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Effects of 6-hydroxydopamine and 6-hydroxydopa on Development of Behavior[1]

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MORGAN, D. N., J. H. McLEAN AND R. M. KOSTRZEWA. *Effects of 6-hydroxydopamine and 6-hydroxydopa on development of behavior.* PHARMAC. BIOCHEM. BEHAV. 11(3) 309–312, 1979.—Rats treated at birth with 6-hydroxydopamine (6-OHDA) (60 μg/g, IP) or 6-hydroxydopa (6-OHDOPA X2) (60 μg/g, IP at birth and 48 hr later) exhibited increases in general activity throughout the initial 5 weeks after birth, with peak activity occurring around 20 days postnatally. Activity changes in the 6-OHDOPA×2 group appeared to be due to increased exploratory behavior (ambulation, climbing, rearing, sniffing), while the 6-OHDA changes appeared to be due to the increased self-directed behavior (eating, grooming, scratching). Despite these behavioral differences there was no obvious difference between treated groups in norepinephrine (NE) levels in the various brain regions, i.e., all treatments resulted in a reduction in neocortical and hippocampal NE and an elevation in cerebellar NE. These findings suggest that noradrenergic neurons may be altered to different degrees by each agent in more discrete brain regions than were tested, or that other neurotransmitter systems may be more selectively altered by either of the drug treatments. Because striatal dopamine was unaltered in any of the groups, however, there is reason to question a previously suggested link between minimal brain dysfunction (MBD) and dopamine depletion in the neonatal brain.

6-Hydroxydopamine Development Hyperactivity Catecholamine 6-Hydroxydopa Norepinephrine Neonatal activity

NEONATAL injections of 6-hydroxydopamine (6-OHDA) have been shown to alter the development of central catecholaminergic neurons by causing destruction of many immature pathways [10]. Neonates treated with 6-OHDA showed regional changes in norepinephrine (NE) levels throughout the brain. While hypothalamic levels of NE were unaltered, cortical levels were reduced and brainstem levels of NE were elevated [16]. The resulting behavioral effects of neonatal 6-OHDA treatment were subtle, with few or no apparent gross changes; in fact, several studies have demonstrated marked neurochemical alterations but have failed to detect changes in behavior [6, 15, 16]. Other studies have reported decreased exploratory activity and increased autonomic activity [14], and reduced foot shock-induced suppression of water licking [18]. Further, Shaywitz, Yager and Klopper [19] found that 6-OHDA-treated neonates, pretreated with desmethylimipramine (DMI), showed marked increase in activity levels, peaking at Day 20, and then declining towards control in the next one to two weeks. In addition, treated animals exhibited learning deficits in a shuttle box avoidance task. These behavioral alterations led the authors to propose their treatment serve as an animal model for minimal brain dysfunction (MBD) in children. Because of the relatively selective depletion of brain dopamine (DA) with their dosage regimen of 6-OHDA, it was felt that brain DA depletion was responsible for this behavioral pattern.

A similar animal model was produced when 6-hydroxydopa (6-OHDOPA) was administered to neonatal rats [12]. These animals, when tested in adulthood, exhibited less shock-induced aggression, impaired performance on a passive avoidance task, and increased general activity. While DA was unaltered, depletion of NE was found in the cortex, hippocampus, and spinal cord, and elevation of NE was shown in the brainstem and cerebellum. Since the neonatal 6-OHDOPA treatment resulted in activity increases in adulthood, one might also predict increased activity in immature animals.

METHOD

Animals

Litters of Sprague-Dawley albino rats were treated within four hours of birth with either the diluent saline (0.85%)—ascorbic acid (0.1%), 6-OHDA (60 μg/g, IP, free base) (Regis Chemical Co., Chicago, IL), or 6-OHDOPA (60 μg/g, IP, free base) (Regis Chemical Co., Chicago, IL). Half of the

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6-OHDOPA litters received only the Day 1 injection (6-OHDOPAxl) and the other half received a second identical injection 48 hr later (6-OHDOPAx2). Each group contained four litters of approximately equal numbers of males and females, ranging in size from six to nine pups. Animals were housed in a reversed light-dark cycle (on at 2200 hr, off at 0800 hr) and were weaned at 25 days of age by removing the mother from the group cage.

Procedure

Behavioral observations. Starting on Day 5 after birth and continuing through Day 35, 11 activity sessions, separated by three-day intervals, were held for each litter of rat pups. Each session was held between 1230 and 1630 hr in a fully lighted and quiet environment. Nine 2½-gallon aquaria were arranged on a shelf so they could be scanned by an observer, with one rat pup per aquarium. The floor of each aquarium was covered with a thin layer of sawdust. Also, one to two food pellets were present in each aquarium. Observing one litter at a time, each subject was scanned at the beginning of every minute during the 15-min session, with the observer recording the on-going behavior. Animals were scored on nine mutually exclusive categories: sleeping, inactivity (standing or sitting), ambulating, climbing, rearing, eating, sniffing, grooming, and scratching. Activity responses (which included all behaviors except sleeping and inactivity) were counted across trials for a general activity analysis. These seven active behaviors were further divided into two mutually exclusive categories: exploratory and self-directed behaviors. The exploratory responses included ambulating, climbing, rearing, sniffing, and the self-directed behaviors, eating, grooming, and scratching. All sessions were run blind by the same observer.

Tissue sampling and biochemical analysis. All animals were sacrificed by decapitation one week after the last behavioral session (six weeks of age) and the brains were dissected immediately into cerebellum, anterior cortex, hippocampus, hypothalamus, and striatum. Right cardiac atria and ventricles were removed from additional similarly treated animals for analysis of peripheral effects at 14, 21, and 28 days of age. Tissues were immediately frozen on dry ice and stored at −50°C. Later analysis for catecholamine content was done using the hydroxyindole fluorometric method of Hogans [7,17]. Briefly, tissues were homogenized in acidified butanol, extracted into phosphate buffer, and oxidized with iodine. NE content was determined at 385/485 nm, activation/emission wavelengths (uncorrected), and DA at 320/380 nm on an Aminco-Bowman Spectrophotofluorometer.

Statistical analysis. Behavioral data were analyzed using Analysis of Variance (ANOVA), with a two-factor mixed design with repeated measures on one factor. Animals were randomly deleted to provide equal N's for each group. Dunnett's t-test [20] was used to compare treatment groups with the control group for each day, as well as for each category of behavior. Dunnett's t-test was also used in the biochemical analysis.

RESULTS

Behavioral Analysis

Groups of rats treated with 6-OHDA or 6-OHDOPA did not differ from the control group in terms of mortality or alterations in body weight. The mean number of active responses during the 11 15-min sessions is shown in Fig. 1A. Sources of variance for groups, $F(3,100)=4.51$; trials, $F(10,1000)=38.81$; and groups × trials interaction, $F(30,1000)=2.95$, were all significant ($p<0.01$). Post-ANOVA Dunnett's t-test showed 6-OHDA, $t(4,100)=3.24$, and 6-OHDOPAx2, $t(4,100)=2.93$, groups to be more active overall than controls ($p<0.01$). The effect of 6-OHDOPAxl treatment was not significantly different from control for any of the behavioral measures and is therefore not included in the figures. Tests of significance comparing each experimental group to the control group were run at each three-day interval and revealed the 6-OHDA group to be significantly lower in activity than the control group at Day 5 and significantly higher at Days 14, 17, 20, 23, 26, 29, and 32 ($p<0.01$). The 6-OHDOPAx2 animals were significantly more active than controls on Day 5 ($p<0.05$), and on Days 8, 23, 29 and 32 ($p<0.01$).

The two categories of active behaviors, exploratory behaviors (ambulating, climbing, rearing, sniffing), and self-directed behaviors (eating, grooming, scratching) are shown in Figs. 1B and 1C. In both categories, the ANOVA was significant for groups, $F(3,100)=2.89$, $p<0.05$ and $F(3,100)=10.39$, $p<0.01$, respectively; trials, $F(10,1000)=56.21$, $p<0.01$ and $F(10,1000)=17.06$, $p<0.01$, respectively; and interaction effects, $F(30,1000)=1.60$, $p<0.05$ and $F(30,3000)=3.27$, $p<0.01$, respectively. The exploratory behavior post-ANOVA Dunnett's t-test showed no significant effects for 6-OHDA when compared to saline; however, the 6-OHDOPAx2 group was significantly higher than controls in exploratory behavior, $t(4,1000)=2.64$, $p<0.05$. When comparing treatment groups to the control group at each three-day interval, activity of the 6-OHDA subjects was found to be significantly lower than controls on Day 5 ($p<0.01$) and significantly higher than controls on Day 14 ($p<0.05$). On no other days was activity of the 6-OHDA group different from that of controls. However, activity of the 6-OHDOPAx2 group was significantly higher than controls on Days 5, 8, 32 ($p<0.05$), and 23 ($p<0.01$).

The self-directed behavior's post-ANOVA Dunnett's t-test showed significantly higher responses than controls for both 6-OHDA and 6-OHDOPAx2, $t(4,100)=2.82$, $p<0.01$ and $t(4,100)=4.48$, $p<0.01$, respectively. When compared across days, the self-directed behavior of 6-OHDA-treated animals was significantly higher than controls on Day 17, 20, 23, 32, 35 ($p<0.05$), and on Day 29 ($p<0.01$), with an obvious peak in activity around Day 20. Self-directed behavior of the 6-OHDOPAx2 group was higher than controls only at Day 8 ($p<0.05$).

Biochemical Analysis

Treatment of neonatal rats with either 6-OHDA or 6-OHDOPA resulted in marked alterations of NE content of the brain when animals were sacrificed at six weeks of age (Fig. 2). In the neocortex, NE levels were reduced approximately 40% in the 6-OHDA and 6-OHDOPAxl groups, and 72% in the 6-OHDOPAx2 group. Also, in the hippocampus, treatment with 6-OHDOPA or 6-OHDA resulted in a 65 to 88% respective reduction in NE levels. However, in agreement with previous findings, cerebellar content of NE was elevated approximately 55% after 6-OHDA and 6-OHDOPAx2 treatment and by 104% after a single neonatal 6-OHDOPA treatment. Neither hypothalamic NE nor striatal DA was altered by any of the treatments.

Peripheral effects of the neurotoxic agents on sympathe-

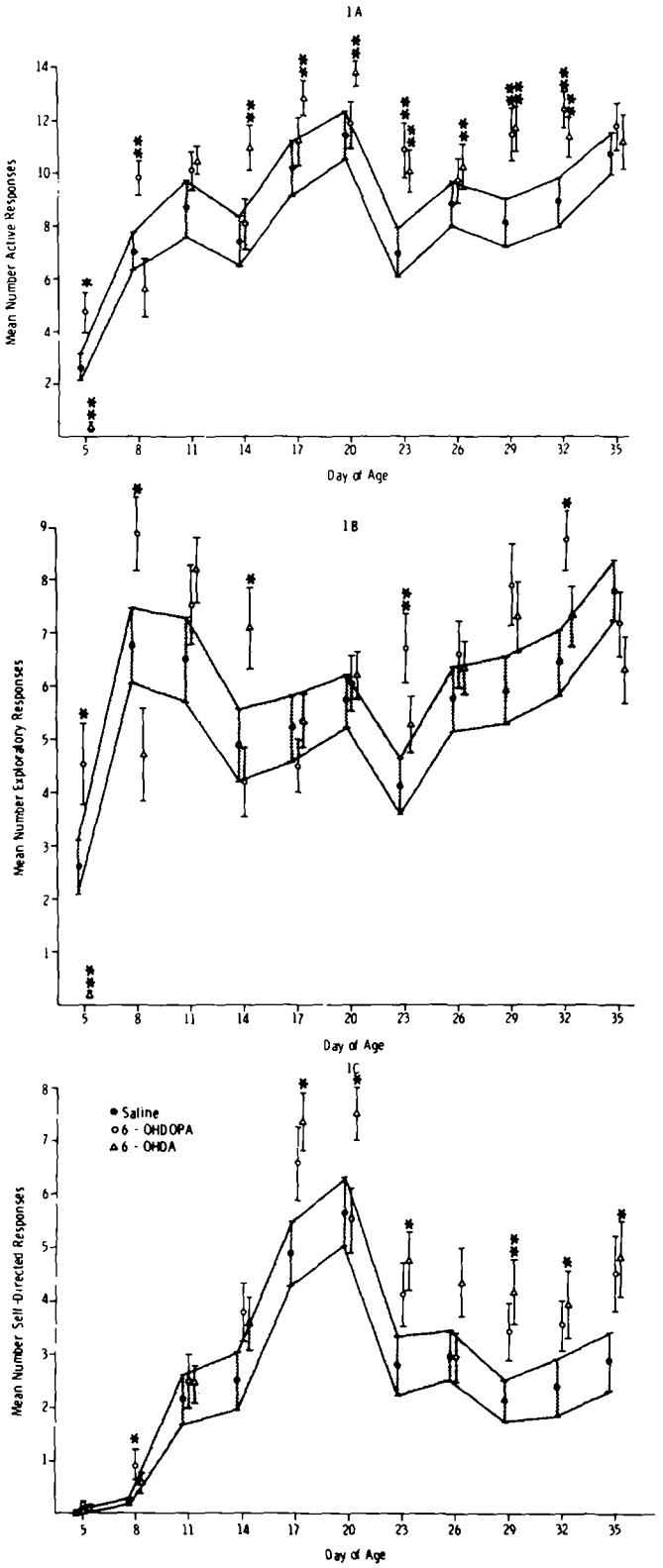

FIG. 1A. Mean (± 1 SEM) of active responses per 15 min session for animals treated neonatally with saline, 6-OHDA (60 μg/g, IP) or 6-OHDOPA×2 (60 μg/g, IP) (N=26 per group). One SEM above and below the saline group is shaded to emphasize that the comparisons of interest are each treated group vs control. *Level of significance compared to control, $p<0.05$; **$p<0.01$. (1B) Mean (± 1 SEM) number of exploratory responses. (1C) Mean (± 1 SEM) number of self-directed responses.

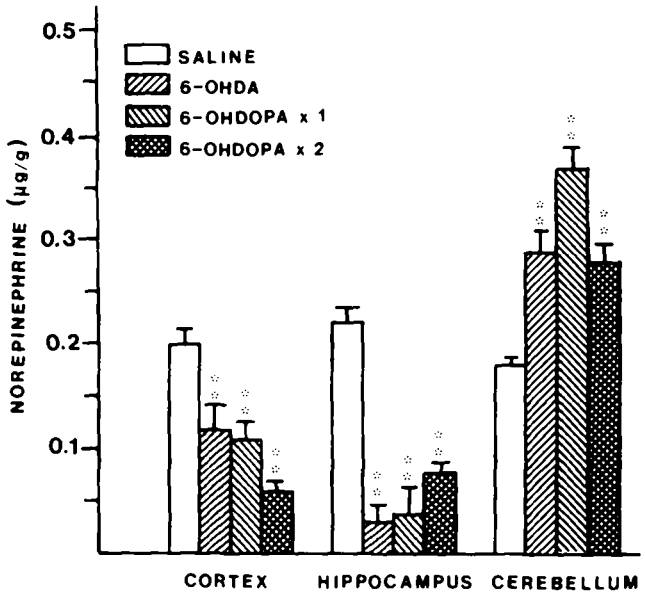

FIG. 2. NE content of neocortex, hippocampus, and cerebellum of rats treated neonatally with saline, 6-OHDA, 6-OHDOPAxl, or 6-OHDOPAx2 (all 60 μg/g, IP) and sacrificed at six weeks of age. Each column represents the mean ± 1 SEM of seven or eight animals. *$p<0.05$; **$p<0.01$.

tic neurons were determined by assay of NE content of the heart. It was found that 6-OHDA decreased atrial NE at two weeks by 35%, while all treatments reduced cardiac ventrical NE content by 44 to 76% at two weeks. At Days 21 and 28, NE levels were still reduced in the ventricle of 6-OHDA and 6-OHDOPAx2 groups, respectively. Atrials levels were reduced only in the 6-OHDOPA group at 28 days.

DISCUSSION

Examination of the behavior of all groups pointed out several interesting findings. Both 6-OHDA and 6-OHDOPA increased general activity in neonatally treated rats; however, there are some subtle differences in these two agents. When general activity measures were broken down into exploratory-vs-self-directed behaviors, it became evident that the increased activity of the 6-OHDA-treated animals was due primarily to self-directed behavior increases, with only slight increases in exploratory behavior; whereas, the activity increases of the 6-OHDOPA-treated animals were due almost exclusively to increases in exploratory behavior. Further examination of the data revealed the previously reported [13] peak of general activity around Days 15–20. Whereas the previous reports of a peak in arousal were based on locomotor behavior as measured in stabilimeter cages [13], the increase in the present study was evident only in the self-directed activity data. The marked inactivity on Day 5 for the 6-OHDA group has been reported previously [15,19] and is apparently a characteristic of this treatment.

The neurochemical findings of this study are in agreement with previous work done with 6-OHDA and 6-OHDOPA. Both these agents, when given neonatally, produced elevated NE in the cerebellum, reduced NE in the cortex and hippocampus, and produced no change in NE levels in the

hypothalamus [9, 12, 21]. Resultant peripheral effects, a depletion of NE in the early stages after treatment with a recovery pattern emerging as the rat matures, are also consistent with previous findings [11, 16, 21].

It is interesting that anatomical lesions of the cortex and hippocampus have been reported to produce hyperactivity [1]. It has been suggested that various forebrain structures such as the cortex and hippocampus serve as inhibitory or modulatory centers over brainstem reticular regions which are excitatory [3,4]. Hence destruction of the cortex or hippocampus would result in disinhibition of the more caudal excitatory areas and a more active organism would be produced. While it has been suggested that the forebrain inhibitory control over the reticular formation is cholinergic [2], projections to the forebrain from the locus coeruleus are noradrenergic. Thus, destruction of these NE nerve terminals by 6-OHDA or 6-OHDOPA results in loss of forebrain control of brainstem excitation and the net effect is increased activity. While Campbell and his associates have apparently abandoned their notion that activity fluctuations during development reflect the caudal to rostral maturation of the brain [5], the present data are not inconsistent with this position.

The present neurochemical findings, however, cannot account for the differential effects on activity by 6-OHDA and 6-OHDOPA, as these agents produced similar NE alterations. This implies that if NE were responsible for the differences in self-directed and exploratory behavior, it must have been in brain areas not assayed in this study. Or alternately, perhaps neurotransmitters other than NE were selectively altered by these agents. While other neurotransmitters cannot be ruled out, previous research has shown that serotonin levels are unaltered in these same brain regions by neonatal 6-OHDOPA treatment [6]. And finally, since striatal DA was unaltered by either 6-OHDA or 6-OHDOPA, one must question the suggestion that early hyperactivity, and hence MBD, is due to a functional deficiency in brain DA [19]

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