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Effects of Apomorphine on Escape Performance and Activity in Developing Rat Pups Treated with 6-Hydroxydopamine (6-OHDA)[1]

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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(3) 371–377, 1980.—The effects of apomorphine and escape learning were examined in normal developing rat pups and littermates preferentially depleted of brain dopamine by the intracisternal administration of 6-hydroxydopamine (6-OHDA) at 5 days of age, a treatment which resulted in a rapid and permanent reduction in brain dopamine to concentrations 12–29% of littermate controls while norepinephrine was not significantly altered. At 19 days of age both 0.1 and 1.0 mg/kg doses of apomorphine increased general motor activity in normal but not 6-OHDA treated pups (though these pups were significantly hyperactive prior to apomorphine). At 26 days only the 1.0 mg/kg dose increased motor activity in both normal and 6-OHDA pups. Exploratory activity at 30 days in both normal and 6-OHDA pups was first reduced then abolished by progressive doses of apomorphine. Stereotyped activity was increased by 0.1 and 1.0 mg/kg apomorphine at 19 days in both normal and 6-OHDA pups. By 26 days, apomorphine no longer produced intense stereotypies in normal pups, but did effect such responses in 6-OHDA treated animals. Administration of apomorphine resulted in a disruption of escape performance in a T-maze and shuttle box in normal pups only at 1.0 mg/kg but disrupted performance in 6-OHDA treated animals at both 0.1 and 1.0 mg/kg dosages. These results indicate a peak effect of apomorphine on general motor activity at three weeks of age in normal pups. Our results also suggest that apomorphine will disrupt escape learning, effects that appear to be correlated with the apomorphine induced increase in motor activity.

Apomorphine Escape performance Activity 6-Hydroxydopamine

EVIDENCE from several lines of investigation supports the notion that the period of intense hyperexcitability observed in the developing rat pup between two-three weeks of age is related to the development of central catecholaminergic mechanisms [2, 6, 7, 8, 11, 17, 21, 22, 23]. Administration of the neurotoxin 6-hydroxydopamine (6-OHDA) to neonatal rat pups has been employed by a number of investigators [13, 14, 26, 29, 30] to further delineate the role of brain catecholamines on behavioral arousal. Preferential reduction of brain dopamine may be accomplished by pretreating the 5 day old rat pup with desmethylimipramine (DMI) followed by the intracisternal administration of 6-OHDA, a treatment that results in the development of hyperactive motor behavior and significant impairment in T-maze and shuttle box avoidance performance.

Administration of amphetamine [27] and methylphenidate [28] to 6-OHDA treated rat pups results in a significant attenuation in hyperactivity and improvement on avoidance tasks, a finding which may be explained by invoking the concept of receptor supersensitivity. In normal rat pups, brain dopaminergic pathways act to inhibit facilitory noradrenergic influences on activity. When these inhibitory pathways are damaged by administration of 6-OHDA to the 5 day old rat pup, then the facilitory pathways predominate, and the observed effect is one of hyperactive motor behavior. The remaining dopaminergic receptors, though damaged, are supersensitive and as a result, administration of amphetamine and the subsequent release of catecholamines (both dopamine and norepinephrine) will produce a more pronounced effect on supersensitive dopamine receptors than on the less damaged but normally sensitive noradrenergic receptors. The net effect, then, is for dopaminergic pathways to be activated, thus, inhibiting the excitatory influences of the noradrenergic systems. This so called "dual

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transmitter hypothesis" has been discussed at length by Antelman and Caggiula in other contexts [1].

In order to further explore the role of central dopaminergic mechanisms in the development of behavioral arousal we have investigated the effects of the dopaminergic receptor agonist, apomorphine, on the activity and learning performance of normal developing rat pups and littermates treated with 6-OHDA. Specifically, two major questions are addressed: (1) The ontogeny of the behavioral response to apomorphine in control animals. (2) The development of receptor supersensitivity in 6-OHDA treated animals.

METHOD

Animals

Sprague-Dawley rat pups with mother were obtained from Charles River, Inc., Wilmington, MA at 24 hr (± 12 hr) of age and individually housed in clean plastic cages (30×32×10 cm) with sawdust bedding. Mothers and pups were housed under fluorescent lighting conditions (16 G.E. 40 W fluorescent bulbs) with 12 hours of light (lights 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. Weights were recorded at the time of intracisternal 6-OHDA administration and again at weekly intervals. 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. Pups were numbered at the time of intracisternal injection by toe punch and randomized as experimental or control according to a table of random numbers.

Experimental Groups

The animals were divided into six experimental groups and each litter was composed of four rat pups treated with 6-OHDA at 5 days of age and four vehicle treated littermates. Twelve litters were utilized yielding cell sizes of 14–16 animals per cell. An equal number of 6-OHDA and vehicles received either saline or apomorphine in doses of 0.1 or 1.0 mg/kg body weight. Each litter contained 1–2 pups in every experimental group. 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 (such as activity at 19 days, T-maze at 21 days etc.).

Pharmacological Depletion of Brain Catecholamines with Intracisternal 6-hydroxydopamine (6-OHDA)

The 6-hydroxydopamine HBr was purchased from Regis Chemical Company (Chicago, IL) and was used without further purification. It was prepared immediately prior to use in a 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. Intracisternal injections were administered by flexing the neck of the infant rat and injecting 100 μg (calculated as free-base) in 20 μl of solution via a precalibrated microsyringe (Hamilton) with a 27 ga needle inserted immediately beneath the occiput. (Petroleum jelly was applied to the neck prior to injection to minimize leakage). All pups received intracisternal injections. The experimental group received doses of 6-OHDA and littermate controls received the saline solution containing 0.4 mg/ml of ascorbic acid (vehicle).

Determination of Brain Catecholamines

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 1 minute after death. Frozen brains were stored at −70°C and biochemical determinations performed within 2–3 weeks of sacrifice. Dopamine and norepinephrine were analyzed by fluorometric techniques modified after procedures described previously by Roth and Stone [25] and Boadle-Biber and others [3].

Determination of Activity

Apparatus. The activity was recorded in a soundproof room 2.5×4 meters in size, illuminated by six 150 W infrared heat lamps (General Electric Co.) mounted on tracks 2 m high on each wall. Ambient temperature ranged between 25–27°C. Activity was recorded via a television camera (Panasonic, Model No. WV0261, equipped with a 12.6 mm F 1.4 lens) that was placed in the center of the room at a height of 2 m. The camera was coupled to an Hitachi time-lapse recorder (Model No. FV-512U), and a Vicon date/time display generator, (Model No. 240T), as well as the Setchel-Carlson monochrome video monitor (Model No. 6M-912). Plastic cages, each 20×50×15 cm deep with bottoms painted black with non-reflecting paint and each equipped with a water bottle and food pellets, were placed on the floor of the room so that the entire array of nine cages was viewed simultaneously on the video monitor. When the rat pups reached 2 weeks of age, a wire mesh covering was placed over the cages to prevent the animals from escaping.

Procedure. Activity was determined at 13, 19 and 26 days of age 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. Apomorphine (or saline) was administered subcutaneously and recording was begun immediately and continued for 1 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 of 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 min periods throughout the 60 min observation period. For example, we scored the animals' activity from 0–5 min, 10–15 min, 20–25 min 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.

Activity was always scored in a blind fashion and thus, activity for any animal was scored without knowledge of whether that animal was a treated or control. This was accomplished in several ways: (1) Randomizing the animals as they were placed in the boxes, (2) Scoring the rats by number but not breaking the code until the scoring was complete. Activity was scored by the same observer and repeated scoring yielded a reliability of activity measurements with an interobserver correlation of 0.9.

An animal was considered to exhibit activity if any

movement of any kind was observed. The duration of these movements was determined by activating an electric timer (Standard Electric Time, Model No. 11-2, Springfield, MA) at the onset of any movement, and stopping the timer when the movement ceased. The cumulative duration of movements for each 5 min interval was thus obtained, and the percentage of time that the animal was active during each observation period noted.

Stereotyped Activity

Stereotyped activity was coded using a scoring system modified after that described by Costall and associates [10]. 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

Exploratory activity was determined at 30 days of age utilizing a procedure described initially by File [15]. The apparatus employed was constructed from 1 cm thick plywood and was 45×45×45 cm high. The floor was raised 16 cm from the very bottom of the box and contained four equally spaced holes each 3 cm in diameter. The interior of the box was painted dull black. The box rested on a Plexiglas base and was illuminated from beneath the Plexiglas by two 40 W fluorescent light bulbs. One of four objects was placed beneath each hole. The experiments were performed 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 sec the pan was removed and observations begun each minute and continued for a 5 min 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.

Determination of Escape Performance

T-maze. Escape learning in a T-maze was determined at 21 days of age, 15 min after subcutaneous administration of apomorphine. The T-maze was constructed of opaque Plexiglas with a floor of stainless steel rods 1 mm in diameter. The main part of the T was 31 cm long and each cross piece was 16 cm long; all parts of the maze had an internal width of 9 cm and a height of 11 cm, and the 1 mm diameter stainless steel rods were separated by 5 mm. The rat was placed in the long arm of the T and the experiment initiated by raising the starting gate which activated an electromagnetic switch and thus, resulted in the conductance of a 2 mA current delivered via a shock generator/scrambler (BRS/LVE Model No. SGS-004). In order to escape the shock, the rat had to traverse the maze into a safe compartment. The safe compartment was on the side opposite to that the rat showed a preference for in a trial run. He was allowed 30 sec to complete this task and if unsuccessful, was manually placed in a safe compartment where he remained for a 30 sec period. 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 Instrumental 8RC01A, South Braintree, MS). A total of 20 trials was recorded for each rat. Results were coded and analyzed utilizing analysis of variance.

Shuttle box performance. Escape learning in a shuttle box was determined at 28 days of age. The shuttle box was constructed of opaque Plexiglas with a floor of stainless steel rods and consisted of two compartments separated by a 5 cm high hurdle, each compartment 20×14×17 cm high. The starting compartment was painted black while the goal compartment was painted white and was illuminated by a flashlight bulb. The experiment was initiated by placing the rat in the black painted compartment. A starting switch was activated which simultaneously sounded a bell for a 1 sec period. Five seconds later a 2.5 mA current was delivered via a shock generator/scrambler (BRS/LVE Model SGS-004, Tech Serv Inc., Beltsville MD), by way of stainless steel rods 2 mm in diameter and separated by 1.5 cm. The floor of the goal compartment was covered by a smooth Plexiglas sheet. When the animal crossed into the goal compartment, a light switch was activated, stopping the timer (Sigma Instruments, Model B8RC01A). The time required to avoid the shock (escape latency) was noted from the onset of the bell and a total of 20 trials performed with each animal. The results were coded and analyzed using analysis of variance.

RESULTS

General Motor Activity

Total motor activity was measured over the one hour observation period at 13, 19 and 26 days of age. Four way analysis of variance indicated significant main effects for age, $F(2,62)=18.8$, $p<0.001$, 6-OHDA treatment, $F(1,30)=10.9$, $p<0.005$, and apomorphine, $F(2,30)=25.2$, $p<0.001$. The interaction between age, 6-OHDA and apomorphine was significant as well, $F(4,62)=2.53$, $p<0.05$, and results are indicated in Fig. 1. No significant differences were observed in activity at Day 13, but Scheffe post hoc comparisons indicated that at 19 days of age apomorphine at both dosages increased activity in normal pups ($p<0.005$) but had no significant effect in 6-OHDA animals. At 26 days of age the low dose of apomorphine had no effect on either normal or 6-OHDA pups. However, the high dose increased activity in both normal ($p<0.005$) and 6-OHDA animals ($p<0.001$).

Exploratory Activity

Both the number of exploratory head dips, $F(5,179)=9.47$, $p<0.001$, and the duration of explorations, $F(5,179)=9.62$, $p<0.001$, were significantly different between groups, as shown in Fig. 2. Exploratory activity determined at 30 days of age, was almost completely abolished by the high dose of apomorphine in both normal and 6-OHDA animals. Both the number of explorations, as well as the duration of the exploratory behavior were first re-

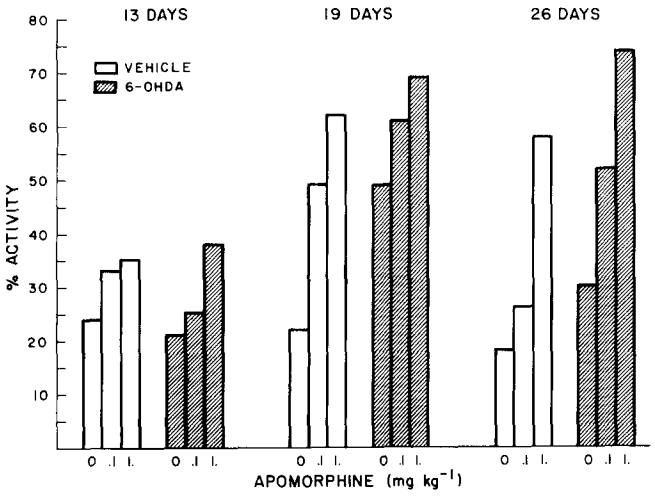

FIG. 1. Effects of apomorphine on general motor activity in normal and 6-OHDA pups. Apomorphine increased activity in normal but not 6-OHDA treated animals at 19 days. Dosages of 1.0 mg/kg increased activity in both normal and 6-OHDA pups at 26 days. Activity is represented as the percentage of the hour-long observation period that the pups exhibited activity.

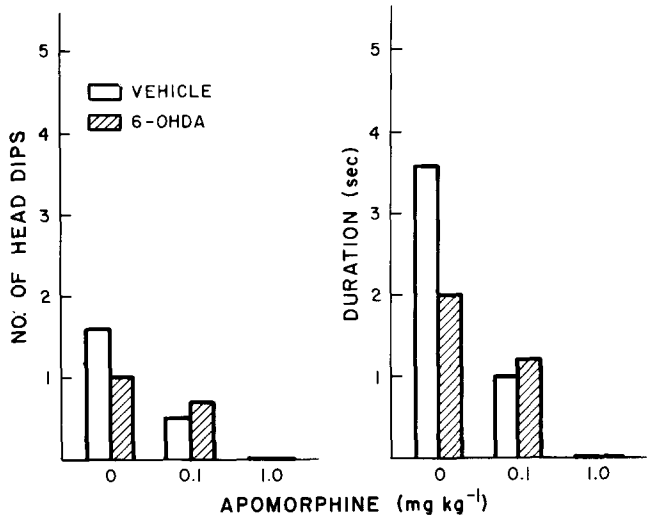

FIG. 2. Effects of apomorphine on exploratory activity in a hole box. Without apomorphine both vehicle and 6-OHDA pups demonstrated similar levels of exploratory activity. Administration of 0.1 mg/kg apomorphine reduced both the number of head dips over the five minute observation period as well as the total duration of head dips, while the higher dose (1.0 mg/kg) abolished exploratory activity in both normal and 6-OHDA pups.

duced by 2/3 then completely abolished by the high dose of apomorphine. The explorations per second were unaffected by the agent, averaging 0.46/second and 0.48/second in control and 6-OHDA without apomorphine and 0.51 and 0.56/second after apomorphine. These differences were not significantly changed from control values.

Stereotyped Activity

The effects of apomorphine on stereotyped activity are

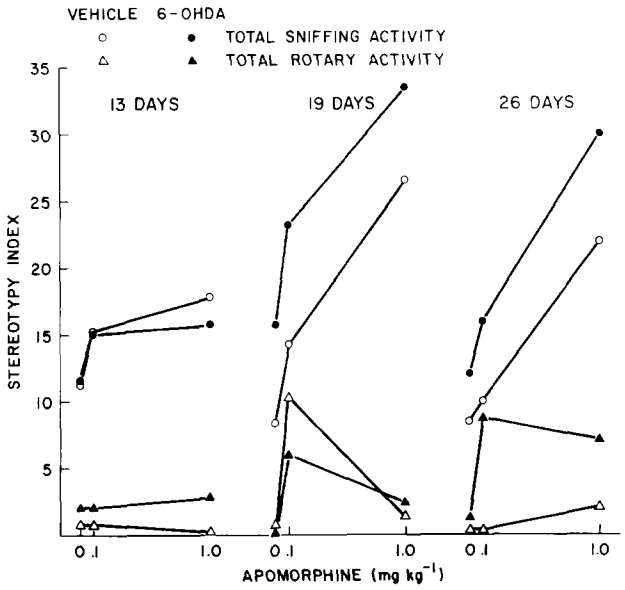

FIG. 3. Effects of apomorphine on stereotyped activity. The stereotypy index reflects both the duration and intensity of the activity. Sniffing was considerably more frequent than rotary activity. At 13 days, sniffing occurred at the same frequency in normal and 6-OHDA treated animals. Beginning at 19 days, apomorphine increased the frequency of sniffing in both normal and 6-OHDA pups, and the slope of the increase was similar. Rotary activity occurred much less frequently than did sniffing, and was not observed except in pups who had received apomorphine. At 19 days, low dosages increased rotary activity in both vehicle and 6-OHDA pups. However, by 26 days, 0.1 mg/kg as well as 1.0 mg/kg of apomorphine increase rotary activity in 6-OHDA pups compared to controls.

shown in Fig. 3. At 13 days no significant differences were observed between 6-OHDA and vehicles nor were there any significant effects due to apomorphine for either sniffing, $F(5,35)=1.31$, $p>0.1$, or for rotary activity, $F(5,38)=0.78$, $p>0.1$, though clearly sniffing was considerably more frequent than was rotary activity.

By 19 days of age significant differences were observed for both sniffing, $F(5,35)=4.89$, $p<0.01$, and rotary activity, $F(5,35)=2.82$, $p<0.05$. Apomorphine increased sniffing activity in a dose dependent fashion. Thus, in vehicle treated animals the low dose of apomorphine increased sniffing by 1.7 times while the higher dose increased this activity by greater than three fold. Comparable values for 6-OHDA treated animals were an increase of 1.5 times at the low dose and a two fold increase at the higher dose. Rotary activity was increased by more than 12 times by the 0.1 mg/kg dose of apomorphine in the vehicle animals while the higher dose resulted in less than a two fold increase. In 6-OHDA pups, the low dose increased rotary activity six fold, while the high dose increased rotary activity by a factor of two.

Both sniffing, $F(5,35)=3.07$, $p<0.01$, and rotary activity, $F(5,35)=3.12$, $p<0.01$, were significantly different at 26 days also. The administration of apomorphine to both vehicle and 6-OHDA pups at 26 days produced an almost identical dose dependent increase in sniffing activity as that observed at 19 days. However, the vehicle and 6-OHDA treated pups differed significantly in their development of rotary activity in response to apomorphine. Vehicle treated pups failed to ex-

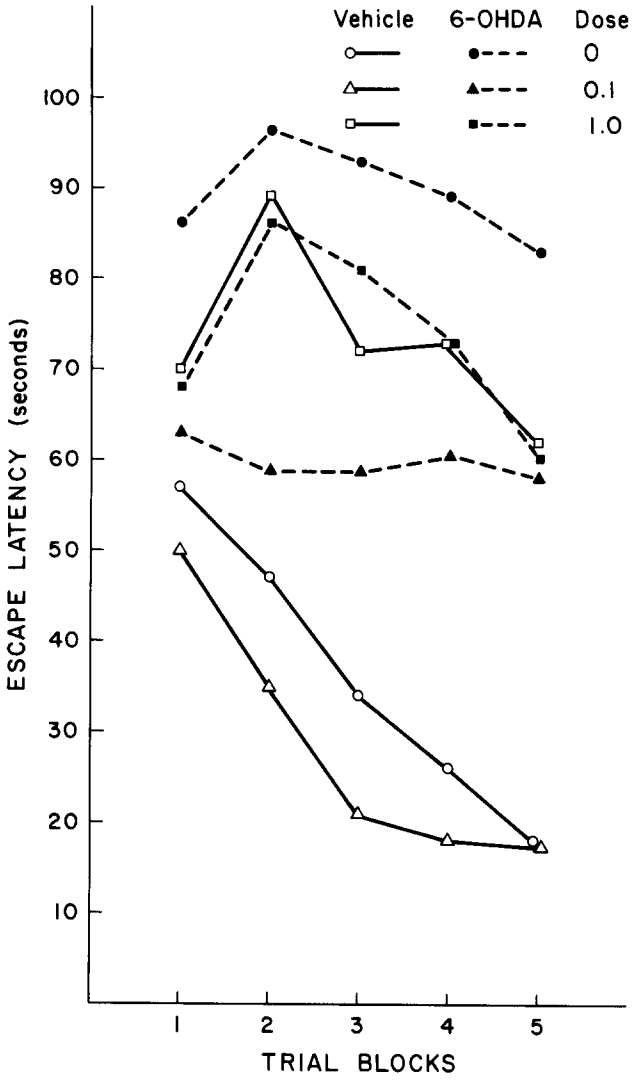

FIG. 4. Effects of apomorphine on avoidance performance in a T-maze at 21 days of age. Escape latency (seconds) is shown for the mean of blocks of 4 trials each. Vehicle treated pups not given apomorphine, or administered 0.1 mg/kg, demonstrated acquisition of the task over the 20 trial blocks. Vehicle pups given 1.0 mg/kg apomorphine and all 6-OHDA pups failed to acquire the task.

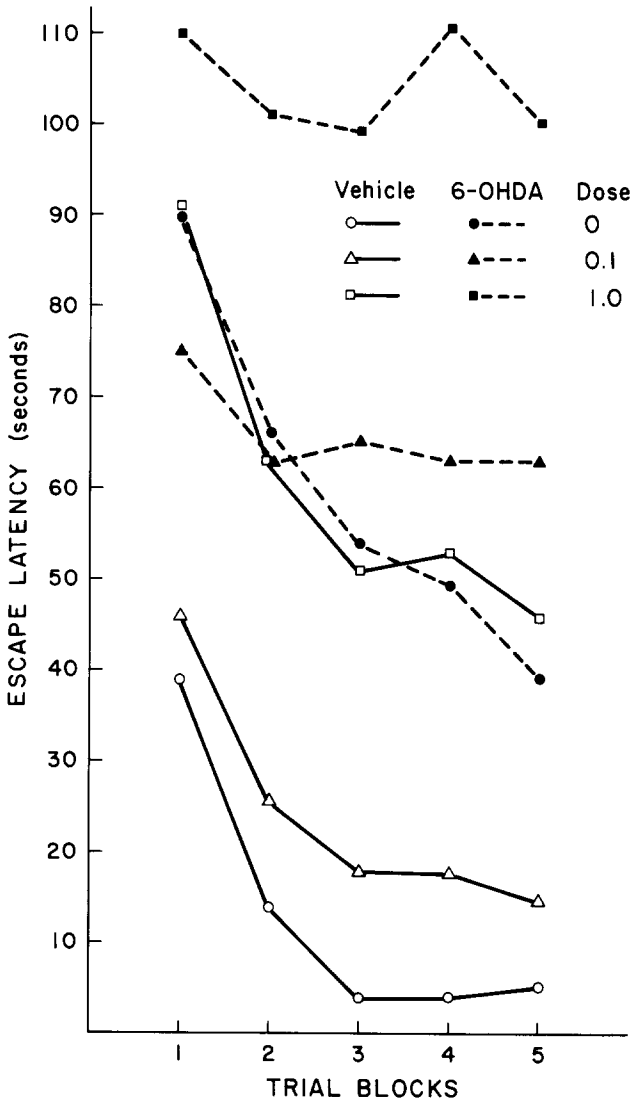

FIG. 5. Effects of apomorphine on shuttle box avoidance performance at 28 days of age. Results are mean escape latencies (seconds) for blocks of 4 trials. All vehicle pups exhibit a decrease in escape latency over trials, though 1.0 mg/kg apomorphine significantly prolongs escape latency. At this age 6-OHDA pups also acquire the task, but both 0.1 and 1.0 mg/kg apomorphine disrupted avoidance performance in 6-OHDA animals.

hibit any significant increase in rotary activity at this age, but 6-OHDA treated animals developed an almost seven fold increase in rotary activity on the low dose and a greater than five fold increase on the higher dose.

Escape Performance

Escape performance determined at 21 days in a T-maze demonstrated significant effects due to 6-OHDA, $F(1,46)=8.41$, $p<0.01$, and apomorphine, $F(2,46)=3.19$, $p<0.05$, and results over 5 blocks of 4 trials each are shown in Fig. 4. Normal pups administered the low dose of apomorphine or none at all exhibited similar learning patterns over the trial blocks. They required 50–60 sec during initial trials but rapidly learned to traverse the maze in less than 20 sec. In contrast normal pups given the high dose of apomorphine traversed the maze in 70 sec initially and never improved their rate over time. Scheffe post hoc comparisons indicate significant differences between low and high doses, ($p<0.025$). Pups treated with 6-OHDA did not learn the maze and this effect was not influenced by apomorphine.

Escape learning in a shuttle box at 28 days (Fig. 5) demonstrated significant effects from 6-OHDA, $F(1,42)=14.5$, $p<0.001$, and apomorphine, $F(2,42)=5.34$, $p<0.01$. Normal pups at this age required 40 sec in initial trials but rapidly learned to perform the task within 5 sec. Pups given low doses also learned rapidly. However, larger doses of apomorphine significantly impaired shuttle box performance compared to both non-treated ($p<0.005$) and low dose control pups ($p<0.05$). At this age the 6-OHDA pups

TABLE 1
BRAIN CATECHOLAMINE CONCENTRATIONS

	Apomorphine dose mg/kg	DA	NE
Vehicle	0	100	100
	0.1	99	94
	1.0	114	102
6-OHDA	0	24*	95
	0.1	12*	102
	1.0	29*	96

Concentrations expressed as percentage of control. For dopamine this averaged 761 ± 85.2 (mean ± SEM) and for norepinephrine 325 ± 24.9. Both values expressed as ng/g wet weight and were corrected for recovery. Each cell represents the mean of analysis of 6 animals.

*Significantly different from control, $p<0.001$.

demonstrated acquisition of learning, requiring 90 sec initially but 40 sec at the end of the task. However, this learning curve was disrupted with both low and high doses of apomorphine.

Catecholamine Concentrations

Concentrations of dopamine and norepinephrine in the vehicle treated group were not altered by apomorphine (Table 1). Brain dopamine concentrations in 6-OHDA treated pups were reduced by 76% but administration of apomorphine had no additional effect on these already depleted values. Norepinephrine concentrations were not altered by either 6-OHDA or apomorphine.

DISCUSSION

Our results indicate that in normal developing rat pups, apomorphine induced increases in general motor activity emerge by 19 days of age. At this time both the low and high dose resulted in an increase in activity, though a clear dose related effect was not observed. At 26 days, only the high dose resulted in an increase in activity. Stereotyped activity followed a similar pattern, with apomorphine producing an increased sniffing activity in a dose dependent fashion at 19 and 26 days of age. Rotary activity at 19 days was increased approximately six times more by the low dose than by the high dose; by 26 days apomorphine exerted little effect on this activity, however. Exploratory activity in a hole box at 30 days was reduced by the low dose and abolished by the higher dose.

In contrast, pups treated with 6-OHDA failed to demonstrate an increase in general motor activity after apomorphine until 26 days, and then only to the higher dose. However, the already increased activity levels of 6-OHDA animals at 19 days could have produced a ceiling effect that would tend to mask any increases in activity that might result from apomorphine administration.

Increases in stereotyped activity were observed as early as 19 days in 6-OHDA pups, an effect manifest upon both sniffing and rotary activity. In contrast to normal pups, 6-OHDA animals continued to exhibit increases in rotary activity at 26 days as well. Again, like their normal littermates, exploratory activity in treated pups was reduced by apomorphine.

While some investigators [16, 17, 19] have observed effects of apomorphine as early as the first week of life, other studies suggest that responses are not observed until the second or third week of life [18,24].

Most recently, Reinstein and associates [24] examined the effects of apomorphine (1 and 10 mg/kg) during the first month of postnatal life. Using a time sample method of scoring, they observed the pups for two hours after injection of apomorphine. No effect was observed at 7 days. However, by 14 days apomorphine increased sniffing activity in a dose dependent fashion and this finding was observed at 3–5 weeks of age as well. Interestingly, general activity was not intensified until 3 weeks of age, and declined at 4 and 5 weeks.

In general, the portions of our study involving normal rat pups have confirmed and extended these observations although the doses used by us were lower by a factor of 10. Thus, we, like Reinstein *et al.* observed an apomorphine induced increase in general activity beginning at 3 weeks of age (19 days in our study). We did not observe a reduction in activity at 14 days, a phenomenon noted by Reinstein *et al.* only at the 10 mg/kg dose. Furthermore, like Reinstein, we also observed a peak effect of apomorphine at 19 days of age in normal rat pups. Slight, but not statistically significant increases in sniffing were observed by us at 13 days, and the increase was seen only at the high (1 mg/kg) dose.

Supersensitivity may be defined as a decrease in the amount of an agonist required to elicit a particular biological response and would be suggested by a differential response to apomorphine between the normal and 6-OHDA treated pups. Such an effect was not observed for either general motor activity or exploratory activity, but was evident for stereotypies at 26 days of age when small amounts of apomorphine (0.1 mg/kg) given to 6-OHDA pups produced an accentuation in sniffing activity, but had little effect in normal pups. This differential response was even more pronounced for rotary activity. Thus, at 26 days the low dose of apomorphine had little effect on rotary activity in normal pups but the same 0.1 mg/kg dose resulted in a six fold increase in rotary activity in pups treated with 6-OHDA.

Supersensitivity of the postjunctional type, characteristically develops gradually and slowly over a time course measured in weeks [20]. Furthermore, postjunctional supersensitivity lacks agonist specificity, and thus, one would expect to demonstrate supersensitivity with agents other than apomorphine. In a previous study [27] utilizng a different dopaminergic agonist, amphetamine, a reduction in activity was observed only in 6-OHDA treated pups and this was noted to occur during the fourth week of life. This result adds further support for the notion that the phenomenon of receptor supersensitivity after 6-OHDA in the developing rat pup requires more than 14 days to emerge. Furthermore, Creese and Iversen [12] have noted supersensitivity to apomorphine in 90 day old rat pups who had been treated with intraventricular 6-OHDA during early neonatal life. However, their paradigm did not incorporate desmethylimipramine pretreatment prior to the intraventricular 6-OHDA injection, and thus, the animals were depleted of both dopamine and norepinephrine, rather than solely the depletion in brain dopamine effected in our procedure.

The effects of apomorphine on avoidance learning are complex. In adult rats, apomorphine can increase as well as decrease conditoned responding, and these effects can also be demonstrated using operant procedures involving either positive or negative reinforcement [4,5]. Furthermore,

apomorphine has been shown to possess both positive reinforcing and aversive properties in the same animals in the same test session [31]. In general, these effects have been interpreted as reflecting an apomorphine induced increase in motor behavior accompanied by a facilitation in the stereotyped execution of the learned response. An alternate hypothesis incorporates the notion of a role for brain dopamine in memory formation [9].

Our results indicate that in normal animals the high dose of apomorphine will significantly disrupt avoidance learning at both 21 and 28 days while in 6-OHDA pups both low and high dosages disrupt performance. This effect appears to be correlated with the apomorphine induced increase in motor activity observed at comparable ages. Such findings may be interpreted as suggesting that administration of apomorphine resulted in an increase in non-purposeful, non-goal directed activity, and this was subsequently reflected in an inability to traverse the maze, or quickly and correctly avoid the shock. Support for such a notion is found in the results of the exploratory activity test where low dosages of apomorphine reduced and high dosages abolished the exploratory activity.

REFERENCES

1. Antelman, S. M. and A. R. Caggiula. Norepinephrine-dopamine interactions and behavior. *Science* **195:** 646–653, 1977.
2. Bloom, F. E. The formation of synaptic junctions in developing rat brain. In: *Structure and Function of Synapses*, edited by G. D. Pappas and D. P. Purpura. Raven Press, New York, 1972.
3. Boadle-Biber, M., J. Highes and R. H. Roth. Acceleration of noradrenaline biosynthesis in the guinea pig vas deferens by potassium. *Br. J. Pharmac.* **40:** 702–718, 1970.
4. Broekkamp, C. L. E. and J. M. van Rossum. Effects of apomorphine on self-stimulation behavior. *Psychopharmacology* **34:** 71–80, 1974.
5. Butcher, L. L. Effects of apomorphine on free-operant avoidance behavior in the rat. *Eur. J. Pharmac.* **3:** 163–166, 1968.
6. Campbell, B. A., L. D. Lytle and H. C. Fibiger. Ontogeny of adrenergic arousal and cholinergic inhibitory mechanisms in the rat. *Science* **166:** 635–636, 1969.
7. Campbell, B. A. and P. D. Mabry. Ontogeny of behavioral arousal: A comparative study. *J. comp. Psychol.* **81:** 371–379, 1972.
8. Campbell, B. A. and P. D. Mabry. The role of catecholamines in behavioral arousal during ontogenesis. *Psychopharmacology* **31:** 253–264, 1973.
9. Colpaert, F. C., W. F. M. Van Bever and J. E. M. Leysen. Apomorphine: Chemistry, Pharmacology, Biochemistry. In: *International Revue of Neurobiology*, edited by C. C. Pfeiffer and J. R. Smythe. **19:** 1976, pp. 225–268.
10. 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:** 89–111, 1977.
11. Coyle, J. T. Biochemical aspects of the catecholaminergic neurons in the brain of the fetal and neonatal rat. In: *Dynamics of Degeneration and Growth in Neurons*, edited by K. Fuxe, L. Olson and Y. Zotternman. Oxford: Pergamon Press, 1974, pp. 425–434.
12. Creese, I. and S. D. Iversen. Blockage of amphetamine induced motor stimulation and stereotypy in the adult rat following neonatal treatment with 6-hydroxydopamine. *Brain Res.* **55:** 369–382, 1973.
13. Eastgate, S. M., J. J. Wright and J. S. Werry. Behavioral effects of methylphenidate in 6-hydroxydopamine-treated neonatal rats. *Psychopharmacology* **58:** 157–162, 1978.
14. 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:** 195–208, 1979.
15. File, S. E. Are central cholinergic paths involved in habituation of exploration and distraction? *Pharmac. Biochem. Behav.* **4:** 695–702, 1976.
16. Holmgren, B., R. Urba-Holmgren and M. Valdes. Spontaneous and amphetamine induced head-shaking in infant rats. *Pharmac. Biochem. Behav.* **5:** 23–28, 1976.
17. Kellogg, C. and P. Lundberg. Ontogenic variations in response to L-DOPA and monoamine receptor-stimulating agents. *Psychopharmacology* **23:** 187–200, 1972.
18. Lal, S. and T. Sourkes. Ontogeny of stereotyped behavior induced by apomorphine and amphetamine in the rat. *Archs int. Pharmacodyn.* **202:** 171–182, 1973.
19. Lal, S. and F. Feldmuller. Effect of amphetamine and apomorphine on brain monoamines and behaviour in the immature and young adult rat. *Archs int. Pharmacodyn. Ther.* **2:** 239–251, 1975.
20. Langer, S. Z. Denervation supersensitivity. In: *Handbook of Psychopharmacology, Vol. 2*, edited by L. L. Iversen, S. D. Iversen and S. H. Snyder, Principles of Receptor Research, New York: Plenum Press, 1975, pp. 245–280.
21. Loizou, L. A. and P. Salt. Regional changes in monoamines of the rat brain during postnatal development. *Brain Res.* **20:** 467–470, 1970.
22. Moorcroft, W. H., L. D. Lytle and B. A. Campbell. Ontogeny of starvation-induced behavioral arousal in the rat. *J. comp. physiol. Psychol.* **75:** 59–67, 1971.
23. Olson, L. and A. Seiger. Early prenatal ontogeny of central monoamine neurons in the rat: Fluorescence histochemical observations. *Z. Anat. Entwicklungsgesch* **137:** 301–316, 1972.
24. Reinstein, D. K., D. McClearn and R. L. Isaacson. The development of responsiveness to dopaminergic agonists. *Brain Res.* **150:** 216–223, 1978.
25. Roth, R. M. and E. A. Stone. The action of reserpine on noradrenergic biosynthesis in sympathetic nerve tissue. *Biochem. Pharmac.* **17:** 1581–1590, 1968.
26. 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.
27. 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.
28. 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.* **7:** 463–469, 1978.
29. 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:** 133–138, 1977.
30. 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:** 163–166, 1978.
31. Wise, R. A., R. A. Yokel and H. DeWit. Both positive reinforcement and conditioned aversion from amphetamine and from apomorphine in rats. *Science* **191:** 1273–1274, 1976.