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Failure of Amphetamine Isomers to Decrease Hyperactivity in Developing Rats

JAMES T. CONCANNON AND MARTIN D. SCHECHTER

Department of Pharmacology, Northeastern Ohio Universities, College of Medicine
Rootstown, OH 44272

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CONCANNON, J. T. AND M. D. SCHECHTER. *Failure of amphetamine isomers to decrease hyperactivity in developing rats.* PHARMAC. BIOCHEM. BEHAV. **17**(1) 5–9, 1982.—Possible amphetamine-induced changes in locomotor activity were investigated in developing rats administered intracisternal injections of 6-hydroxydopamine (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 44% of control levels, whereas norepinephrine levels were not significantly reduced. In normal and 6-OHDA-treated pups activity increased from moderately low levels at 15 days of age to moderately high levels at 25 days of age. However, 6-OHDA-treated rats were hyperactive at 20 days of age. At 25 days, activity in both groups was equal and declined to levels typical for adults. Administration of graded doses of *d*- and *l*-amphetamine generally increased activity in both groups of rats, with *d*-amphetamine being more potent than *l*-amphetamine. Furthermore, no dose of either amphetamine isomer decreased activity in 6-OHDA-treated, hyperactive rats. Hence, no convincing evidence was found for a "paradoxical calming" effect of amphetamine in hyperactive rats, supporting other recent reports. These results suggest that the neonatal DA-depleted rat does not provide an accurate model system for pre-clinical investigation of the human hyperkinetic syndrome.

Hyperactivity 6-Hydroxydopamine Dopamine *d*-Amphetamine *l*-Amphetamine Activity
Developing rats Hyperkinesis Attentional deficit disorder Animal models

A LARGE number of investigators have recently attempted to model the human hyperkinetic syndrome by administering the neurotoxin 6-hydroxydopamine (6-OHDA) to neonatal rat pups and observing the course of their behavioral activity developmentally in this dopamine-depleted preparation [5–7, 9, 14, 16, 19, 23, 24, 26]. These attempts have met with varying degrees of success in terms of: (a) producing several of the cardinal features (e.g., overactivity, impaired learning ability, and decreased "attention" [15]) of the hyperkinetic syndrome [18]; (b) bearing some temporal relationship to the pathogenesis of hyperkinesis in humans [19] and, most importantly, (c) decreasing the hyperactivity by the administration of psychostimulant medication [20,21]. This last criterion, comprising the so-called "paradoxical calming" effect of psychostimulants, has been most difficult to fulfill in the 6-OHDA model (e.g., [5, 6, 11, 16, 26]), i.e., it is very difficult to find a dose of stimulant that will simultaneously increase activity in normal animals, yet decrease activity in 6-OHDA treated pups (cf. [20,21]). Therefore, the aim of the present investigation was to examine the ability of a wide range of doses of both *d*- and *l*-amphetamine to decrease hyperactivity in 6-OHDA-treated hyperactive pups. This effort represents an attempt to replicate the "paradoxical calming" effect of *d*-amphetamine in 6-OHDA-treated hyperactive pups [20] and is the first attempt to decrease hyperactivity by *l*-amphetamine administration [1] in 6-OHDA-treated pups.

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 pups with an equal number of males and females whenever possible. On occasion, a litter with less than 8 pups was fortified by addition of animals culled from other litters born on the same day. 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 (0.4 mg/ml ascorbate in saline) injection. Activity was determined at 15, 20, 25, and 30 days of age, between the hours of 1300 and 1600, using the time-sampling technique described in detail by Shaywitz *et al.* [19].

Starting at 15 days of age, and at 5 day intervals thereafter, 6-OHDA- or vehicle-treated rat pups were removed from

the mother and administered an intraperitoneal injection of *d*- or *l*-amphetamine or an equal volume of physiological (0.9%) saline. The *d*-amphetamine doses, used in separate groups of animals, toxin- or vehicle-injected (ns=10–13), were 0.125, 0.25, 0.50, 1.0, and 2.0 mg/kg; *l*-amphetamine doses were 0.25, 0.50, 1.0, 2.0, and 4.0 mg/kg. In addition, one vehicle-injected (n=27) and one 6-OHDA-injected (n=25) group of animals received a saline injection. Assignment to treatment (*d*-, *l*-amphetamine or saline) was random with the assigned animal always receiving the same treatment.

Thirty min after injection, pups were again removed from the mother and were individually placed in 33×27×17 cm clear plastic cages for behavioral observation. Each cage was scanned each minute and the behavior was recorded at that particular moment, according to one of the following mutually-exclusive categories [19]: sleeping, inactive (lying motionless), ambulating, climbing, rearing, eating, drinking, sniffing, grooming, scratching. 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 at least 90% agreement on activity categorization detailed in Shaywitz *et al.* [19]. 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*- or *l*-amphetamine or saline).

Drugs and Dosage Rationale

6-OHDA HBr (Aldrich Chemical Co., Milwaukee, WI), 100 μg, calculated as free base, was prepared fresh daily and administered as previously described [5] over a 30-sec injection interval. The vehicle-treated littermate control group received an equivalent volume of the 6-OHDA vehicle. Both 6-OHDA and its vehicle were administered 60 min after an intraperitoneal injection of 20 mg/kg DMI HCl (as base), to produce selective depletion of central dopamine levels [5,19].

d-Amphetamine sulfate (Sigma Chemical Co., St. Louis, MO) or *l*-amphetamine (S.K.F. Laboratories, Philadelphia, PA) was dissolved in physiological saline and administered intraperitoneally in a volume of 0.005 ml/g; control animals within the same litters received an equivalent volume of the saline vehicle. The doses of *d*-amphetamine were within the range used by Shaywitz *et al.* [20] for altering activity in both 6-OHDA and normal rats between the ages of 12 and 30 days, i.e., to produce overactivity in normal rat pups and "paradoxical calming" in 6-OHDA treated, DA-depleted pups. The doses of *l*-amphetamine were twice those of *d*-amphetamine, since *d*-amphetamine has been reported to be between 2 and 10 times more potent in increasing activity than is *l*-amphetamine [2,25].

Biochemical Determinations

All animals were sacrificed by decapitation at 35 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 were homogenized in 5 ml of 0.05 M $HClO_4$ and 300 μl dihydroxybenzylamine (DBA), the internal standard, with a Brinkman (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 was added to a conical reaction vial containing 100 mg of acid-washed alumina and 1 ml Tris buffer, pH=8.6 (3.0 M). The alumina was shaken and washed as described by 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 a 100 μl loop injection and Micropak MCH-10 reverse phase column and model LC-2A electrochemical detector with glassy carbon electrode set at 720 mV vs an AgCl electrode. Mobile phase consisted of 3 parts citric acid (0.1 M) to 2 parts Na_2HPO_4 (0.1 M) made with 0.3 mM in sodium octylsulfate and 10% acetonitrile.

Data were analyzed using a Varian CDS 111L data system based on the peak and ratio of dopamine and norepinephrine to the internal standard DBA in tissue samples multiplied by the ratio of peak areas of DBA to norepinephrine and dopamine standards.

Statistical Methods

Measurements of each category of activity were calculated as percentage of occurrence during 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 [20], only the category of "Total Activity" was analyzed by using a 2 (Intracisternal Injection: 6-OHDA vs vehicle) × 11 (Drug Treatment) × 4 (Age) mixed unweighed means analysis of variance (ANOVA), with Intracisternal Injection and Drug Treatment as between-group factors and Age representing the repeated measure. Subsequent to this analysis, all between-group comparisons were conducted utilizing Duncan's Multiple Range Test. Similar results were obtained using completely between-group two-way ANOVAs at each level of the repeated measure (i.e., Age). Throughout the experiment $p \leq 0.05$ was considered to be statistically significant.

RESULTS

Activity

Depicted in Table 1 is the mean absolute and percentage of "Total Activity" [19] as a function of age and type of brain injection for animals administered saline prior to behavioral observations. The results show an increase in activity for both groups from moderately low values (about 35%) at 15 days of age to moderately high values (about 70%) at 25 days of age. In addition, 6-OHDA-treated pups are approximately 70% more active than vehicle-injected rats at 20 days of age, although this heightened activity returns to control levels at 25 days of age, the peak of activity for both groups. After 25 days of age, both groups show a slight decline from the peak of activity to levels fairly typical for adults [4,19].

Presented in Fig. 1 a–d are the data representing the responsiveness to *d*- and *l*-amphetamine as a function of age in 6-OHDA vs vehicle-treated rats. A 2×11×4 ANOVA of these data revealed simple main effects of Dose, $F(10,259)=12.61$, $p<0.001$ and Age, $F(3,777)=14.37$, $p<0.001$, and Age × Dose, $F(30,777)=3.89$, $p<0.001$, and Age × Dose × Treatment, $F(30,777)=1.39$, $0.05<p<0.10$ interactions. The results of subsequent comparisons of most interest, generated by using Duncan's Multiple Range Test, are presented in Fig. 1 a–d with the aid of symbols. Transient hyperactivity was present in 20-day-old 6-OHDA-treated rat pups at a time when their body weights were essentially normal. Activity was increased above the vehicle/saline baseline by most doses of *d*-amphetamine at most ages and

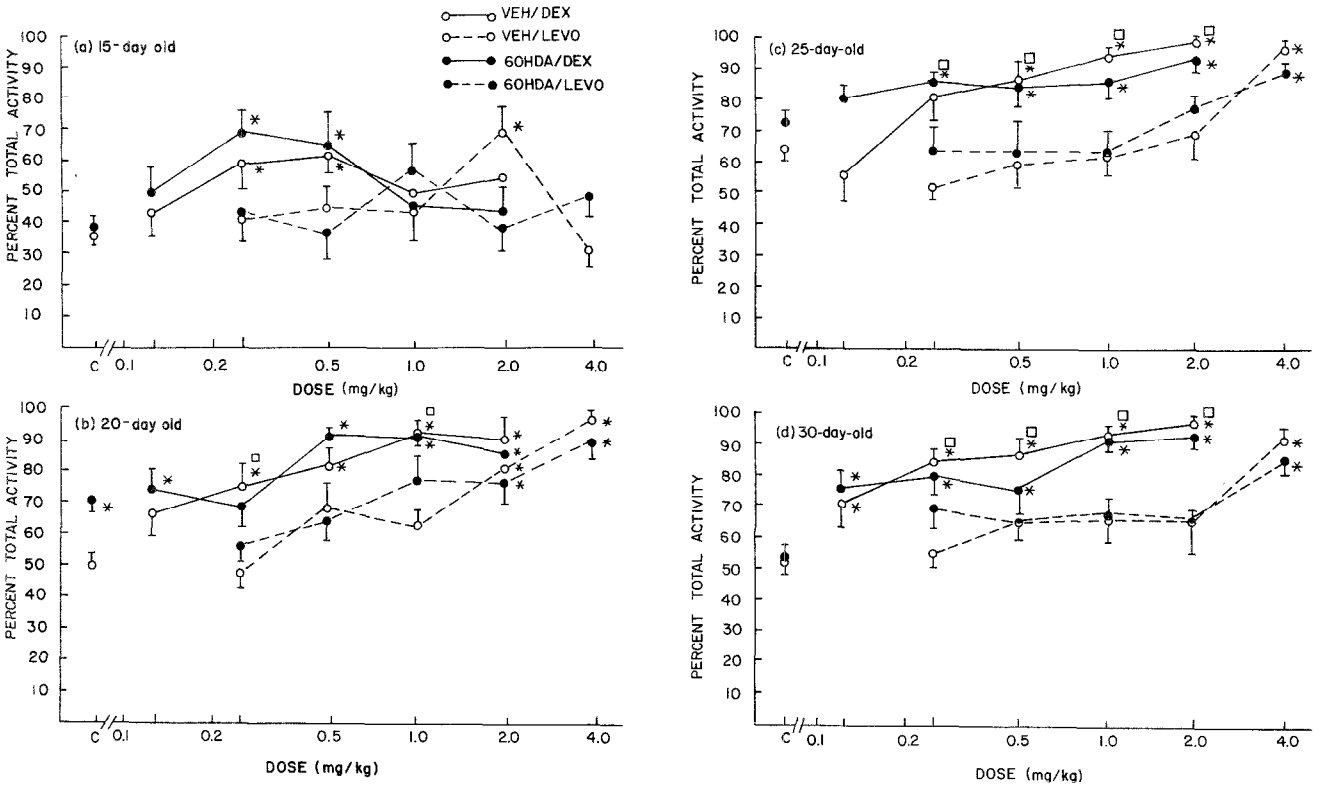

FIG. 1. a–d. Mean (±S.E.M.) percent total activity for (a) 15-, (b) 20-, (c) 25-, and (d) 30-day old rat pups. Ordinate: total activity represented as a percent of total observations during a 60-min period. Abscissa: doses of either *d*- (0.125, 0.25, 0.50, 1.0, 2.0 mg/kg) or *l*-amphetamine (0.25, 0.50, 1.0, 2.0, 4.0 mg/kg). Isolated points on the left side of each panel represent activity in control group (C) animals injected with saline either after neonatal vehicle (open circle) or 6-OHDA (closed circle) treatment. *Differs from vehicle-saline animals, $p<0.05$; □vehicle-injected animals receiving *d*-amphetamine differ from vehicle-injected animals receiving equal mg/kg dose of *l*-amphetamine, $p<0.05$ using Duncan's Multiple Range Test.

by only the highest two doses of *l*-amphetamine. Perhaps most importantly, no dose of either *d*- or *l*-amphetamine that increased activity in vehicle-injected animals reduced activity below the 6-OHDA saline-treated baseline. (Therefore, these latter comparisons are not indicated in the figure.) Hence, there is little indication of a "paradoxical calming" effect of either amphetamine isomer in the dose ranges utilized.

Dopamine/Norepinephrine Concentrations

Analysis of whole-brain concentrations in the 6-OHDA treated rats sacrificed at 35 days of age revealed a mean dopamine concentration (±S.E.M.) of 315.88±39.74 ng/mg tissue and in vehicle-treated rats a mean dopamine concentration of 717.29±30.98 ng/gm tissue. This represents a 56.0% depletion which is statistically significant. Norepinephrine levels were 360.42±11.96 ng/gm and 394.80±13.61

TABLE 1

MEAN ABSOLUTE TOTAL ACTIVITY AND PERCENT TOTAL ACTIVITY FOR ANIMALS ADMINISTERED SALINE PRIOR TO TESTING

Treatment Group	Age (Days) 15	20	25	30
Vehicle/saline	21.6 (36)*	30.0 (50)	38.4 (64)	31.2 (52)
6-OHDA/saline	22.8 (38)	42.6 (71)	43.2 (72)	31.8 (53)

*Indicates percent total activity (e.g., for vehicle/saline group on day 15: 21.6/60×100=36%).

ng/gm for 6-OHDA vs vehicle-treated rats, respectively. This represents an 8.7% depletion which was not statistically significant.

DISCUSSION

The present results show that neonatal administration of 6-OHDA produces developmental hyperactivity in rat pups at 20 days of age, although this heightened activity returns to control levels at 25 and 30 days of age. These findings are in agreement with those of Shaywitz *et al.* [19]. In addition, both *d*- and *l*-amphetamine were shown to increase activity in normal rat pups and *d*-amphetamine was more potent in this respect [2]. However, no dose of either isomer decreased activity in 6-OHDA-treated rats below the 6-OHDA/saline baseline. Rather, doses of amphetamine effective in normal animals either had no effect on or increased activity in 6-OHDA-treated pups [5, 6, 16]. Indeed, the only evidence for a "normalizing" effect of amphetamine was that 0.25 mg/kg *d*-amphetamine, a dose which increased activity in 20-day-old normal rats, returned activity to the control level in 6-OHDA-treated rats, although it did not decrease activity relative to the 6-OHDA/saline baseline. Whether this isolated finding represents a sub- or supersensitivity to *d*-amphetamine remains unresolved at this time. Resolution of this issue will ultimately require selective regional administration of 6-OHDA coupled with regional DA levels and DA receptor-binding studies in developing rats.

Production of developmental hyperactivity by neonatal 6-OHDA administration supports the conclusions of a number of previous reports [5, 7, 9, 14, 16, 19–21, 24, 26]. As previously mentioned [5], some reports show a return of hyperactivity to control levels at 25 and 30 days of age [16, 19, 20], whereas others show that hyperactivity continues well beyond weaning and into pubescence [7, 9, 14, 17, 21]. One determinant of continued hyperactivity in adulthood may be very substantial DA depletion [13], although this variable was not systematically studied in this investigation.

Our findings, however, again question whether psychostimulants will decrease 6-OHDA-induced hyperactivity through the mechanism of dopamine supersensitivity [5]. First of all, the paradoxical response to psychostimulants detected by Shaywitz *et al.* [20,21] was not strikingly dose-responsive, and no paradoxical response was detected in the present study. Secondly, a calming response to *d*-amphetamine has been achieved in underweight, overactive developing mice not receiving any other type of brain insult [12]. Thirdly, no weight loss data were reported by Shaywitz *et al.* [20,21] when they found calming responses to psychostimulants although studies reporting no significant weight loss by 6-OHDA failed to evidence calming responses to stimulants in 6-OHDA-treated developing rats [5,16]. Thus, calming responses to psychostimulants may occur by some mechanism other than DA neuronal supersensitivity in 6-OHDA treated rats, e.g., decreased body weight [3, 10, 12, 22]. Hence, the young DA-depleted rat does not represent a good animal model of childhood hyperkinesis since the resultant overactivity is not attenuated by clinically-effective doses of psychostimulants [5].

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