-specific ontogenesis in the rat. *Devl Neurosci.* **2**: 195–200, 1979.