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The Effects of l-, d-, and Parahydroxy-Amphetamine on Locomotor Activity and Wall Climbing in Rats of Different Ages[1]

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BAUER, R. H. *The effects of l-, d-, and parahydroxy-amphetamine on locomotor activity and wall climbing in rats of different ages.* PHARMAC. BIOCHEM. BEHAV. 13(2) 155–165, 1980.—In the first experiment, 15, 17, 21, 36, 90, and 275-day-old rats were injected with either physiological saline, 0.5, 1.0, 4.0, 8.0, or 16.0 mg/kg of l-amphetamine. In Experiment 2, rats of these ages were injected with saline or comparable doses of d-amphetamine. Starting immediately after the injection, photo-cell crossings and wall climbing were recorded during 15-min intervals for a total of 4 hours. In general, photo-cell crossings in 15-day-old rats were increased more by low doses than higher doses. In 17 and 21-day-old rats, the peak in the dose response curve gradually shifted toward higher doses, until, at 36 days of age, low doses produced no significant change in photo-crossings and the highest doses produced the maximum increase. In the two oldest ages, the dose-response curves for photo-crossings were the typical inverted U function. In adults, d-amphetamine had a more potent action on photo-cell crossings than l-amphetamine. However, in other ages, l-amphetamine appeared to be slightly more potent than d-amphetamine or there were no potency differences between the two isomers. Low doses of both d- and l-amphetamine increased wall climbing in the three youngest ages but higher doses were without effect. In 36-day-old rats, wall climbing was slightly increased by 4.0 mg/kg of d-amphetamine but no dose of either isomer altered wall climbing in adults. Since amphetamine appears to produce behavioral changes by acting on catecholamines, the age-dependent behavioral effects of amphetamine may be due to maturation of central nervous system catecholaminergic neurons. However, involvement of other neurotransmitter systems can not be excluded. In Experiment 3, parahydroxy-amphetamine (1.0, 4.0, and 16.0 mg/kg) did not significantly alter photo-cell crossings or wall climbing in 15, 17, 21, 36, or 90-day-old rats. Because parahydroxy-amphetamine has only peripheral effects, it appears likely that central actions are responsible for the age-dependent behavioral effects of l- and d-amphetamine.

Amphetamine Development Aging Motor activity Rats Catecholamines

HISTOCHEMICAL and biochemical studies indicate that catecholamine-containing neurons in the brain of altricial species, such as the rat, gradually mature from birth to about puberty. At birth, catecholamine containing cell bodies in the lower brain stem appear to be nearly fully developed. As the animal grows older, axons from these cell bodies grow in a rostral direction and innervate successively higher structures. In the rat, cortical innervation occurs at about 45 days of age (for reviews see [17,21]).

Since catecholaminergic neurons change during development, drugs which alter behavior by acting on these neurons would be expected to have different behavioral effects in immature and mature animals. In one of the first studies to examine the behavioral effects of a catecholaminergic drug in immature and mature animals, various doses of d-amphetamine (0.25, 1.0, 2.0, 4.0, and 8.0 mg/kg) altered stabilimeter cage activity of 10, 15, 20, 25, and 90-day-old rats to a comparable degree [3]. More recent studies have shown that in approximately 30-day-old rats d- and dl-amphetamine (2.0, 5.0, and 10.0 mg/kg) have only a slight effect on open-field activity, but in 20-day-old and adult rats the activity changes from these doses are similar [1,18].

Some of the discrepant developmental findings with amphetamine may be due to procedural differences. Total stabilimeter cage activity was recorded during a 2-hour session, starting immediately after the injection [3], whereas open-field activity was recorded for a shorter duration (5 or 8 min) starting 15 or 30 min after drug administration [1,18]. In perhaps the only developmental studies to examine locomotor activity at various times after d-amphetamine administration, 1.0, 2.0, and 10.0 mg/kg produce a U-shaped temporal function in 15-day-old rats, i.e., increased activity during the first hour, a decrease during the next hour, and an increase during the third hour [5,16]. Injection of these doses in 30-day-old rats results in only a slight increase in the middle of the session. In other studies with adults, across time

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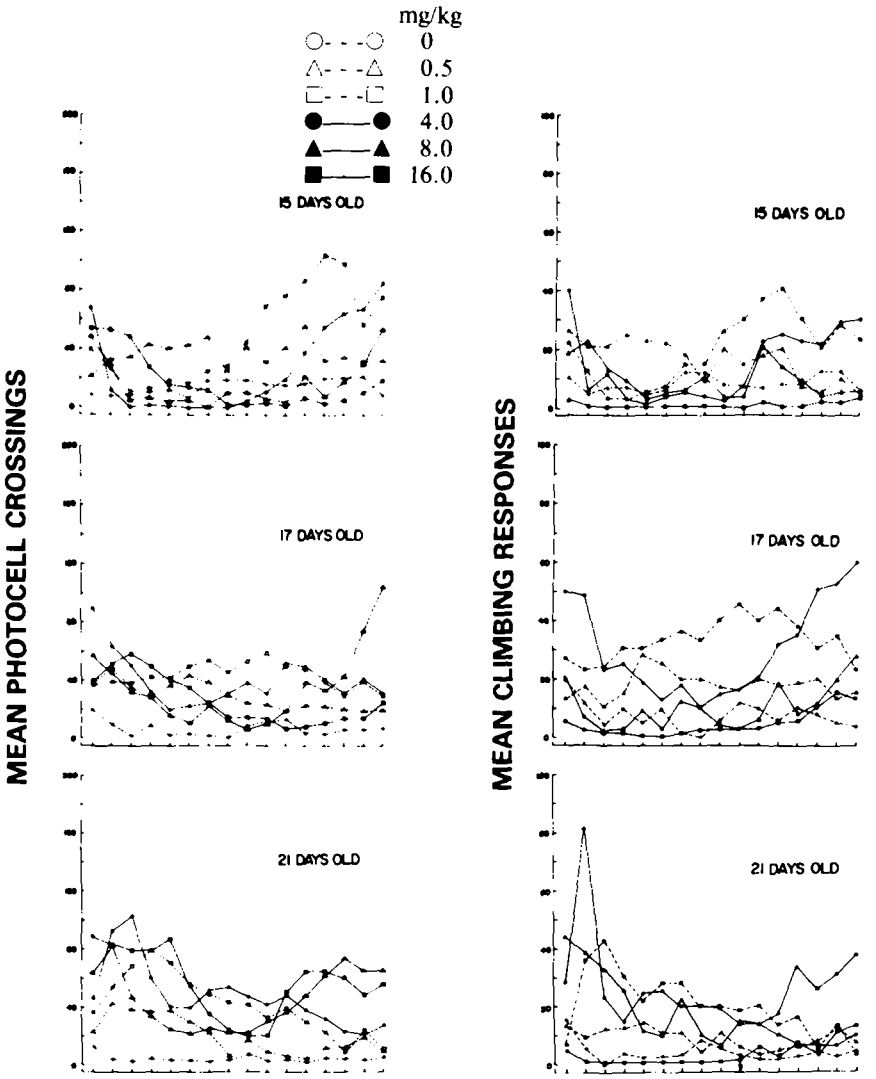

FIG. 1. The mean number of photo-cell crossings (left panels) and the mean number of rearing responses (right panels) for six different ages as a function of dose and time.

higher doses (5.0 and 10.0 mg/kg) result in a U-shaped response but lower doses (0.5 and 1.0 mg/kg) increase locomotor activity [11,22]. Therefore, the dosage administered and the time at which motor activity is examined may be critical variables in developmental psychopharmacological studies.

The type of behavioral response examined may also be a factor in developmental psychopharmacological studies. The responses recorded by stabilimeter cages are thought to be different from those recorded in the open-field and photo-cell chambers, and the use of stabilimeter cages in drug research has recently been criticized [23]. Furthermore, the developmental changes in stabilimeter cage activity are different from the developmental activity changes recorded in the open-field and photo-cell chambers [4,6].

When examining some, if not all, behavioral responses in immature and mature animals, it is important to equate the animal's size with the apparatus size. For example, when recording rearing responses in automated apparatus (completing the circuit between a metal floor and metal on the side walls above the floor), if the metal on the side walls is low enough for young animals to close the circuit by rearing, adults could close the circuit by touching their body against the wall. On the other hand, if the metal on the walls is high enough for adults to close the circuit by rearing, it may be physically impossible for young animals to complete the circuit. For this reason, in the present studies, the apparatus size was equated with the mean snout to rump length of each age examined.

Development of central and/or peripheral neurons may be responsible for the age-dependent effects of d- and l-amphetamine, because these drugs act on catecholaminergic neurons in both the central and peripheral nervous system [2,13]. Comparing the behavioral effects of centrally acting drugs with peripherally acting drugs is a standard technique for separating central and peripheral drug effects. Due to the relative inability to cross the blood-brain barrier, parahydroxy-amphetamine has minimal central nervous system effects, but has the same potency as d- and l-amphetamine in peripheral nervous system [2,13]. If development of central neurons plays a major role in the age-related behavioral effects of d- and l-amphetamine, one would expect the behavioral effects of d- and l-amphetamine to change during development, whereas parahydroxy-amphetamine would be expected to produce comparable

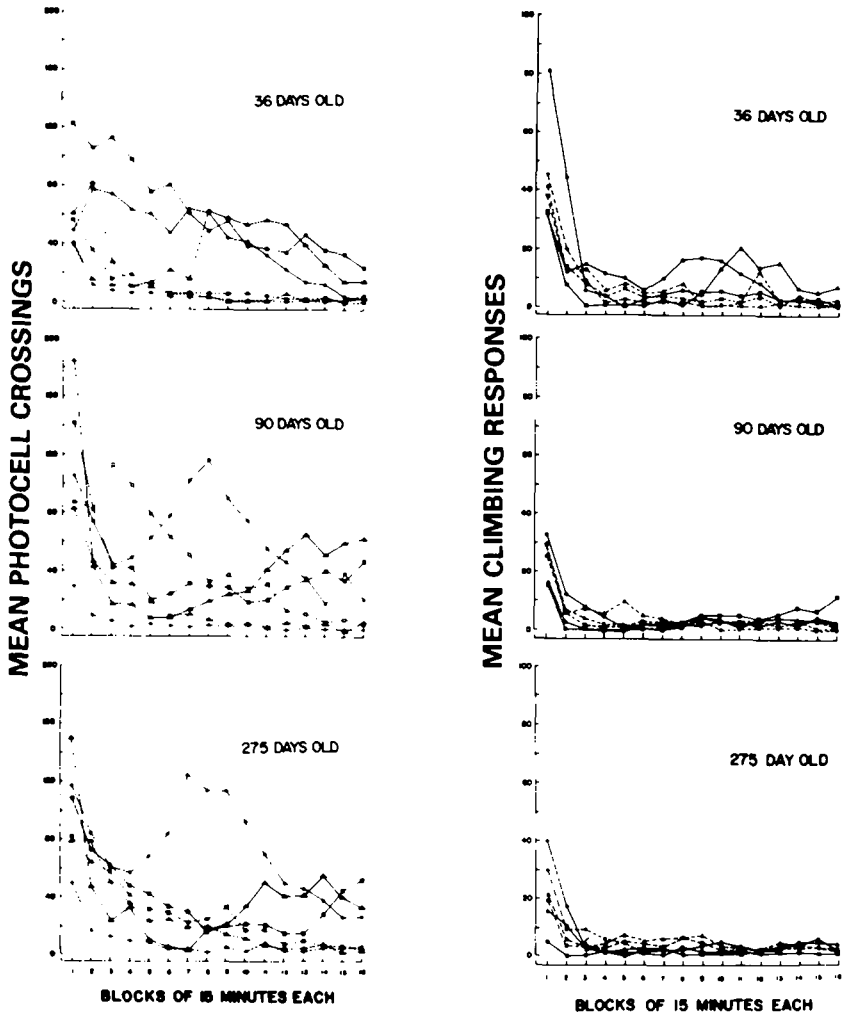

FIG. 1. continued.

changes in all ages. On the other hand, if maturation of the peripheral nervous system is of primary importance, these three drugs would be expected to result in comparable changes during development.

The major purpose of the present studies was to examine the effects of l-amphetamine (Experiment 1), d-amphetamine (Experiment 2), and parahydroxy-amphetamine (Experiment 3) on photo-cell crossings and wall climbing in immature and mature rats as a function of time after drug administration. In each of these experiments, starting immediately after the drug injection, photo-cell crossings and wall climbing were recorded during 15-min intervals for a total of 4 hours.

EXPERIMENT 1

The behavioral changes induced by l-amphetamine are thought to be primarily due to increased release and reduced reuptake of both norepinephrine and dopamine [7, 11, 19]. Therefore, l-amphetamine was administered in Experiment 1.

METHOD

Subjects and Experimental Design

Experimentally naive, male Sprague-Dawley rats (Rattus norvegicus) served as subjects (N=396). These animals were offspring of breeding stock obtained from the Department of Microbiology at the University of California, Los Angeles. Shortly after birth each litter was reduced to 8 pups. The rats had free access to Purina Rat Chow and water throughout the experiment. The colony room had a 14-hour light-cycle from 6:00 a.m. to 8:00 p.m. The colony room and experimental room were maintained at 23 ± 1°C.

The rats were either 15, 17, 21–22, 36–37, 90–100, or 275–285 days of age at the time of testing. For brevity, only the youngest age of each group will be referred to hereafter. These animals were administered either physiological saline, 0.5, 1.0, 4.0, 8.0, or 16.0 mg/kg of l-amphetamine sulfate (n=11 per each age and each dose group) immediately prior to receiving a 4-hour test session. For each animal, motor activity and wall climbing responses during 15-min intervals were determined. Therefore, the experimental design was a 6 (age)×6 (dose)×16 (blocks of 15 min) complete factorial.

Apparatus

The apparatus consisted of 5 activity chambers. Except for the dimensions, the chambers were identical. The two oldest ages were tested in a 45×45×45-cm box constructed of 0.6-cm Plexiglas. Aluminum sheets (32×45 cm) were attached to the inside walls 13.0-cm above the aluminum floor. An orthogonal light-photo cell system was placed 4.0-cm

above the aluminum floor and midway between the side walls. All apparatus dimensions for younger groups varied according to the mean snout to rump length of each age [26]. The apparatus dimensions for younger rats were reduced as follows: 15-day-old, 61%; 17-day-old, 57%; 21-day-old, 48%; and 36-day-old, 33%.

Each activity chamber was placed inside a sound resistant box constructed of 2-cm plywood. A fan mounted on the outside wall of each box provided ventilation and masked extraneous sounds. Photo-cell crossings and wall climbing, i.e., completing the circuit between the floor and aluminum on the walls, were recorded on counters and event recorders.

Procedure

Rats tested at 21 days of age or younger were housed with their littermates and mothers in standard cages (28×23×24 cm) with sawdust shavings until tested. These animals were randomly assigned to groups with the constraint that their eyes were open (approximately 95% have their eyes open by 15 days of age). Rats tested at 36 days of age and older were weaned at 21 days of age and 8–10 rats were placed in group cages (60×35×23 cm) with wire mesh floors. At 36, 90, or 275 days of age, cages were randomly selected and the animals in the selected cages were tested within 2 days of each other. This selection procedure for the older groups eliminated intermixing animals in different cages for purposes of maintaining the same number of rats in a cage.

The rats were weighed and then given an intraperitoneal injection of the appropriate dose. The drug solutions were mixed such that 0.01 cc/g of body weight was injected. The bottles containing the solutions were coded, so the experimenter did not know the dose being injected until the experiment was completed. Immediately after the injection, the animals were placed in the appropriate sized activity chamber for a total of 4 hours. Testing began at either 9:00 a.m. or 1:30 p.m. After testing, the tail of each rat was marked with a felt tip pen, and they were returned to their home cage. The apparatus was cleaned with a damp paper towel after each rat was tested.

RESULTS AND DISCUSSION

Photo-Cell Crossings

For each animal, the number of photo-cell crossings during 15-min intervals was determined. The mean number of photo-cell crossings during each interval are shown in the left panels of Fig. 1 as a function of age and drug dose. A 6 (age) ×6 (dose) × 16 (blocks of 15-min intervals) mixed analysis of variance of photo-cell crossings showed that the main effects for age, $F(5,360)=2.32$, $p<0.05$, dose, $F(5,360)=30.79$, $p<0.001$, and blocks, $F(15,5400)=51.20$, $p<0.001$, were significant (for all statistics reported, the criterion for significance was $p<0.05$). A significant age×dose interaction, $F(25,360)=3.74$, $p<0.001$, suggests that the dose-response curves differed as a function of age (see the upper panel of Fig. 2). A significant dose×blocks interaction, $F(75,5400)=7.50$, $p<0.001$, and inspection of Fig. 1 indicate that the changes accross blocks were dependent upon the dose administered. Moreover, a significant 3-way interaction, $F(375,5400)=3.26$, $p<0.001$, suggests that the drug induced changes in photo-cell crossings that occur across blocks differed as a function of age.

The finding that some doses of l-amphetamine produce a U-shaped function in 15-day-old rats and adults supports previous findings with d- and l-amphetamine [5, 11, 22]. The present findings show, in addition, that a U-shaped temporal function occurs in other ages and that across ages the doses which produce such a curve differ. While the same dose in 90 and 275-day-old rats generally results in similar behavioral changes, it should be noted that even in these ages there were some behavioral differences.

Comparison of the mean number of photo-crossings during the 4-hour session within each age showed that the youngest rats given 1.0 mg/kg of l-amphetamine were more active than those given saline, 8.0, or 16.0 mg/kg of l-amphetamine. Tukey's (a) test was used to make all individual comparisons [27]. In the 17-day-old group, saline controls were less active than their same aged counterparts treated with 1.0, 4.0, and 8.0 mg/kg, and the 4.0 mg/kg group made more photo-crossings than the 0.5 and 16.0 mg/kg groups. At 21 days of age, rats given the four highest doses were more active than saline controls, and the 0.5 mg/kg group was less active than the 16.0 mg/kg group. The 36-day-old rats injected with saline, 0.05, and 1.0 mg/kg were less active than their same aged counterparts given the three highest doses. The 90-day-old rats given saline were less active than those given doses of 1.0 mg/kg and greater, and these aged animals given 0.5 mg/kg made fewer photo-crossings than rats given 1.0, 4.0, and 8.0 mg/kg. In the oldest group, rats treated with saline made fewer crossings than those administered doses greater than 1.0 mg/kg and those given 4.0 mg/kg were more active than rats given other doses.

Tukey's test indicated that the total number of photo-cell crossings for saline controls of different age groups were not significantly different. Since the baseline activity rate was comparable for the various ages, comparison of each drug dose across ages appears warranted. When injected with 0.5 mg/kg of l-amphetamine, 36-day-old rats made fewer photo-cell crossings than 15-day-old rats. When given 1.0 mg/kg, the 36-day-old animals were less active than 15, 17, 21, and 90-day-old groups given this dose. The youngest group given 4.0 mg/kg was significantly less active than 17, 90 and 275-day-old groups given the same dose. Treatment with 8.0 mg/kg resulted in a lower number of photo-cell crossings in 15-day-old rats than 21 and 90-day-olds. The two youngest groups injected with the highest dose made significantly fewer crossings than 21 and 36-day-old groups.

In general, the present findings indicate that from infancy to adolescence the peak in the dose-response curve gradually shifts toward higher doses (see the upper panel of Fig. 2). After 36 days of age, the peak is shifted toward lower doses. The finding that lower doses of l-amphetamine have relatively little effect on photo-cell crossings in 36-day-old rats supports previous findings in the open-field [1,18] and suggest that this age is insensitive to low doses of l-amphetamine. Since the greatest activity increases in 36-day-old rats occurs with the highest doses of amphetamine, this age is apparently more tolerant to l-amphetamine than other ages. l-Amphetamine is thought to induce behavioral changes by acting primarily on catecholamines [7, 11, 19] and, therefore, the age-dependent behavioral effects of this drug may be due to maturational changes in catecholaminergic neurons.

Wall Climbing

The right panels of Fig. 1 present the mean number of wall climbing responses during 15-min intervals as a function of

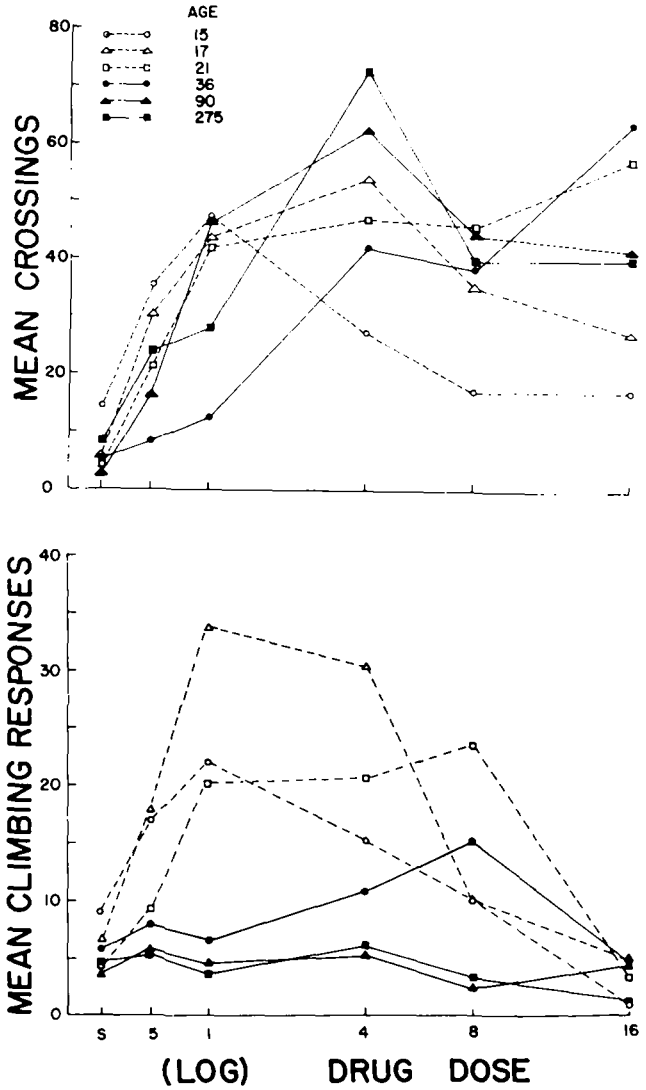

FIG. 2. The mean number of photo-cell crossings (upper panel) and rearing responses (lower panel) for the 4-hour session for six ages as a function of drug dose.

age and drug dose. A 6 (age)×6 (dose)×16 (blocks of 15 min intervals) mixed analysis of variance of the number of climbing responses revealed that the main effects for age, $F(5,360)=15.09$, $p<0.001$, dose, $F(5,360)=11.45$, $p<0.001$, and blocks, $F(15,5400)=33.89$, $p<0.001$, were significant. A significant dose×age interaction, $F(25,360)=2.79$, $p<0.001$, and inspection of the lower panel of Fig. 2 indicate that the dose-response curves differed as a function of age. The dose×blocks interaction was significant, $F(75,5400)=3.29$, $p<0.001$, suggesting that the changes across blocks were dependent on the dose administered. Furthermore, a significant 3-way interaction, $F(375,5400)=3.90$, $p<0.001$, suggests that the drug-induced changes in climbing that occurred across time differed among the age groups.

Tukey's tests were computed on the mean number of climbing responses during the 4-hour session for each age group. In the youngest rats, saline controls and the 16.0 mg/kg group made significantly fewer responses than the 0.5, 1.0, and 4.0 mg/kg groups and the 1.0 mg/kg group made more than the 8.0 mg/kg group. In 17-day-olds, the 1.0 and 4.0 mg/kg group made more than the two highest dose groups. At 21 days of age, 1.0, 4.0, and 8.0 mg/kg groups made more climbing response than saline controls and the highest dose group, and the 8.0 mg/kg group made more than the 0.5 mg/kg group. In the three oldest ages, there were no significant differences among drug doses.

Comparison of saline controls of different ages showed that the mean number of climbing responses during the total session were not significantly different. Therefore, the mean number of climbing responses for different ages given the same dose were compared by Tukey's test. There were no significant age differences in animals given 0.5 and 16.0 mg/kg. When given 1.0 mg/kg, the three youngest ages made significantly more responses than the three oldest ages and 17-day-olds made more than 15 and 21-day-olds. In groups treated with 4.0 mg/kg, climbing was greater in 17 and 21-day-old animals than the two oldest ages and 17-day-old rats made more than 15 and 36-day-olds. The 21-day-old animals given 8.0 mg/kg made more responses than the two youngest and two oldest groups given this dose. The finding that lower doses of l-amphetamine increased climbing in infants but produced virtually no change in adults provides further support for the suggestion that the behavioral effects of l-amphetamine differ in immature and mature rats.

Comparison of the upper and lower panels of Fig. 2 suggest that in the youngest age the dose response curves for photo-crossings and climbing were fairly similar. In 17 and 21-day-old rats, however, the portion of the curves for lower doses were similar but higher doses appear to reduce climbing more than photo-crossings. In the three oldest ages, the dose response curves for photo-crossings and climbing appear quite different. Thus, at least for photo-crossings and climbing, the type of response examined after l-amphetamine treatment can yield quite different developmental changes.

EXPERIMENT 2

d-Amphetamine is considerably more potent than l-amphetamine in increasing locomotor activity [24]. This potency difference has been attributed to the greater action of d-amphetamine on norepinephrine than l-amphetamine [23]. However, this potency difference has been questioned [12]. Nevertheless, other pharmacological evidence suggests that changes in locomotor activity are mediated by noradrenergic neurons [9,24].

In the rat, development of noradrenergic neurons appears to precede development of dopaminergic neurons by approximately 15 days; complete development of noradrenergic and dopaminergic neurons occurs at about 35 and 55 days of age, respectively (see [21]). In light of these developmental differences and the potency differences between l- and d-amphetamine, the age-related behavioral effects of d- and l-amphetamine may differ. Therefore, in Experiment 2, photo-cell crossings and climbing responses were examined in rats of various ages following d-amphetamine.

METHOD

Subjects, Apparatus and Procedure

The rats were 15, 17, 21, 36–37, 90–100, and 275–285-days-old with the same characteristics as those described in Experiment 1. The apparatus was the same as in Experiment 1. The animals were injected with either physiological saline, 0.5, 1.0, 4.0, 8.0, or 16.0 mg/kg of d-amphetamine sulfate ($n=11$ per each age and each dose group). Other procedures were the same as those in Experiment 1.

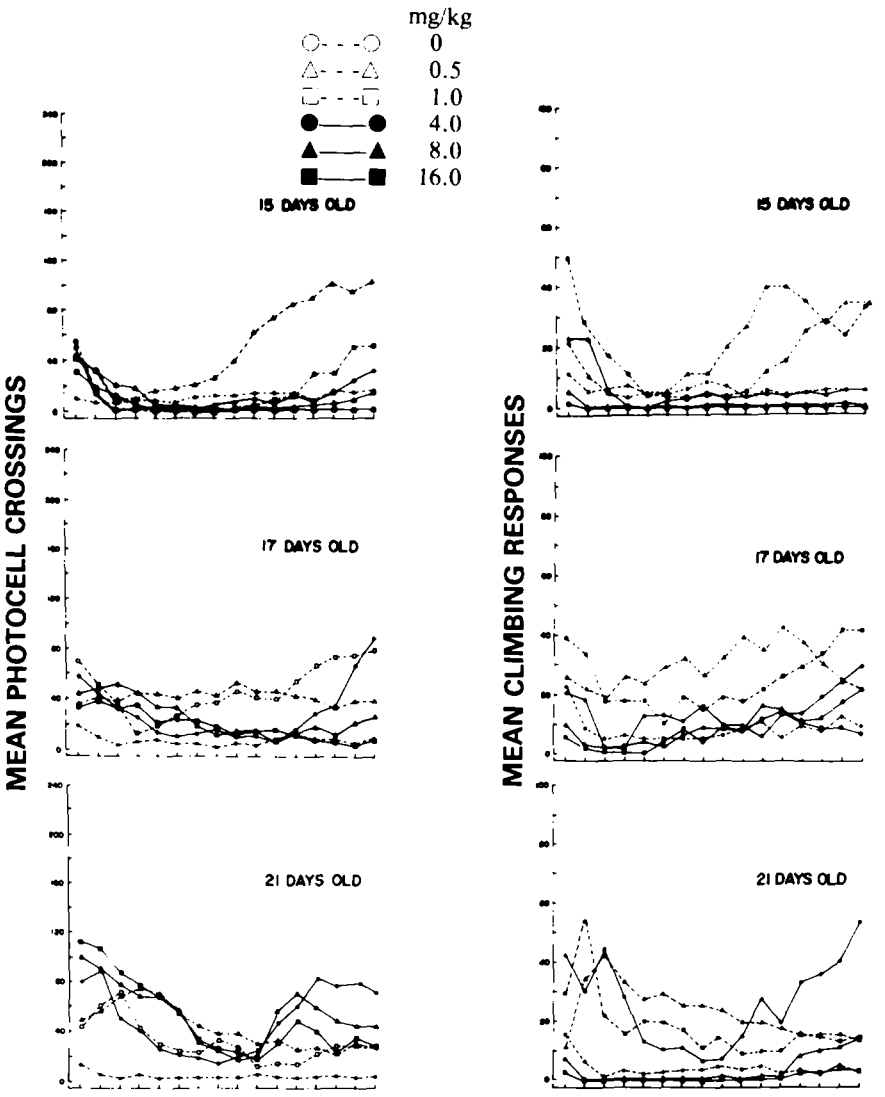

FIG. 3. The mean number of photo-cell crossings (left panels) and rearing responses (right panels) for different ages as a function of drug dose and time.

RESULTS AND DISCUSSION

Photo-Cell Crossings

The number of photo-cell crossings for each rat during 15 min intervals was determined (see the left panels of Fig. 3) and these counts were analyzed by a 6 (age)×6 (dose)×16 (blocks) mixed analysis of variance. The main effects for age, $F(5,360)=13.65$, $p<0.001$, dose, $F(5,360)=31.05$, $p<0.001$, and blocks, $F(15,5400)=89.66$, $p<0.001$, were significant. A significant dose × age interaction, $F(25,360)=11.58$, $p<0.001$, indicates that the dose response curves differed as a function of development (see the upper panel of Fig. 4). The left panels of Fig. 3 suggest that the changes in photo-cell crossings across blocks differed as a function of drug dose; this is supported by a significant interaction between dose and blocks, $F(75,5400)=6.80$, $p<0.001$. A significant 3-way interaction, $F(375,5400)=4.80$, $p<0.001$, and inspection of the left panels of Fig. 3 suggests, furthermore, that the drug induced changes across blocks were dependent of the animals' age.

Comparison of the mean number of photo-cell crossings during the total session showed that in the youngest age group 0.5 mg/kg of d-amphetamine increased crossings more than all other doses. At 17 days of age, the 0.5 and 1.0 mg/kg groups were significantly more active than the saline and 16.0 mg/kg groups. In 21-day-olds, saline controls were significantly less active than all other dose groups. In the 36-day-old animals, (a) the 16.0 mg/kg group was significantly more active than all other groups, (b) the 8.0 mg/kg group was more active than the saline, 0.5, and 1.0 mg/kg groups, and (c) the 1.0 and 4.0 mg/kg groups were more active than the saline and 0.5 mg/kg groups. At 90 days of age, the 0.5, 1.0, and 4.0 mg/kg groups were more active than the other dose groups. In the oldest rats, 1.0 and 4.0 mg/kg increased crossings more than doses of saline, 0.5, and 16.0 mg/kg and the 8.0 mg/kg group was more active than saline controls.

Comparison of the mean number of photo-cell crossings during the total session in different aged saline controls revealed no signficant differences. In animals treated with 0.5 mg/kg of d-amphetamine, the 36-day-old group made signifi-

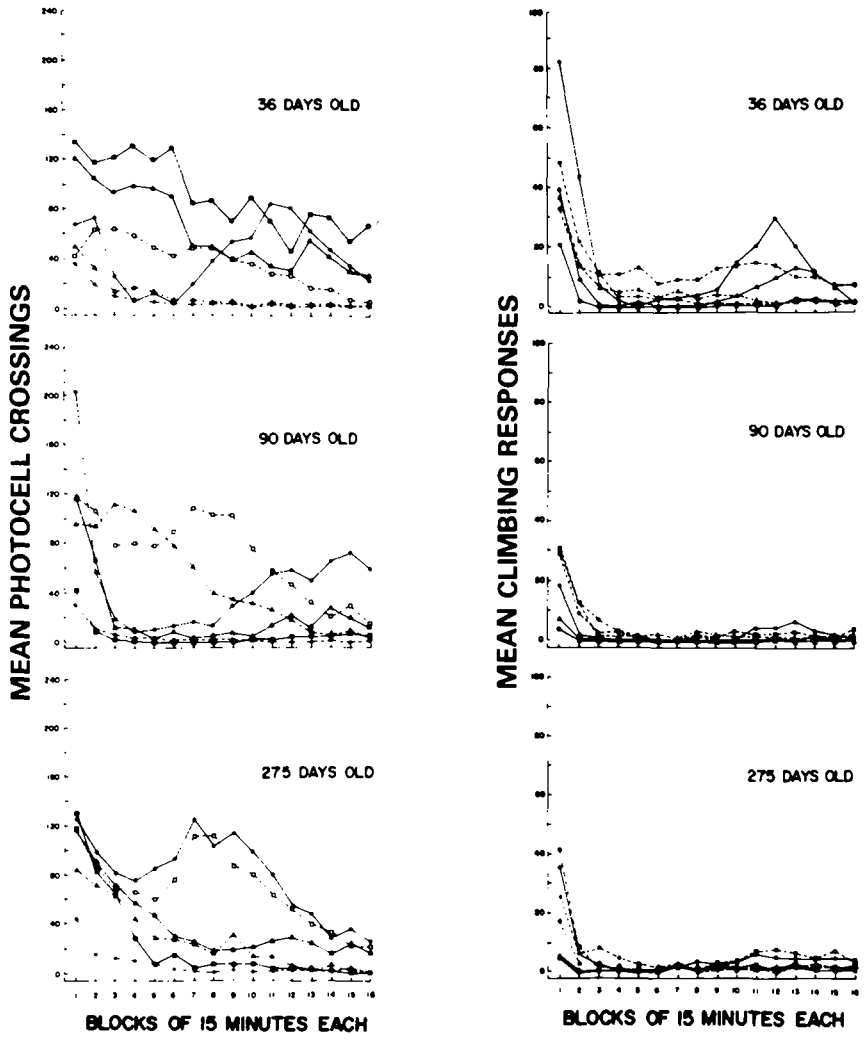

FIG. 3. continued.

cantly fewer crossings than 15, 17, 21, and 90-day-old groups. When given 1.0 mg/kg, photo-crossings were higher in the two oldest groups as compared to younger groups and the 17-day-old rats made more crossings than the youngest group. In rats injected with 4.0 mg/kg, the four oldest ages made significant more crossings than the youngest age and the 21 and 275-day-old animals made more than the 17-day-olds. When injected with 8.0 mg/kg, the 21 and 36-day-old rats made significantly more crossings than 15, 17, and 90-day-old groups, and the oldest group made more than the youngest. When given the highest dose, 21 and 36-day-old rats made more crossings than all other ages and the 36-day-old rats were more active than 21-day-olds.

The results of Experiments 1 and 2 are basically similar. That is, from 15 to 36 days of age the peak in the dose response curve is gradually shifted toward higher drug doses. In adults the peak is once again at relatively lower doses. At least for locomotor activity, the 36-day-old rats appear to be very insensitive to low doses and to be very tolerant of high doses. General observation of the animals after the testing session also suggested that high doses were quite debilitating in adults but were much less so in younger rats. These developmental changes provide further support for the suggestion that there would be maturational differences in response to amphetamine.

Wall Climbing

The right panels of Fig. 3 present the mean number of climbing responses during 15-min intervals as a function of age and drug dose. A 6×6×16 mixed analysis of variance revealed that the main effects for age, $F(5,360)=20.45$, $p<0.001$, dose, $F(5,360)=24.15$, $p<0.001$, and blocks, $F(15,5400)=58.56$, $p<0.001$, were significant. A significant age×dose interaction, $F(25,360)=3.61$, $p<0.001$ and inspection of the lower panel of Fig. 4 show that the dose-response curves differed as a function of development. A significant dose × blocks interaction, $F(75,5400)=4.97$, $p<0.001$, suggests that the temporal changes in climbing were dependent on the dose administered. Furthermore, inspection of the right panels of Fig. 3 suggests that the drug induced changes that occurred across blocks are dependent on the developmental stage of the animal; this suggestion is supported by a significant 3-way interaction, $F(375,5400)=2.48$, $p<0.001$.

For each age, Tukey's test was used to compare the mean number of climbing responses of different dose groups dur-

ing the 4-hour session. The 15-day-old rats injected with 0.5 and 1.0 mg/kg of d-amphetamine made more responses than their same aged counterparts given other doses and those injected with 1.0 mg/kg made more than those treated with 8.0 or 16.0 mg/kg. In 17-day-olds, rats injected with 0.5 and 1.0 mg/kg made more climbings than their same aged counterparts given other doses. In 21-day-old rats, climbing was higher following treatment with 0.5, 1.0, and 4.0 mg/kg than all other doses. In 36-day-old rats, 4.0 mg/kg increased climbing significantly more than the highest dose. d-Amphetamine did not significantly alter climbing in the two oldest ages.

Comparison of saline controls of different ages showed that there were no significant differences in the mean number of climbing responses for the total session. Comparison of different ages treated with 0.5 mg/kg of d-amphetamine showed that more climbings were made by the three youngest ages than the three oldest ages. When administered 1.0 mg/kg, climbings were higher in the three youngest groups than the two oldest groups and 17-day-old rats made more than 36-day-olds. In rats injected with 4.0 mg/kg, 21 and 36-day-old rats made significantly more than 15, 90, and 275 and 21 more than 15 and 17-day-olds. There were no significant age differences in animals given the two highest doses.

The wall climbing data of Experiment 2 generally support the findings of Experiment 1. In the three youngest ages d-amphetamine, particularly at lower doses, increased climbing but higher doses did not alter climbing. In adolescents, 4.0 mg/kg slightly increased climbing but in adults d-amphetamine did not significantly alter climbing. This developmental pattern suggests that catecholamines may play a role in climbing of infant rats but catecholamines do not appear to be involved in climbing of adults.

Comparison of the upper and lower panels of Fig. 4 shows that the dose-response curves for photo-cell crossings and climbing were more similar in infants than adults. Developmental differences in the dose-response curves for photo-crossings and climbing indicate that, at least for d- and l-amphetamine, the type of behaviors examined can be a major factor in developmental psychopharmacological studies.

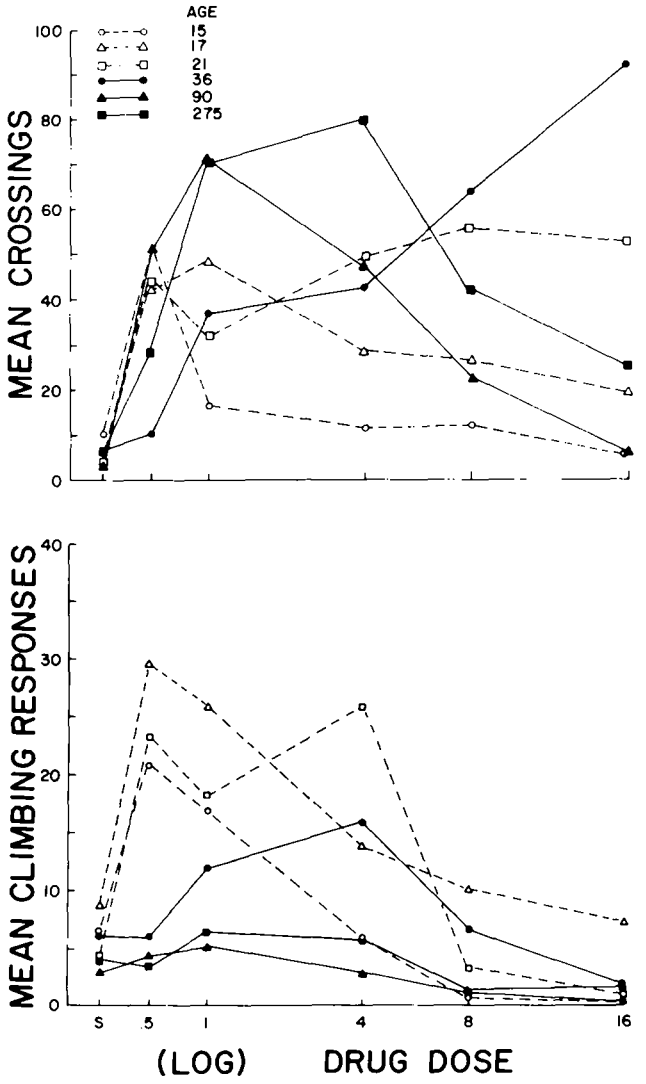

FIG. 4. The mean number of photo-cell crossings (upper panel) and rearing responses (lower panel) for the total session as a function of age and dose.

COMPARISON OF EXPERIMENTS 1 AND 2

Since the only major difference between Experiments 1 and 2 was the type of amphetamine isomer administered, the results of these two experiments were compared.

Photo-Cell Crossings

Within each age group, the total number of photo-cell crossings for comparable doses of l- and d-amphetamine were compared by Tukey's test (in all comparisons between l- and d-amphetamine, Tukey's tests were computed using the mean of the mean squared error variance of the 6×6×16 analyses of variance reported in Experiments 1 and 2). In the youngest group, rats given 1.0 mg/kg of l-amphetamine made significantly more photo-crossings than their same age counterparts injected with a comparable dose of d-amphetamine. In 17-day-old rats, 4.0 mg/kg of l-amphetamine increased crossings significantly more than 4.0 mg/kg of d-amphetamine. There were no significant differences between comparable doses of the two drugs in 21-day-old rats. In 36-day-old animals, the two highest doses of d-amphetamine increased crossings more than the two highest doses of l-amphetamine. Photo-cell-crossings were higher in 90-day-old rats given 0.5 and 1.0 mg/kg of d-amphetamine than these doses of l-amphetamine, but 90-day-old rats given the highest dose of d-amphetamine were less active than those given the highest dose of l-amphetamine. The oldest rats injected with 1.0 mg/kg of d-amphetamine were more active than those injected with a comparable dose of l-amphetamine.

In support of previous findings with adult rats [24], photo-crossings were increased more by low doses of d-amphetamine than comparable doses of l-amphetamine, but photo-crossings were reduced more by higher doses of d-amphetamine than l-amphetamine. Thus, for locomotor activity of adults, d-amphetamine is more potent than l-amphetamine [24]. However, a comparable potency difference was not found in 15, 17, and 21-day-old rats. In 36-day-old rats the two highest doses of d-amphetamine increased photo-cell crossings more than the two highest doses of l-amphetamine. Thus, there is a potency difference in adolescent rats, but this difference only occurs at very high doses.

Increased locomotor activity from l- and d-amphetamine is thought to be due to an action on norepinephrine, and stereotyped behavior, with a concommitant reduction in locomotor behavior, is thought to be due to a dopaminergic action [7, 11, 16, 19, 24]. Thus, at least for locomotor behavior, the noradrenergic and dopaminergic action of l- and d-amphetamine seem to be more similar in the three youngest ages than in adults. In 36-day-old rats the noradrenergic action of these drugs appear to predominate over their dopaminergic action. The earlier development of noradrenergic neurons, as compared to dopaminergic neurons [21], may be the underlying basis for the developmental differences in potency between l- and d-amphetamine.

Wall Climbing

For each age group, the mean for the total number of climbing responses for comparable l- and d-amphetamine doses were compared by Tukey's test. There were no significant differences between comparable doses of these drugs in 15, 36, 90, and 275-day-old rats. In 17-day-old rats given 0.5 mg/kg, climbings were higher in rats given d-amphetamine than those given l-amphetamine, but at this age climbing was higher in rats given 4.0 mg/kg of l-amphetamine than this dose of d-amphetamine. More climbings were made by 21-day-old rats given 0.5 mg/kg of d-amphetamine than the same dose of l-amphetamine, but at this age more climbings were made by animals given 8.0 mg/kg of l-amphetamine than this dose of d-amphetamine. Since in 17 and 21-day-old groups lower concentrations of d-amphetamine increased climbing more than l-amphetamine but higher concentrations of l-amphetamine increase climbing more than d-amphetamine, it appears that at these ages d-amphetamine is more potent than l-amphetamine. While the transmitter(s) involved in wall climbing is not known, the presumed more potent action of d-amphetamine on norepinephrine [11, 19, 24] suggests that, at least in 17 and 21-day-old animals, norepinephrine may play a slightly greater role than dopamine. The more rapid development of noradrenergic neurons [21] may be responsible for the behavioral difference in 17 and 21-day-old rats as compared to other ages.

EXPERIMENT 3

The behavioral changes produced by l- and d-amphetamine may be due to their action in the central and/or peripheral nervous system, because these drugs act on both [2,13]. Parahydroxy-amphetamine is equipotent with l- and d-amphetamine in the peripheral nervous system but, due to the relative inability to cross the blood-brain barrier, the central actions of parahydroxy-amphetamine are minimal [13]. For this reason, comparison of l- and d-amphetamine with parahydroxy-amphetamine has been used to separate central and peripheral effects. To examine the possibility that the age-dependent changes in response to l- and d-amphetamine are due to peripheral actions, in Experiment 3 photo-cell crossings and climbing were recorded in different ages following injection of parahydroxy-amphetamine.

METHOD

Subjects, Apparatus and Procedure

Rats with the same characteristics as those described in Experiment 1 served as subjects. The apparatus was the same as the two previous experiments. Rats of 15, 17, 21, 36, or 90 days of age were injected with either 1.0, 4.0, or 16.0 mg/kg of parahydroxy-amphetamine (n=11 per each age and each dose group). Other procedures were like those described in Experiment 1.

RESULTS AND DISCUSSION

For each animal the number of photo-cell crossings during 15 min intervals was determined and these counts were analyzed by a 4 (dose)×5 (age)×16 (blocks) mixed analysis of variance. Since saline controls of Experiments 1 and 2 were very similar, in analyses of Experiment 3, rats injected with saline in Experiment 1 served as controls. The main effect for blocks was significant, $F(15,3000)=3.03$, $p<0.001$, but the main effect for drug dose and all interactions involving dose were not significant.

A 4×5×16 mixed analysis of variance of the number of wall climbing responses revealed a significant main effect for blocks, $F(15,3000)=3.21$, $p<0.01$, but no significant effects involving drug dose.

These findings with parahydroxy-amphetamine suggest that ontogenetic effects of l- and d-amphetamine are due to maturation of the central nervous system and not to maturation of the peripheral nervous system.

GENERAL DISCUSSION

Considerable evidence indicates that catecholaminergic neurons in the central nervous system of the rat mature from birth to about puberty [17,21]. In light of the evidence that amphetamine produces behavioral changes by acting on central nervous system catecholamines, it appears that the differential behavioral effects of l- and d-amphetamine in immature and mature animals are due to some aspect of neural maturation. Catecholamine release, reuptake, and monoamine oxidase, the primary catabolic enzyme of catecholamines, are all known to exhibit developmental changes [17,21]. Amphetamine acts presynaptically by increasing the release and reducing the reuptake of norepinephrine and dopamine [7, 11, 24] and at higher doses there is also evidence that amphetamine inhibits monoamine oxidase [7]. Therefore, age related differences in catecholamine release, reuptake, and/or monoamine oxidase may be the underlying basis for the differential behavioral effects of amphetamine as a function of development. Since catecholaminergic axons grow in a rostral direction during development [17], incomplete synaptogenesis of catecholamine neurons may also be responsible for maturational changes in response to amphetamine. Catecholamine receptor sites on the postsynaptic membrane are thought to be functional prior to synaptogenesis [17], suggesting that maturational differences in receptor sensitivity are not responsible for the present behavioral results. However, when receptor sites are first innervated by presynaptic terminals, they may be hypersensitive. For this reason, developmental changes on the postsynaptic membrane may also play a role in the present findings.

Although catecholamine development may be the underlying basis for the age-dependent behavioral effects of amphetamine, maturation of neurons which release other purported neurotransmitters may also be involved. Serotonergic, cholinergic, and catecholaminergic neurons are known to develop at about the same rate [17,21]. Higher doses of l- and d-amphetamine (>5.0 mg/kg) release acetylcholine and serotonin in adults [11,25] and acetylcholine release occurs with lower doses in immature rats than adults [25]. There-

fore, the possibility that l- and d-amphetamine—particularly at higher doses—may produced differential behavioral effects in immature and mature rats by acting either directly or indirectly on serotonin and/or acetylcholine cannot be excluded.

In the rat, the blood-brain barrier for some substances appears to gradually develop until adult status is attained at about 25 days of age [28]. However, following systemic injections, brain d-amphetamine levels are similar in infant, adolescent, and adult rats [8], suggesting that the blood-brain barrier for d-amphetamine develops quite early. In addition, if the blood-brain barrier for amphetamine was not developed, parahydroxy-amphetamine may have central effects in young animals. As shown in Experiment 3, parahydroxy-amphetamine did not alter locomotor activity or climbing. Thus, at least with the ages used in the present study, it appears that development of the blood-brain barrier does not play a critical role in developmental psychopharmacological studies with amphetamine.

The results of Experiments 1 and 2 show that across time some l- and d-amphetamine doses result in a U-shaped function, other doses initially increase activity followed by a subsequent decline, whereas other doses are without effect. The doses which result in such temporal changes also appear to differ as a function of the animal's maturity. Low to moderate doses of d-, l-, and dl-amphetamine are thought to increase locomotor activity by excitation of noradrenergic neurons [9, 11, 19, 24]; higher doses produce a variety of stereotyped responses, such as sniffing, and head turning, which are incompatible with locomotor activity [11, 19, 24]. These stereotyped responses are thought to be due to excitation of dopaminergic neurons [11, 16, 19, 24]. Thus, heightened locomotor activity at certain times after amphetamine may be due to a predominantly noradrenergic action, whereas lower locomotor activity may be due to a predominantly dopaminergic action. Differences in the rate at which norepinephrine and dopamine containing neurons mature [17,21] may account for the finding that the doses which produce a U-shaped temporal curve or higher doses which did not alter locomotor activity differed across ages.

Depletion of central nervous system catecholamines is also a factor which could account for the reduced activity seen at certain times after high amphetamine doses. After injections of higher doses of d- and l-amphetamine, catecholamine release exceeds biosynthesis; consequently stores of central nervous system catecholamines are gradually depleted [4,20]. Drug induced catecholamine depletion leads to decreased locomotor activity and sedation [11]. Furthermore, drug induced catecholamine depletion is more rapid and more prolonged in immature rats than adults [15]. Slower biosynthesis, poor storage mechanisms, and reduced reuptake are all factors which may contribute to the more rapid depletion of catecholamines in immature animals than adults [15]. Thus, a developmental differential between catecholamine synthesis and depletion may be responsible for some of the maturational differences found in the present study.

d-Amphetamine metabolism is reported to be slower in immature rats than adults [10]. If metabolic differences are primarily responsible for the ontogenetic consequences of amphetamine, then across time the behavioral effects of amphetamine would be expected to change at a gradual rate but the rate of change should be slower in immature rats than adults. In Experiments 1 and 2, photo-cell crossings and climbing, at least in the early portion of the session, do not appear to decrease at a slower rate in young rats than adults. In addition, in the present and previous reports some amphetamine doses result in a U-shaped temporal function in infants and adults [5,16]. The activity increase at the end of the recording session is difficult to account for on the basis of amphetamine metabolism, because more amphetamine should be metabolized at the end of the session than earlier in the session. Thus, both the present and previous findings appear to be inconsistent with the possibility that developmental differences in amphetamine metabolism alone are primarily responsible for the age-dependent temporal changes induced by amphetamine.

In conclusion, results of the present studies indicate that d- and l-amphetamine have differential behavioral effects in immature and mature rats, as a function of (a) drug dose, (b) time after administration, and (c) the type of behavioral response examined. These differential behavioral effects may be due to some aspect of catecholamine maturation, such as release, reuptake, monoamine oxidase, or synaptogenesis. However, other developmental changes, such as maturation of cholinergic and/or serotoninergic neurons, amphetamine metabolism, and catecholamine depletion may also play a role in these age-related behavioral effects. On the basis of the present evidence, the relative importance of these factors cannot be specified. Nevertheless, the present findings are consistent with the hypothesis that the behavioral effects of amphetamine are dependent on development of the central nervous system.

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