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Effects of Apomorphine on Appetitive Conditioning in 6-Hydroxydopamine Treated Rat Pups[1]

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WELDON, D. A., R. S. WOOL, M. H. TEICHER, B. A. SHAYWITZ, D. J. COHEN AND G. M. ANDERSON. *Effects of apomorphine on appetitive conditioning in 6-hydroxydopamine treated rat pups.* PHARMAC. BIOCHEM. BEHAV. 17(6) 1281–1284, 1982.—Rat pups were treated on postnatal day 5 either with the combination of desmethylimipramine (DMI) and 6-hydroxydopamine (6-OHDA) to produce depletion of brain dopamine, or with control injections of saline. Two days later they were presented a novel anise odor paired with intraoral baby formula, and on the next day were tested for preference for the novel odor. Before conditioning and testing, animals were treated with either apomorphine (0.05 mg/kg) or isotonic saline. Performance of the conditioned appetitive response was impaired in dopamine depleted animals. In DMI/6-OHDA treated pups, apomorphine administration prior to conditioning produced an improvement in performance, but drug treatment prior to testing had no effect. In normal pups, apomorphine administration either before conditioning or testing produced impaired performance at testing.

6-Hydroxydopamine Attention deficit disorder Hyperactivity Developmental psychobiology Apomorphine Dopamine depletion Associative learning

TREATMENT of neonatal rats with the combination of desmethylimipramine (DMI) and 6-hydroxydopamine (6-OHDA) produces a profound and permanent reduction in brain dopamine (DA) and results in a constellation of behavioral effects during development. These effects include hyperactivity during the peak period of behavioral arousal that usually appears at postnatal day 15 (P 15) and disappears by P 30 [9, 12, 20, 28, 30, 34], and failure to habituate this activity in a novel environment [26,33]. Furthermore, rats tested at P 20 and later in both avoidance and escape tasks are impaired in learning performance [14, 25, 27, 29].

The behavioral syndrome described above for rat pups has many similarities to the clinical syndrome of attention deficit disorder with hyperactivity (ADD) observed in children, a disorder characterized by excessive general activity in comparison with other children of the same age, difficulty in sustaining attention, and impulsive behavior [1, 31, 36]. Though some have questioned the specificity of the response [22], most investigators have noted that children with this disorder respond to the stimulants methylphenidate and amphetamine with a reduction of their activity and an improvement in attention [4, 35, 36].

Psychostimulant administration also reverses many of the effects of neonatal DA depletion in infant rats. Amphetamine treatment reduces the activity of DA depleted rats during their hyperactive phase at doses that cause an increase in the activity levels of controls [28,32]. Methylphenidate administration produced a similar improvement in DA depleted animals in an escape task [27]. Controls in these studies, however, responded to these drugs with a performance deficit. Two studies (using different depletion procedures), however, have found an increase in activity after methylphenidate [8] and amphetamine [20].

Since the DA depletion procedure used in the above studies produces a 60–80% depletion of brain DA levels without significant effects on norepinephrine levels, it is parsimonious to attribute the behavioral effects to dysfunction of DA systems. One hypothesis for the behavioral effects of DA depletion has been that a DA system, perhaps the mesolimbic DA pathway, exerts tonic inhibitory influences upon excitatory noradrenergic systems in the brain [30]. According to this notion, termed the dual transmitter hypothesis by Antelman and Caggiula [3], the hyperactivity is attributed to an absence of DA mediated inhibition, and thus

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the reversing effects of methylphenidate and amphetamine can be explained by their known facilitating actions at DA synapses [18,19]. The paradoxical effects of amphetamine and methylphenidate in DA depleted pups are explained by assuming that dopaminergic receptors in the DA depleted rats become supersensitive and thus the net drug effect is more inhibitory in those animals. Since these psychostimulants also affect noradrenergic synapses the facilitation of activity in controls is consistent with the model.

If the paradoxical effects of amphetamine and methylphenidate in DA depleted pups are attributable to the differential effects of the drugs on DA receptors, then administration of the DA receptor agonist apomorphine should produce different effects in depleted and control pups. One purpose of the present study, therefore, was to test the effects of apomorphine administration on learning performance in young rat pups. In addition, the present experiment was designed to test the effects of the drug administration either before conditioning, before testing, or both. The task used to test these effects involved a classical conditioning paradigm in which pups are exposed to a novel odor paired with intraoral milk presentation and then are later tested for conditioned preference to the novel odor. Previous research has demonstrated the validity of this learning task with very young rats, i.e., that the resulting preference for the odor is indeed due to the pairing of the stimulus with the reinforcer and is not due to pseudoconditioning [5,13]. By using this task, it was also possible to determine whether the deficits in performance shown by 6-OHDA treated neonates in aversive learning paradigms would generalize to an appetitive learning situation.

METHOD

Design

The present study used a 2×2×2 factorial design with DA depletion (6-OHDA or saline treatment), drug administration before conditioning (apomorphine or saline) and drug administration before testing (apomorphine or saline) as factors.

Subjects

Eighty-two Long-Evans rat pups from 9 litters born in the Hamilton College vivarium were used in the experiment. Except for the days of treatment and testing, animals were housed in litters with their mothers in pine bedding in standard plastic maternity cages. Mothers had access to food and water ad lib, and the vivarium was maintained at a temperature of 21±3°C on a 12 hr light-dark cycle (lights on at 0600 hrs).

Apparatus

The conditioning chamber was 8 cm high × 10 cm in diameter, and was situated under a heat lamp. An aquarium air pump was connected by polyethylene tubing to a valve. The valve allowed air to flow into either a flask containing 5 ml anise extract in 100 ml of water, or a flask containing 100 ml of water alone. From these flasks tubing was connected to the conditioning chamber.

The acrylic testing chamber was 30×20×10 cm i.d., with a floor made of wire screening and was situated above a 30×20 cm tray. Both the floor of the test chamber and the tray were segmented into 15×20 cm sections by lines drawn on their surfaces. An incubator consisting of a 40×27×32 cm glass aquarium equipped with a 200 W incandescent light coupled to a thermostat was used to maintain the environment at 31±1°C.

Procedure

Dopamine depletion and cannulation. At 5 days of age, pups were separated from their mothers, toe punched for identification, and randomly assigned to one of the eight experimental groups. Pups assigned to dopamine depletion groups were administered 20 mg/kg of DMI IP in a volume of 0.1 cc. One hr after this injection, the animals received an intracisternal injection of 100 μg of 6-OHDA HBr in a volume of 20 μL. This procedure has been shown to produce rapid depletion of brain DA [6,24]. Control pups received saline vehicle for both injections. After these treatments, animals were returned to their mothers.

When 6 days of age, pups were again separated from their mothers and were fitted with intratongue cannulae under ether anesthesia. Cannulation was accomplished by inserting a 26 μm diameter piano wire (beveled at one end) through the ventral jaw and posterior tongue of the animal [11]. A cannula made of PE-10 tubing with a flare at one end was then twisted onto the protruding wire. The wire was then drawn out of the mouth, pulling the cannula into place with its flared end resting in the posterior tongue. The cannula was then cut to the length of the pup, and the animal was maintained in the incubator at 31±1°C until the next day.

Conditioning. The day after cannulation pups were administered 0.05 mg/kg apomorphine HCl (Sigma) IP in 0.1 cc of physiological saline or an equivalent volume of the saline vehicle, depending upon their experimental group. After 30 min, each animal was placed in the conditioning chamber with the temperature maintained at 35±1°C. In each of five 1-min cycles, each pup received 20 sec of anise scented air paired with the infusion of 0.03 ml of infant formula (Enfamil), followed by 40 sec of unscented air. After this 5 min conditioning period, the pup was replaced in the incubator; when all pups had been conditioned, their cannulae were removed and the animals were returned to their mothers.

Testing. When 8 days of age, pups were placed in the incubator and maintained at 31±1°C for 4 hr prior to testing to ensure hunger. Thirty min prior to testing, each animal was administered either 0.05 mg/kg apomorphine HCl in 0.1 cc physiological saline or an equivalent volume of saline alone, depending upon the group designation. For testing, 3 ml of anise extract were pipetted onto one side of the tray situated under the test chamber floor, and each animal was placed onto the middle line and observed for a 5 min period. Performance was measured as the percentage of time that the pup spent over the side scented with the conditioned anise odor, discounting the time spent straddling the center line. The side of the apparatus under which the conditioned odor was placed was changed from trial to trial. After testing, pups were returned to their mothers.

Biochemical analyses. On the day following testing animals were sacrificed by decapitation. Brains were extracted and quick-frozen on dry ice within 1 min of death. Frozen brains were stored at −70°C and dopamine and norepinephrine were analyzed by high performance liquid chromatographic techniques [2].

RESULTS

Figure 1 illustrates the mean percentage of time spent

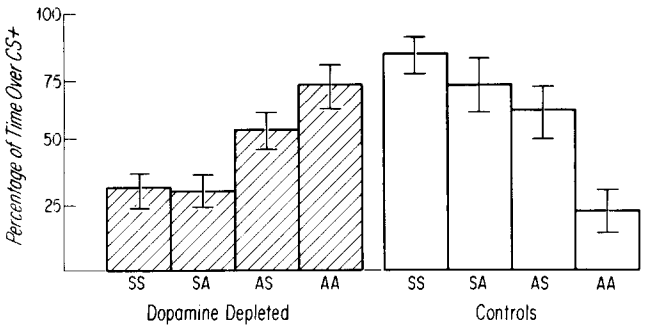

FIG. 1. Mean percentage of a 5 min period spent over the conditioned odor for pups treated with saline prior to both conditioning and testing (SS), saline prior to conditioning and apomorphine prior to testing (SA), apomorphine prior to conditioning and saline prior to testing (AS), and apomorphine prior to both conditioning and testing (AA). Data from DA-depleted pups are shown on the left and controls are on the right.

TABLE 1

BRAIN CATECHOLAMINE CONCENTRATIONS

Group	Dopamine	Norepinephrine
Controls (combined)	100% (353.8 ± 107.2 ng/g)*	100% (379.0 ± 61.9 ng/g)
6-OHDA Treated		
SS	20.3†	84.7
AS	21.4†	87.4
SA	22.5†	79.7
AA	17.5†	85.0

*Concentrations are expressed as percentages of control values. Numbers in parentheses refer to mean concentrations±SEMs.
†Significantly different from controls, $p<0.001$.

over the conditioned stimulus for the eight experimental groups. Data were analyzed using a 2×2×2 analysis of variance, and tests for simple main effects were made using Newman-Keuls pairwise comparisons.

Overall, DA depleted animals spent less time over the conditioned odor than did controls, $F(1,74)=7.41$, $p<0.01$. For DA depleted pups, apomorphine treatment before conditioning caused a significant improvement in the learning performance, $F(1,74)=18.38$, $p<0.001$. For controls, apomorphine treatment before testing produced a significant decrease in the percentage of time spent over the conditioned stimulus, $F(1,74)=22.08$, $p<0.001$. Apomorphine treatment before testing did not affect learning performance for DA depleted pups, but it produced a performance impairment in controls, $F(1,74)=11.06$, $p<0.005$. The triple interaction of DA depletion, drug prior to conditioning, and drug prior to testing approached but did not attain statistical significance, $F(1,74)=3.79$, $0.06>p>0.05$.

Table 1 indicates the DA and norepinephrine depletion levels of the four 6-OHDA treatment groups. DA levels were determined to be between 17.5–22.5% of the control values; norepinephrine levels were not significantly different from control values.

DISCUSSION

The present results show that apomorphine administration prior to conditioning reverses the performance impairments exhibited by DA depleted animals in an appetitive learning task. The finding that neonatal 6-OHDA treatment produces impairments in an appetitive conditioning task extends the previous observations of aversive conditioning deficits in these animals [14, 25, 27, 29]. In addition, the deficits were observed in pups as early as 8 days of age, whereas previous reports had measured learning impairments in animals at least 20 days of age. In learning experiments involving two choices, naive animals exhibit performance at chance levels (i.e., 50%). Naive animals in the current experimental test situation, however, spend 40% of their time over the novel anise extract (Wool, Shaywitz and Teicher, in preparation); the odor appears, therefore, to be aversive. In this context, DA depleted animals treated with saline before conditioning behave as if they had not experienced conditioning at all.

The fact that apomorphine in a low dose reversed the performance deficit in DA depleted rats is particularly interesting since higher doses of the drug (0.1 and 1.0 mg/kg) disrupt learning in shuttle box and *t*-maze tasks [14]. Escape learning in young rats is also disrupted by administration of the dopamine agonist bromocriptine [29]. Since DA autoreceptors are considered to be inhibitory on DA synthesis [23], and DA depletion produces learning deficits, it is most sensible to consider the reversing effect of apomorphine in the present study to be mediated by postsynaptic receptors in the 6-OHDA treated animals. These results imply that tonic dopaminergic stimulation is required for normal learning performance.

Although dopamine is apparently required for normal performance to occur in the present experimental task, the specific process in which it is involved remains to be identified. Adult rats with lesions of the mesotelencephalic dopaminergic system show sensory neglect to visual, auditory and olfactory stimuli [15, 16, 17]. Olfactory sensory deficits were not specifically examined in the current study, but the pattern of results does not support anosmia as an explanation of the impaired performance in 6-OHDA treated pups. For example, pups treated with saline before testing obviously did not ignore the olfactory stimulus, since they spent more time away from the side of the apparatus scented with the odor (see Fig. 1). A second behavioral effect of 6-OHDA treatment that might be responsible for the impaired performance in the learning task is motor hyperactivity, but since dopamine depleted pups do not demonstrate hyperactivity until 15 days of age [20, 21, 25, 28, 29, 30], this is an unlikely explanation. In addition, the indication that apomorphine treatment before conditioning produced an improvement in performance when the animals were tested the next day argues against a simple motor deficit as a reason for the behavioral impairment. A third possibility to consider is that pups treated with 6-OHDA might not be responsive to the positive reinforcer. However, Wool, Shaywitz, and Teicher (in preparation) have shown that DA depleted and control pups exhibit comparable behavioral activation [10] in response to milk presentation in the same task used in the present study, so this explanation also appears improbable. Certainly additional research into the associative, attentional, sensory and motor abilities of 6-OHDA treated rat pups will be helpful in interpreting their deficits in appetitive conditioning performance.

In control animals, apomorphine administration prior to conditioning produced a deficit in performance, and similar results have been obtained previously [14]. Normal adult rats have been found to respond to low doses of apomorphine with an increase in conditioned avoidance response acquisition but to high doses with a decrease in performance [7]. Thus, dose-response curves will be helpful in the interpretation of the apomorphine effects in infant rats.

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