Pharmacology Biochemistry & Behavior, Vol. 15, pp. 443–448, 1981, Printed in the U.S.A.

Effects of Bromocriptine in Developing Rat Pups After 6-Hydroxydopamine[1]

BENNETT A. SHAYWITZ[2], SUSAN V. LIPTON, MARTIN H. TEICHER, DONALD J. COHEN, GEORGE M. ANDERSON, DAVID K. BATTER, AND J. GERALD YOUNG

Departments of Pediatrics, Neurology, Psychiatry, Laboratory medicine and the Yale Child Study Center, Yale University School of Medicine, New Haven, CT 06510

Received 14 February 1981

SHAYWITZ, B. A., S. V. LIPTON, M. H. TEICHER, D. J. COHEN, G. M. ANDERSON, D. K. BATTER AND J. G. YOUNG. *Effects of bromocriptine in developing rat pups after 6-hydroxydopamine.* PHARMAC. BIOCHEM. BEHAV. 15(3) 443–448, 1981.—The effects of low (0.5 mg/kg) and high (2.0 mg/kg) doses of bromocriptine (BCR) on activity and escape performance were examined during the first month of postnatal life in normal developing rat pups and littermates treated at 5 days of age with a combination of desmethylimipramine and 6-hydroxydopamine (6-OHDA). Such a procedure resulted in significant reductions in brain dopamine to concentrations 10–20% of vehicle controls while norepinephrine was unaffected. BCR increased general motor activity in vehicle pups at 13 and 19 days but had little effect on more mature animals. Pups who had not received BCR exhibited a decline in activity over the hour long observation period (habituation of activity) but this decline was abolished by both low (0.5 mg/kg) and high (2.0 mg/kg) doses of the agent. Stereotyped activity, particularly at 19 days was increased by BCR in 6-OHDA pups but not in vehicle animals, an effect suggesting denervation supersensitivity. Head dips in a hole box at 30 days of age were not influenced by BCR in vehicle pups but significantly reduced by BCR in 6-OHDA pups, suggesting that BCR might be acting to stimulate inhibitory dopaminergic mechanisms. Escape learning in a T-maze at 20 days and shuttle box at 28 days was disrupted by high doses of BCR in vehicle pups and both doses of BCR in 6-OHDA animals. The similarity with the behaviors observed in the clinical syndrome of attention deficit disorder with hyperactivity prompted a number of investigative groups including our own to suggest that the 6-OHDA model might serve as a useful and convenient paradigm to evaluate pharmacological agents that offer potential in the treatment of this most common disorder. From this perspective we would predict that BCR would have little clinical utility since it both failed to attenuate 6-OHDA induced hyperactivity and tended to disrupt performance in an avoidance learning task.

Bromocriptine (BCR) 6-Hydroxydopamine (6-OHDA) Escape performance Desmethylimipramine Motor activity

THE treatment of neonatal rat pups with the combination of desmethylimipramine (DMI) followed by 6-hydroxydopamine (6-OHDA) results in a constellation of behaviors that have many parallels to the human syndrome of Attention Deficit Disorder with hyperactivity (ADD; [25, 26]). Thus we [20] and others [10, 11, 14, 28, 29] have shown that pups depleted of brain dopamine (DA) exhibit hyperactivity during the period of behavioral arousal, and deficits in escape and avoidance learning. This similarity with the clinical syndrome suggests that the 6-OHDA model may provide a useful and convenient paradigm to evaluate pharmacological agents that offer potential in the treatment of this most common disorder.

Administration of the stimulants, amphetamine [21,28] and methylphenidate [23], reduce the hyperactivity observed in 6-OHDA treated developing rat pups, presumably via central catecholaminergic mechanisms (see Discussion), and it is reasonable to suspect that other pharmacological agents with properties similar to the presumed actions of the stimulants might also exert ameliorative effects on 6-OHDA induced hyperactivity. The dopamine agonist, bromocriptine (2-bromo-a-ergocryptine, BCR) has been shown to reduce locomotor activity in adult mice when given at very low dosages [8], though its effects in the developing organism have not been systematically examined. In order to evaluate the potential utility of BCR in the treatment of ADD we have examined its effects on several types of motor activity and learning in the 6-OHDA model of ADD.

METHOD

Sprague-Dawley rat pups and mother were obtained from Charles River, Inc., Wilmington, MA at 24 hr (±12 hr) of age and individually housed under fluorescent lighting conditions (16 G.E. 40 W fluorescent bulbs) with 12 hours of light (lights

[1]Supported in part by PHS Grants NS 12384, AA 03299, HD 03008, NIMH Clinical Research Center Grant No. 1 P 50 MM 30929, and a grant from the Thrasher Research Foundation.

[2]Address correspondence to Bennett A. Shaywitz, M.D., Yale University School of Medicine, Department of Pediatrics, 333 Cedar Street, New Haven, CT 06510.

on 0700) and 12 hours of darkness at a temperature of 21°C. Litters were culled to 8–9 pups at 5 days of age. Mothers and pups were housed together for the entire experimental period, and food (Purina Chow) and tap water were available ad lib to the dam and her pups. These experiments included approximately equal numbers of male and female rats.

Twelve litters of animals were divided into six experimental groups and each litter was composed of four rat pups treated with 6-hydroxydopamine (6-OHDA) at 5 days of age and four vehicle treated littermates, yielding cell sizes of 7–8 animals per cell. An equal number of 6-OHDA and vehicles received BCR in doses of 0.5 or 2.0 mg/kg body weight. Once a rat pup received a particular dose for one behavioral observation (for example, activity at 13 days of age) he received this dose for all subsequent behavioral tasks. BCR was prepared by dissolving the drug in ~0.25 ml of 95% ethanol and diluting the solution with saline. Solutions for controls were prepared with a similar amount of ethanol.

The 6-OHDA HBr was purchased from Regis Chemical Company (Chicago, IL) and was used without further purification. It was prepared immediately prior to use in 0.9% isotonic saline solution containing 0.4 mg/ml of ascorbic acid to prevent oxidation of the 6-OHDA. The solution was kept on ice while in use. Rat pups were given desmethylimipramine (DMI, USV Pharmaceuticals) 20 mg/kg IP one hour prior to 6-OHDA. In earlier experiments [20–22] we also treated littermate controls with DMI but since this agent given alone did not appear to alter either activity or brain catecholamine concentrations we no longer treat the controls with this drug. Intracisternal injections were administered by flexing the neck of the infant rat and injecting 6-OHDA via a precalibrated microsyringe (Hamilton) with a 27 ga needle inserted immediately beneath the occiput. All pups received intracisternal injections—the expermental group received doses of 6-OHDA and littermate controls received the saline solution containing 0.4 mg/ml ascorbic acid (vehicle).

Rats were sacrificed by decapitation at 33 days of age between 9–11 a.m. in order to minimize brain catecholamine variation due to normal circadian periodicity. Brains were removed and frozen on dry ice within one minute after death. Frozen brains were stored at −70°C and biochemical determinations performed within 2–3 weeks after sacrifice. DA and NE were analyzed by high performance liquid chromatographic techniques [1].

Activity was determined at 8, 13, 19 and 26 days of age using procedures detailed elsewhere [24], and measurements were always performed between 1300 and 1600 hours each day to minimize the variation due to circadian periodicity. Rat pups were randomly assigned to one of the nine plastic cages placed on the floor of the room. BCR (or vehicle) was administered subcutaneously prior to placement in the activity cages and recording was begun 30 minutes after injection and continued for one hour. At the conclusion of the taping session, the rat pups were replaced in their home cages and the video tape saved for scoring at a later time. Scoring the activity was accomplished by playing the tape back at a speed equivalent to six times real time, and activity in each rat was determined for alternate 5 minute periods throughout the 60 minute observation period. For example, we scored the animals' activity from 0–5 minutes, 10–15 minutes, 20–25 minutes, etc. We thus had available for analysis six separate measurements of activity for each animal for the hour-long observation period. The mean of these six determinations was then used to obtain the mean activity for the observation period.

Stereotyped activity was coded using a scoring system modified after that described by Costall and associates [6,16]. Both the type of stereotypies, either sniffing or rotary activity, and their intensity were recorded by examining the first minute of every five minute epoch for the hour-long activity recording. Two types of sniffing and rotary activity were distinguished: sniffing (or rotary activity) over a small area, i.e., less than 1/4 of the cage area (given a score of 5) and sniffing (or rotary activity) over wider areas (scored 10). If activity was present for between 0–15 sec of the minute long observation period, it was considered low and scored 1; if present 16–40 sec, moderate, scored 2; and if present greater than 40 sec, high, scored 3. The stereotypy index represented the product of the activity type (5 or 10) and the intensity of the stereotypy (1–3) and was scored for both sniffing and rotary activity separately.

Exploratory activity was determined at 30 days of age utilizing a procedure described initially by File [12]. The apparatus was made of half-inch thick plywood and measured 43 cm square with sides 30 cm high. The floor was elevated 16 cm and a fluorescent light (GE 40 W) was placed so as to illuminate 4 objects placed beneath the floor and visible through 4 holes, each 3 cm in diameter located 11 cm from the corners of the box. The experiments were performed 30 minutes after injection of BCR in a soundproof room and were initiated by placing the rat pup in the center of the box beneath an aluminum pan 10 cm in diameter and 5 cm high. After 10 seconds the pan was removed and observations begun each minute and continued for a 5 minute period. The total number of times that the rat's head dipped below the surface as he peered at one of the objects was recorded, as well as the total time for each minute that the rat was occupied by peering at the objects beneath the holes. As in all experiments, exploratory measures were always performed between 1300 and 1600 hours and results were coded and analyzed utilizing analysis of variance.

Avoidance learning in a T-maze was determined at 20 days of age, 30 minutes after subcutaneous administration of BCR, utilizing procedures described elsewhere [24]. The elapsed time in seconds from start to the rat's entry into the safe compartment (the escape latency) was recorded by means of a light switch (Sigma Instruments 8RCO1A, South Braintree, MA) and the number of correct responses during the total of 20 trials was recorded for each rat. Results were coded and analyzed utilizing analysis of variance.

Avoidance learning in a shuttle box was determined at 28 days of age, 30 minutes after administration of BCR. The apparatus and procedure has been described in detail previously [24].

Statistics

Data was analyzed using two and three way analysis of variance (ANOVA) with programs adopted from Bruning and Kintz [2]. Four way ANOVA were performed with two between, two within factors using a program written by one of the authors (MHT). Post hoc comparisons were analyzed using Scheffe and Dunn's tests [9,15].

RESULTS

Effects on General Motor Activity

A significant main effect emerged for age, $F(3,75)=89.7$, $p<0.001$. Thus, general motor activity in all groups increased from 26.4±1.48% at 8 days, to 51.8±2.11% at 13 days, peaked at 83.8±2.00% at 19 days and declined to

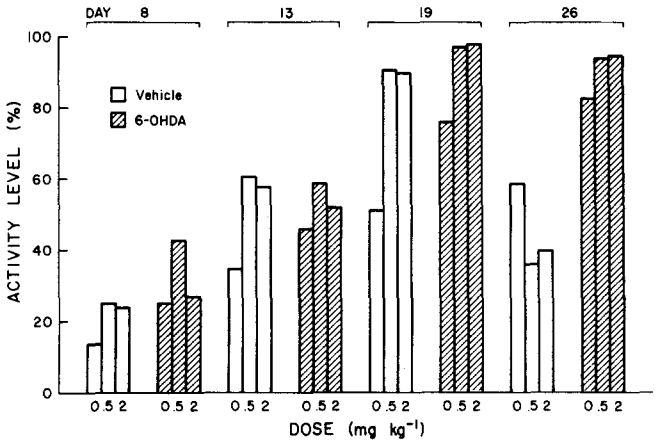

FIG. 1. General motor activity in vehicle and 6-OHDA pups at each age and at each dose of BCR. See text for details.

68.2±2.70% at 26 days (p's for all comparisons <0.001). Over all ages a significant main effect was apparent also for 6-OHDA, $F(1,24)=57.5$, $p<0.001$, with activity averaging 48.9±1.85% in controls compared to 66.2±1.72% in 6-OHDA pups. In addition, a significant main effect was found for BCR, $F(2,24)=15.8$, $p<0.001$, averaging 48.6±2.32% in those non-BCR pups, but increasing to 63.2±2.13% and 60.8±2.29% in pups after 0.5 and 2.0 mg/kg respectively (0 vs 0.5 mg/kg, $p<0.001$; 0 vs 2 mg/kg, $p<0.001$; 0.5 vs 2 mg/kg, NS). Finally, a significant main effect was observed for observations (trials) over the hour long recording period, $F(5,125)=21.3$, $p<0.001$, decreasing from 70.8±2.46% during the first 5 minutes to 49.2±3.58% by the end of the hour.

Significant interactions emerged for age×6-OHDA, $F(3,75)=14.4$, $p<0.001$, with 19 day old and 26 day old 6-OHDA pups being the most active. The age×BCR interaction was also significant, $F(6,75)=3.94$, $p<0.005$, with BCR at both dosages increasing activity at 13 and 19 days but not at 26 days. Figure 1 demonstrates the three way interaction of age×6-OHDA×BCR, $F(6,75)=2.37$, $p<0.05$. First, it is apparent that 6-OHDA pups not treated with BCR are more active than their respective littermate controls at 19 and 26 days. Second, BCR markedly increased the activity of control pups at 13 and 19 days (13: 0 vs 0.5 mg/kg, $p<0.02$; 0 vs 2 mg, $p<0.05$; 19: 0 vs 0.5 mg/kg, $p<0.001$; 0 vs 2 mg/kg, $p<0.001$) and marginally increased the activity of treated pups at 13 and 19 days (0 vs 0.5 mg/kg, $p<0.10$; 0 vs 2 mg/kg, $p<0.05$). However, BCR significantly decreased the activity of control pups at 26 days of age (0 vs 0.5 mg/kg, $p<0.05$; 0 vs 2 mg/kg, $p<0.10$). The interaction of age×BCR×time over the hour long observation period, $F(30,375)=1.47$, $p<0.05$, is shown in Fig. 2. Pups who did not receive BCR exhibited a decline in activity during the hour long observation period (an effect termed habituation of activity [27]). However pups given BCR at either dosage, particularly 19 day olds, showed a much less rapid decline in activity. Pups treated with 6-OHDA also exhibited habituation of activity over the hour long observation that was attenuated by BCR.

Head Dips

Significant interactions were apparent for both the number of head dips, $F(5,48)=4.73$, $p<0.001$, and their duration, $F(5,48)=4.05$, $p<0.005$, and these results are shown in Fig. 3. Both were significantly greater in 6-OHDA pups compared to controls ($p<0.001$, Scheffe test). Administration of BCR had no significant effect on head dips in vehicle pups but both 0.5 and 2.0 mg/kg doses significantly reduced activity in 6-OHDA pups ($p<0.001$, Scheffe test).

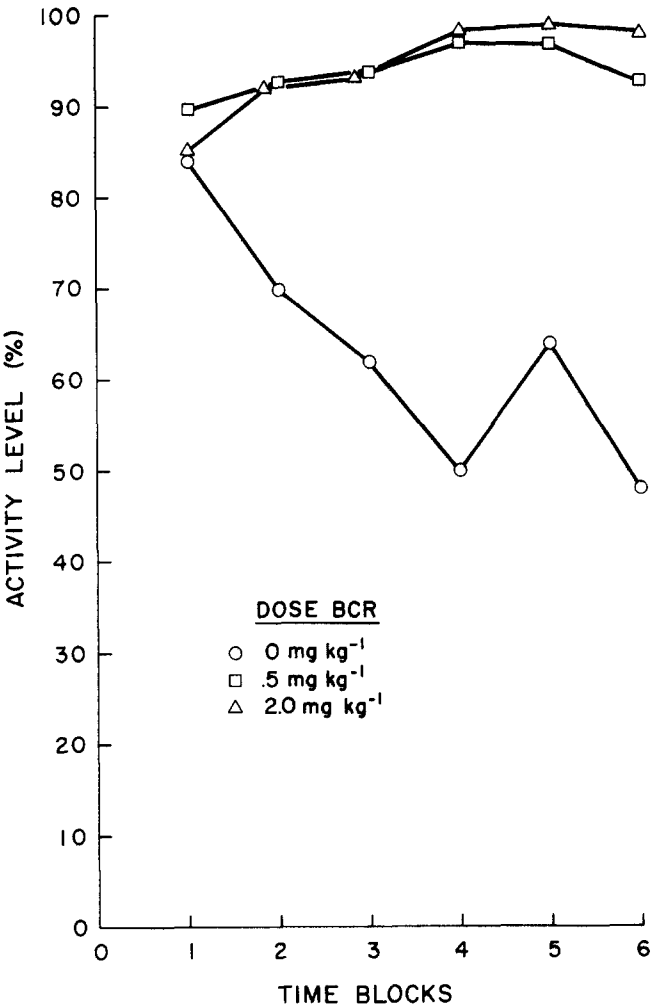

FIG. 2. General motor activity at each dose of BCR at 19 days of age over the hour long observation period (habituation of activity). This figure illustrates habituation of activity in pups who did not receive BCR but no habituation in animals receiving either the low or high BCR dose.

Stereotyped Activity

At 13 days BCR increased sniffing activity in a dose dependent fashion in vehicle treated pups, and this increase was greater than that observed in 6-OHDA animals. Rotary activity was little affected by BCR in either vehicle or 6-OHDA animals. By 19 days BCR more readily influenced both sniffing and rotary activities in 6-OHDA animals, suggesting that at this age BCR might provoke denervation supersensitivity in pups with an already damaged DA system. Thus, sniffing was increased in a dose dependent fashion in 6-OHDA pups, but in vehicles, administration of BCR had little effect. Similarly, rotary activity was increased max-

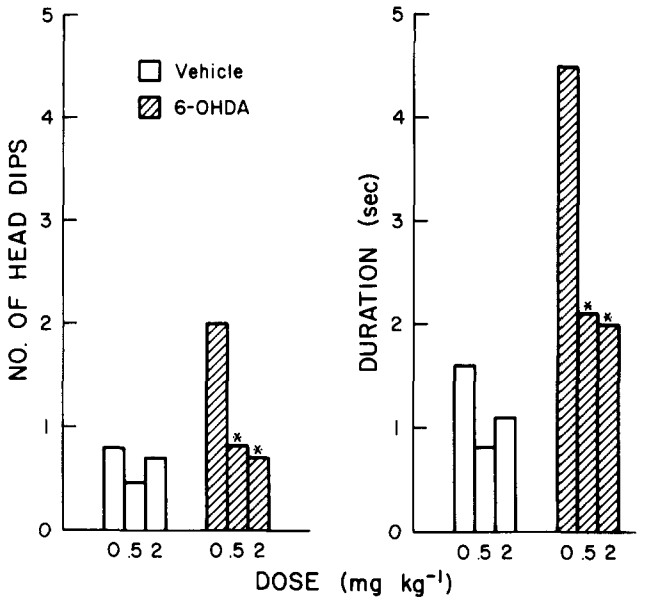

FIG. 3. Exploratory activity in a hole box at 30 days of age. BCR at both high and low doses results in a significant reduction in the number of head dips and the duration of head dips in 6-OHDA pups. *$p<0.001$.

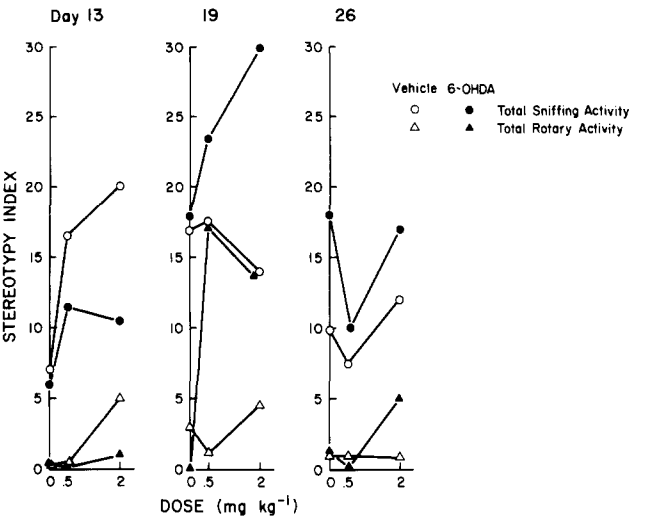

FIG. 4. Stereotyped activity at three ages for vehicle and 6-OHDA pups after BCR. At 19 days both sniffing and rotary activity are increased in 6-OHDA pups after BCR.

imally at 0.5 mg/kg in 6-OHDA pups, but little effect was observed with this dose in vehicles. At 26 days spontaneous sniffing was considerably more frequent in 6-OHDA pups and BCR at low dosages appeared to reduce this activity to that of controls. Spontaneous rotary activity in 6-OHDA pups was comparable to that of vehicles, but the 2.0 mg/kg dose resulted in an increase in this kind of activity in only 6-OHDA animals (Fig. 4).

Escape and Avoidance Learning

When calculated as escape latency in seconds, neither 6-OHDA nor BCR alone exerted a significant main effect in a T-maze at 20 days of age, but their interaction was significant, $F(2,48)=4.34$, $p<0.025$. As shown in Fig. 5 escape latency in vehicle treated pups declines dramatically as trials progress, indicating acquisition of learning. This response, though clearly attenuated, is observed as well in vehicle pups given low doses of BCR. However, vehicle pups given the higher dose of BCR, and all 6-OHDA groups, whether given BCR or not, failed to demonstrate any reduction in escape latency over trials, a response consistent with an inability to acquire escape learning. Escape latency over all trials was reduced by both low and higher doses of BCR in 6-OHDA pups, a finding supported as well by the significant increase in the number of correct responses noted in 6-OHDA pups after both doses of BCR.

A significant main effect was observed for escape latency in the shuttle box at 28 days of age for 6-OHDA, $F(1,24)=11.0$; $p<0.005$, but not for BCR or for the interaction between the two (Fig. 6). When calculated as the number of trials to achieve a criterion of 5 consecutive avoidance responses, a similar 6-OHDA effect was observed as well. In contrast to the results observed in the T-maze, BCR did not appear to produce any improvement in escape or avoidance learning in 6-OHDA pups.

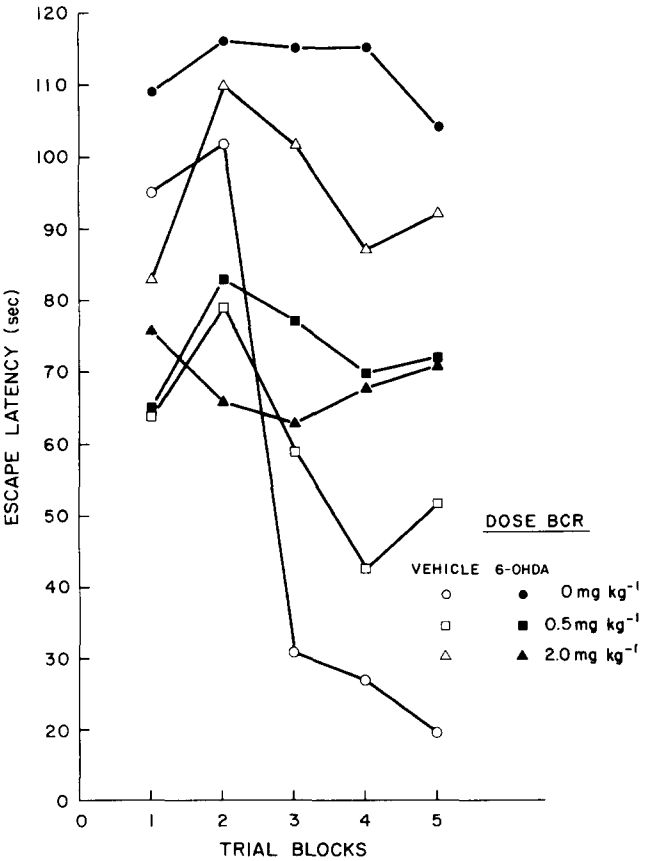

FIG. 5. Escape latency in a T-maze at 20 days for vehicle and BCR treated pups after BCR. In normal pups without BCR, performance time improves over trials, an effect also apparent in vehicle pups after low BCR. However, high BCR in vehicle pups and all 6-OHDA animals fail to improve performance over trials.

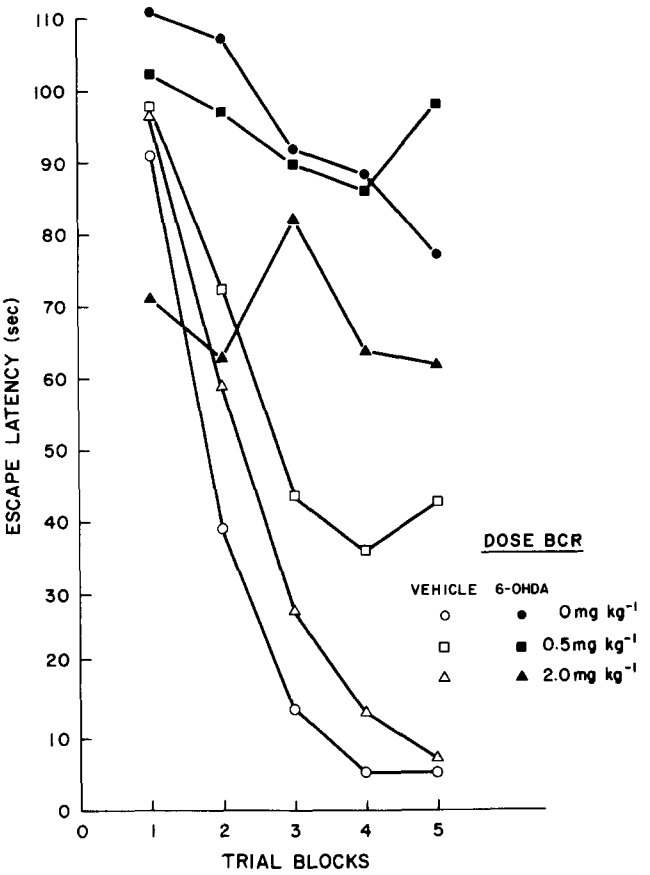

FIG. 6. Escape latency in a shuttle box at 28 days. Vehicle pups improve performance over trials whether receiving BCR or not, but all 6-OHDA groups demonstrate impaired performance.

Brain Catecholamine Concentrations

Concentrations of brain dopamine and norepinephrine at 33 days of age (three days after the last dose of BCR) were not influenced by BCR. Thus pups treated with 6-OHDA at 5 days of age demonstrated significant reductions in brain DA to concentrations 10–20% of vehicle controls. NE was not significantly affected. There were no differences in concentrations for pups receiving 0, 0.5 or 2.0 mg/kg BCR.

DISCUSSION

In these experiments, BCR at both the low and higher dosage increased general motor activity in normal pups at 13 and 19 days but had little effect on more mature animals. It appeared that this increase in activity was directly related to a disruption in the rate of habituation of activity over the hour long period. For example, control pups not given BCR exhibited a sharp decline in activity over the test period but this effect was abolished by BCR. However, pups treated with 6-OHDA exhibited such high baseline levels of activity without BCR that the fact that only a marginal increase in activity was observed in 19 and 26 day old animals might have been due to a ceiling effect. Stereotyped activity, particularly at 19 days, was increased by BCR in 6-OHDA pups but not in control animals, an effect consonant with the notion of denervation supersensitivity. In control animals, escape learning in the T-maze at 20 days was disrupted by both the low and higher dose of BCR and escape learning in the shuttle box at 28 days was disrupted by the low but not by the higher dose.

In just one parameter, that of head dips in a hole box at 28 days (a paradigm which has some similarities to exploratory motor activity), did BCR exhibit the desired effect of reduction in activity although the mechanisms responsible for this BCR induced reduction in activity are difficult to explain. BCR might induce a shift in activity from exploratory to continuous stereotypies. This is not likely since at 26 days, a period most comparable to the age at which exploratory activity was examined, stereotyped sniffing was reduced by BCR, and general motor activity, which to a large extent incorporates stereotypies in its measurement, was not markedly affected by BCR at this age either. Thus the BCR induced reduction in exploratory activity does not seem to be simply an artifact of the measurement technique. Still another possible explanation for the apparently opposite effects induced by BCR on exploratory compared to the other activities measured may be found in the complex nature of the pharmacologic actions of BCR. Thus, it is reasonable to believe that the actions of BCR reflect more than simply direct stimulation of post-synaptic striatal DA receptors. For example, pretreatment with alpha-methylparatyrosine or reserpine inhibits the BCR induced circling in rats, results which suggest a presynaptic component to the actions of BCR [4]. Furthermore, recent evidence now indicates the existence of not only postsynaptic (D2) DA receptors but presynaptic (D3) receptors as well [19]. First proposed by Cools and Van Rossum [3], investigators in a number of laboratories have now shown that presynaptic DA receptors may be activated by low doses of the DA agonist, apomorphine, while higher doses of this agent stimulate postsynaptic receptors [18]. Costall *et al.* [7] have shown that stimulation of the postsynaptic D2 receptor within the mesolimbic DA pathway of the adult rat results in increased motor activity while stimulation of the presynaptic D3 receptor in this same pathway results in decreased activity. In our DMI/6-OHDA model, presynaptic D3 receptors are reduced by 50% while postsynaptic D2 receptors are increased 10–20% (Watanabe, Seeman, and Shaywitz, in preparation). The effects of BCR may prove to be a resultant of stimulation of both postsynaptic (excitatory) and presynaptic (inhibitory) DA receptors, making interpretation of the relative contribution of each difficult to discern. The recent development of pharmacological agents with agonist properties specifically directed towards presynaptic D3 sites [13] may provide an opportunity to more critically examine the role of these receptors on the development of motor activity.

Still another mechanism that might be invoked to explain why BCR reduced exploratory activity in a hole box but increased general motor activity would posit a role for BCR on other central monoaminergic systems. Thus, BCR may influence NE or serotonergic systems [5,27]. More recently Markstein *et al.* [17] have demonstrated that BCR increases turnover of NE in the brain stem and serotonin in cerebral cortex, while decreasing turnover of DA in the striatum and limbic pathways. Such results support the earlier studies of Dolphin *et al.* [8] which demonstrated that the inhibition of activity noted immediately after the administration of large doses of BCR was correlated with an increase in NE turnover in whole brain.

Our results in the T-maze suggest that BCR might reduce escape latency in 6-OHDA pups although this response was

not noted one week later in the shuttle box task. This finding, together with the reduction in hole box activity discussed above provide some support for the use of this agent in the clinical disorder of ADD in children. However, the disruption in escape and avoidance learning in normal pups suggests that any such clinical trial may have potential harmful consequences and should be undertaken cautiously.

REFERENCES

1. Anderson, G. M., D. K. Batter, J. G. Young, B. A. Shaywitz and D. J. Cohen. A simplified liquid chromatographic electrochemical determination of norepinephrine and dopamine in rat brain. *J. Chromat.* **181:** 453, 1980.
2. Bruning, J. L. and B. L. Kintz. *Computational Handbook of Statistics,* Second Ed. Glenview, IL: Scott, Foresman, 1977.
3. Cools, A. R. and J. M. VanRossum. Excitation-mediating and inhibition-mediating dopamine-receptors: A new concept towards a better understanding of electrophysiological, biochemical, pharmacological, functional and clinical data. *Psychopharmacologia* **45:** 243–254, 1976.
4. Corrodi, H., K. Fuxe, T. Hökfelt, P. Lidbrink and U. Ungerstedt. Effects of ergot drugs on central neurons: Evidence for a stimulation of central dopamine neurons. *J. Pharm. Pharmac.* **25:** 409, 1973.
5. Corrodi, H., L. O. Garnebo, K. Fuxe and B. Hamberger. Effect of ergot drugs on central 5-hydroxytryptamine neurons: Evidence for 5-hydroxytryptamine release or 5-hydroxytryptamine receptor stimulation. *Eur. J. Pharmac.* **30:** 172, 1975.
6. Costall, B., D. C. Marsden, R. J. Naylor and C. J. Pycock. Stereotyped behavior patterns and hyperactivity induced by amphetamine and apomorphine after discrete 6-hydroxydopamine lesions of extrapyramidal and mesolimbic nuclei. *Brain Res.* **123:** 89, 1977.
7. Costall, B., D. H. Fortune, S. G. Hui and R. J. Naylor. Neuroleptic antagonism of the motor inhibitory effects of apomorphine within the nucleus accumbens: Drug interaction at presynaptic receptors? *Eur. J. Pharmac.* **63:** 347, 1980.
8. Dolphin, A. C., P. Jenner, M. C. B. Sawaya, C. D. Marsden and B. Testa. The effect of bromocriptine on locomotor activity and cerebral catecholamines in rodents. *J. Pharm. Pharmac.* **29:** 727, 1977.
9. Dunn, O. J. Multiple comparisons among means. *J. Am. statist. Ass.* **56:** 52, 1961.
10. Eastgate, S. M., J. J. Wright and J. S. Werry. Behavioral effects of methylphenidate in 6-hydroxydopamine-treated neonatal rats. *Psychopharmacology* **58:** 157, 1978.
11. 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, 1979.
12. File, S. E. Are central cholinergic paths involved in habituation of exploration and distraction: *Pharmac. Biochem. Behav.* **4:** 695, 1976.
13. Goodale, D. B., D. B. Rusterholz, J. P. Long, J. R. Flynn, B. Walsh, J. G. Cannon and T. Lee. Neurochemical and behavioral evidence for a selective presynaptic dopamine receptor agonist. *Science* **210:** 1141, 1980.
14. Heffner, T., F. Miller, C. Kotake, A. Heller and L. Seiden. Transient of permanent hyperactivity following neonatal 6-hydroxydopamine: A function of brain dopamine depletion. *Soc. Neurosci. Abstr.* **6:** 108, 1980.
15. Keppel, G. *Design and Analysis: A Researcher's Handbook.* Englewood Cliffs, NJ: Prentice-Hall, 1973.
16. Lipton, S. V., J. P. McGough and B. A. Shaywitz. Effects of apomorphine on escape performance and activity in developing rat pups treated with 6-hydroxydopamine (6-OHDA). *Pharmac. Biochem. Behav.* **13:** 371, 1980.
17. Markstein, R., P. L. Herrling, H. R. Burki, H. Asper and W. Ruch. The effect of bromocriptine on rat striatal adenylate cyclase and rat brain monoamine metabolism. *J. Neurochem.* **31:** 1163, 1978.
18. Roth, R. H., P. M. Salzman and M. C. Nowycky. Impulse flow and short-term regulation of transmitter biosynthesis in central catecholaminergic neurons. In: *Psychopharmacology: A Generation in Progress,* edited by M. A. Lipton, A. DiMascio and K. F. Killam. New York: Raven Press, 1978.
19. Seeman, P. Brain dopamine receptors. *Pharmac. Rev.* **32,** 1980, in press.
20. 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, 1976.
21. Shaywitz, B. A., R. D. Yager, J. H. Klopper and J. W. Gordon. Paradoxical response to amphetamine in developing rats treated with 6-hydroxydopamine. *Nature* **261:** 153, 1976.
22. Shaywitz, B. A., J. W. Gordon, J. H. Klopper and D. Zelterman. The effect of 6-hydroxydopamine on habituation of activity in the developing rat pup. *Pharmac. Biochem. Behav.* **6:** 391, 1977.
23. Shaywitz, B. A., J. H. Klopper and J. W. Gordon. Methylphenidate in 6-hydroxydopamine treated developing rat pups. *Archs Neurol.* **35:** 463, 1978.
24. Shaywitz, B. A. and D. E. Pearson. Effects of phenobarbital on activity and learning in 6-hydroxydopamine treated rat pups. *Pharmac. Biochem. Behav.* **9:** 173, 1978.
25. Shaywitz, S. E., D. J. Cohen and B. A. Shaywitz. The biochemical basis of minimal brain dysfunction. *J. Pediat.* **92:** 179, 1978.
26. Shaywitz, S. E., B. A. Shaywitz, D. J. Cohen and J. G. Young. Monoaminergic mechanisms in hyperactivity. In: *Behavioral Syndromes of Brain Dysfunction in Childhood,* edited by M. Rutter. New York: Guilford Press, 1980, in press.
27. Snider, S. R., C. Hutt, B. Stein, A. L. N. Prasad and S. Fahn. Correlation of behavioural inhibition or excitation produced by bromocriptine with changes in brain catecholamine turnover. *J. Pharm. Pharmac.* **28:** 563, 1976.
28. Sorenson, C. A., J. S. Vayer and C. S. Goldberg. Amphetamine reduction of motor activity in rats after neonatal administration of 6-hydroxydopamine. *Biol. Psychiat.* **12:** 133, 1977.
29. 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 system in rat pups. *Psychopharmacology* **57:** 163, 1978.