Bulletin of the Psychonomic Society
1992, 30 (5), 359-362

The development of locomotor response to *d*- and *l*-amphetamine in the infant mouse

DONALD RAY
Mount Union College, Alliance, Ohio
and
Z. MICHAEL NAGY
Bowling Green State University, Bowling Green, Ohio

To study the possible role of catecholaminergic processes underlying the exaggerated expression of behavioral arousal during early development in the mouse, the locomotor activity of 10- to 12-day-old mice following *d*-amphetamine injection was recorded over a 5-h period in a shuttle-cage apparatus. Reliable effects of a 1-mg/kg dose of *d*-amphetamine were observed only in the oldest age group. The relative influences of *d*- and *l*-amphetamine were then assessed at this age. The stereoisomers were approximately equipotent in elevating activity above saline control levels and demonstrated comparable latency and duration of effects. The results support the notion that heightened arousal at this age is mediated by a noradrenergic system.

The ability to moderate reactivity to novel stimuli undergoes dramatic change for most mammalian species during the neonatal period. For example, spontaneous locomotor activity, one measure of behavioral arousal, develops in altricial rodents as an inverted U-shaped function with increasing age. When removed from the home cage and placed into an unfamiliar setting, rats (e.g., Campbell, Lytle, & Fibiger, 1969), mice (e.g., Nagy, Murphy, & Ray, 1975), and hamsters (Campbell & Mabry, 1972) demonstrate a rapid increase in activity beginning shortly after the first week of life, peak levels during the second or third week, and a gradual decrease to adult levels sometime in the second month. This age-related change in locomotor activity may exemplify the idea that young animals show a highly adaptive shift from an "approach" to a "withdrawal" response mode to low-intensity stimuli (Schneirla, 1965). Greater exposure to varied environmental inputs, brought about through heightened activity of the very young animal, might ensure optimal rates of behavioral as well as physiological development. The later decline in general activity levels, despite an improved sensorimotor potential, possibly reflects the organism's emerging capacity for behavioral suppression.

Neurobiological research suggests that these response modes may correspond to at least two distinct but highly integrated brain processes (see Carlton, 1969). Furthermore, the apparent developmental shift from one mode to the other may be the result of a differential maturation rate of these processes: initial activity increases in these species might be the manifestation of the early development of catecholamine (CA) excitatory mechanisms of the lower brainstem (Campbell & Mabry, 1973; Forster & Nagy, 1983), whereas later decreased responding might signal the delayed functional emergence of limbic and/or cortical inhibitory processes (Moorcroft, 1971), at least involving cholinergic (Campbell et al., 1969; Murphy & Nagy, 1976; Ray & Nagy, 1978), serotonergic (Lucot & Seiden, 1986; Mabry & Campbell, 1974; Nagy & Forster, 1982), and gamma-aminobutyric acid (GABA) systems (Murphy, Meeker, Porada, & Nagy, 1979).

Adopting an ontogenetic approach may provide a powerful means for dissociating the respective behavioral roles of excitatory and inhibitory processes. The present investigation focused on the contribution of CA excitatory mechanisms on general locomotor activity of the developing mouse. The study of excitatory function was restricted to observations at ages in this species at which the disinhibitory effects of anticholinergic, antiserotonergic, or anti-GABAergic drugs on locomotor behaviors would be relatively small compared with disinhibitory effects in older mice. Previous studies of the mouse in the shuttle-cage apparatus suggested that, except for a marginal serotonergic influence (Nagy & Forster, 1982), inhibitory processes involving locomotor activities were not in evidence before 13 days of age (Murphy et al., 1979; Ray & Nagy, 1978). Therefore, one purpose of the study was to examine the effects of CA manipulation on locomotor activity over an age range in which infant mice appear to exhibit relatively greater excitatory as opposed to inhibitory capacities. The use of mice 10, 11, and 12 days of age was thought to satisfy this objective. CA alteration was attempted using *d*-amphetamine at a dose reported to result in reliable effects on activity of mice and rats within this age range (e.g., Alleva & Bignami, 1985; Campbell et al., 1969; Sobrian, Weltman, & Pappas, 1975).

A second purpose of the study was to assess the nature of CA involvement in the onset of peak activity levels.

Correspondence should be sent to Z. M. Nagy, Department of Psychology, Bowling Green State University, Bowling Green, OH 43403.

Of primary concern was the degree of dopaminergic (DA) versus noradrenergic (NA) influence on this developmental trend. Spontaneous locomotor activity has been thought to be mediated by a NA system, whereas stereotyped behavior is suggested to be under DA control (North, Harik, & Snyder, 1974). Separating these systems has proved difficult because of the overlapping effects of the drugs used to study them, and this has been especially true for the *d*- and *l*-isomers of amphetamine (e.g., Bunney, Walters, Kuhar, Roth, & Aghajanian, 1975). Although still controversial, the relative potencies of the two isomers may indicate the differential involvement of DA and NA mechanisms. If a behavior is affected by approximately equal low doses of *d*- and *l*-amphetamine, then the behavior may be mediated mainly by the NA system. Conversely, a DA-mediated behavior may be indicated by greater potency of the *d*- relative to the *l*-isomer (Bunney et al., 1975). Although both *d*- and *l*-amphetamine are capable of affecting levels of both norepinephrine and DA, *l*-amphetamine has also been reported to decrease levels of serotonin, and *d*-amphetamine has little such effect in adult rats (e.g., Aulakh, Bhattacharyya, & Pradhan, 1982) and mice (e.g., Zabik, Levine, & Maickel, 1978). Another means of distinguishing NA from DA involvement might relate to the time course of isomer action (Segal, 1975). According to this view, the involvement of a DA component in the control of activity would be manifested by a shorter latency of onset as well as a shorter duration of effect by *d*-amphetamine relative to that of *l*-amphetamine. To test both possibilities, it was necessary to determine the differential influence of these isomers at doses that would allow the assessment of relative potency over an extensive dosage range and to test these effects over a period sufficiently long to permit discovery of any phase differences between drug treatments.

EXPERIMENT 1

Method

Subjects. A total of 96 Swiss-Webster mice (*Mus musculus*), born and raised in the Bowling Green State University Psychology Department mouse colony, served as the subjects in this experiment. The subjects were drawn from litters that had been culled to 8-10 pups the day following birth and, except during testing, were kept with their mothers in 30.4×18×12.8 cm polyethylene cages with wire-grid tops and wood chips on the floor. The colony was maintained at 24±1° C and was on a normal 12:12-h light:dark cycle beginning at 0800 h. Ad-lib food and water were available throughout the study.

Apparatus. Testing was conducted in clear Plexiglas cages measuring 19.4×6.4×9 cm with grid floors of 1-mm-diameter stainless steel rods spaced 4 mm apart center to center and extending parallel to the length of the cage. Red-filtered light sources and photocells, placed 3.4 cm from each end, enabled recording of activity on apparatus located outside the soundproofed, humidity-controlled environmental chamber. The chamber, ventilated with a fan producing an ambient 74-dB noise level, was maintained at 24±1° C and illuminated by two 60-W fluorescent ceiling lights.

Activity counts were registered when a subject crossed alternate photocell beams. Thus, to register one activity count, a mouse had to traverse the distance between photocells. Counts were recorded automatically and printed every 15 min.

Procedure. Separate groups of mice were tested for a single 5.5-h session at 10, 11, or 12 days of age. Using a modified split-litter design, the subjects were assigned to groups such that no more than 1 male and 1 female from a litter were assigned to each age and treatment group. Subject identification was accomplished by toe clipping at 2 days of age.

At the appropriate age, the subjects were removed from the home cage and placed into the activity apparatus. Thirty-two mice of each age, 16 of each gender, were tested for a 30-min adaptation period in the activity cages. On the basis of their activity, mice were later assigned to drug-treatment groups such that the preinjection mean activity levels of the groups were as equivalent as possible. Mice in the drug groups received a single i.p. injection of 1 mg/kg *d*-amphetamine sulfate (Smith, Kline, and French Laboratories) in saline solution, and those in the control groups received saline vehicle injections. Immediately following injections, the animals were returned to the shuttle cages and their activity was recorded for the next 5 h of the session.

After testing, the subjects were returned to their home cages. Activity cages were cleaned with alcohol and checked between each session. Testing was conducted between 0800 and 1700 h during the normal 12-h light cycle. Except for normal maintenance procedures, the mice were not disturbed or handled other than during test sessions.

Results and Discussion

Mean pre- and postinjection activity scores are presented in Figure 1 as a function of age, drug group, and 15-min injection interval. The preinjection data were evaluated by a four-way analysis of variance (ANOVA) with one repeated measure and with age, drug group, gender, and 15-min injection interval as factors. The only reliable effect found was that of age [$F(2,84) = 7.39, p < .01$]. Tukey pairwise comparisons revealed the source of this effect to be a dramatic, though expected (cf. Nagy et al., 1975), increase in activity between 10-11 and 12 days of age ($ps < .01$).

A similar analysis was conducted on the postinjection scores. Activity increased as a monotonic function of age [$F(2,84) = 14.74, p < .001$]. Main effects for drug group [$F(1,84) = 11.37, p < .001$] and 15-min interval [$F(19,1596) = 3.96, p < .001$] were also found. However, of greater interest was a significant age × drug interaction [$F(2,84) = 4.89, p < .05$]. Tests for simple main effects (Kirk, 1968) revealed that amphetamine-induced activity increases at this dose are limited to 12-day-olds

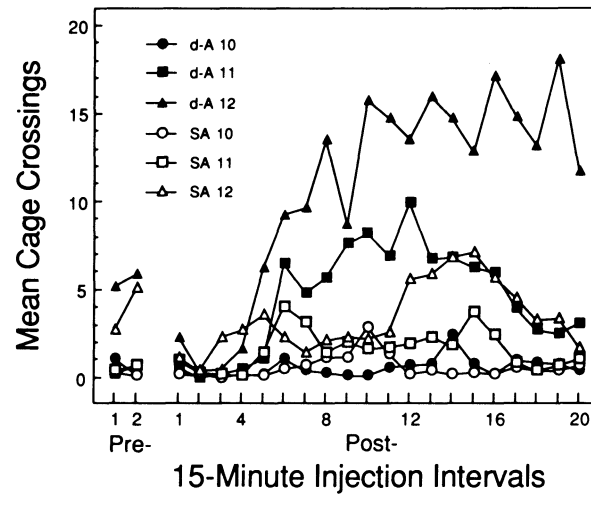

Figure 1. Mean numbers of cage crossings during pre- and postinjection activity tests as a function of age, drug group, and 15-min interval.

[$F(1,84) = 18.87, p < .01$]. As can be seen in Figure 1, the only significant age differences among the amphetamine-treated subjects [$F(2,84) = 4.05, p < .05$] were those associated with increases at 12 days of age ($ps < .05$). Activity remained fairly constant across ages within the saline control condition.

Among the saline groups, age-related changes in activity levels within the narrow age range studied here appear limited mainly to the preinjection periods of testing. However, it is possible that the injection experience per se in the saline groups suppressed activity and masked possible age differences in activity during the remainder of the 5-h session. Among age groups receiving *d*-amphetamine, only the 12-day-old group provided evidence for sensitivity to CA manipulation by a 1-mg/kg dose of *d*-amphetamine. Therefore, we focused on the 12-day-old mouse in Experiment 2.

EXPERIMENT 2

Method

Subjects. Various doses of *d*- and *l*-amphetamine were administered to 144 12-day-old male and female mice. The injection procedures, housing, and testing procedures were the same as described in Experiment 1

Procedure. At 12 days of age, 72 males and 72 females received a 30-min adaptation period in the activity cages. Scores were again used to assign 8 males and 8 females to one of nine drug-treatment groups. Separate groups received i.p. injections of *d*-amphetamine sulfate (0.5, 1.0, 2.0, or 4.0 mg/kg), *l*-amphetamine (1.0, 2.0, 10.0, or 20.0 mg/kg; Smith, Kline, and French Laboratories), or saline vehicle only. The range of dosages selected for study in the two isomers reflects the marked difference in toxicity between them. Following injection, the mice were returned to the activity cages for a 5-h postinjection testing period.

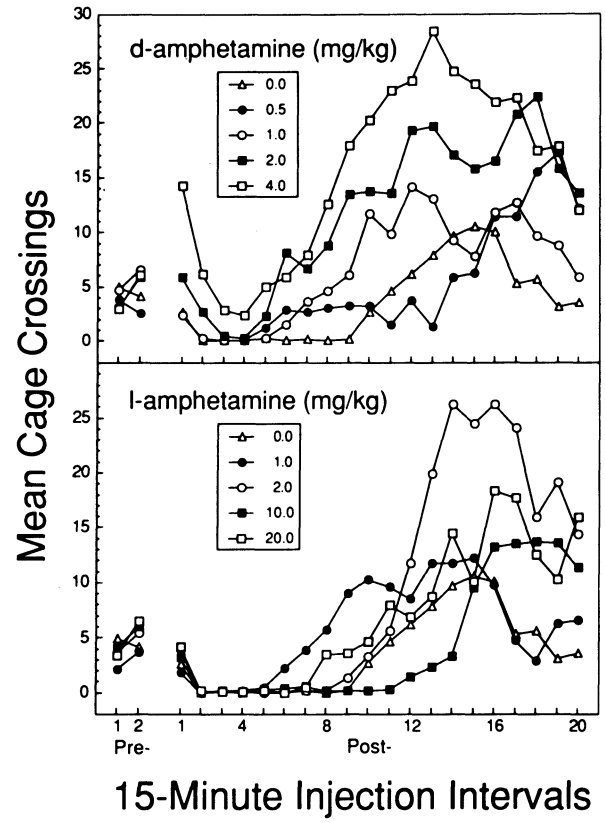

Figure 2. Mean numbers of cage crossings by 12-day-old mice during pre- and postinjection intervals of *d*- (upper panel) and *l*-amphetamine (lower panel) isomers as a function of drug dose and 15-min interval.

Results and Discussion

Figure 2 presents the mean number of pre- and postinjection crossings as a function of drug dose and 15-min injection interval for groups receiving *d*- (upper panel) or *l*-amphetamine (lower panel). Note that crossings for the saline controls are duplicated in both panels for ease of reference. An initial three-way ANOVA with one repeated measure, conducted on the preinjection activity scores with drug group, gender, and 15-min interval as factors, revealed only a marginally significant increase in activity from the first to the second 15-min interval [$F(1,126) = 5.74, p < .05$] for all groups overall. Thus, all groups were equally active prior to the injections.

Because different levels of the two isomers were given, their effects on locomotor activity were assessed by three different ANOVAs. The first compared the postinjection activity levels of mice given either 1.0 or 2.0 mg/kg of *d*- or *l*-amphetamine. This four-way ANOVA, with drug (*d*- or *l*-amphetamine), dose (1.0 or 2.0 mg/kg), gender, and 15-min postinjection interval as factors, indicated significant differences only of 15-min interval [$F(19,1064) = 9.58, p < .0001$] and of the dose × 15-min interval interaction [$F(19,1064) = 1.84, p < .025$], with the higher dosage resulting in significantly higher levels of activity from the 13th-20th 15-min intervals [all $Fs(1,1064) > 13.93$; all $ps < .0001$]. The main effect of drug was not significant ($F < 1.00$), and no interaction at any level with the drug factor approached significance. Thus, there was no evidence of any statistically significant differences in the latency or duration of effect between the *d*- and *l*-amphetamine isomers within either the 1.0- or 2.0-mg/kg doses.

The second ANOVA examined the postinjection effects of *d*-amphetamine, and the third ANOVA examined the effects of *l*-amphetamine on spontaneous locomotor activity. In each case, a three-way ANOVA was conducted, with the main factors in each being dose, gender, and 15-min interval. As the primary interest in each analysis was the relative differences in time course and duration of effect within each isomer, planned comparisons were conducted between the saline control group and each drug dose at each 15-min interval for each amphetamine isomer.

As suggested by examination of the upper panel of Figure 2, following an initial decline in activity during the first hour that probably reflected a consequence of the injection procedure per se, activity increased over the next 2 h as a function of dose and then declined over the last 2 h. The planned comparisons (drug vs. saline control) revealed that the only significant increases in activity due to *d*-amphetamine occurred at the 9th, 17th, and 18th intervals for the 2.0-mg/kg dose (all $ps < .05$) and from the 9th-14th intervals (all $ps < .05$) for the 4.0-mg/kg dose. Although the absolute amount of activity increase

clearly varied as a function of *d*-amphetamine dose, it is also clear that there was little difference among the doses in terms of the latency of their effect. Groups receiving 1.0, 2.0, and 4.0 mg/kg began to exhibit increasingly higher levels of activity at the 6th 15-min interval, at least 45 min earlier than the saline group. The fact that the saline group exhibited a decline in activity following injection that persisted for about 2 h, followed by a 2-h increase in activity, suggests that the initial decline was due to the injection experience, while the subsequent 2-h increase may have been a rebound effect to preinjection levels. Thus, at least some part of the increase in activity displayed by the drug groups may have been due to recovery from the effect of the injection experience per se.

Among the *l*-amphetamine groups depicted in the lower panel of Figure 2, the data suggest somewhat different dose-related activity changes than were obtained for *d*-amphetamine. For example, the highest overall level of activity was displayed by the 2.0-mg/kg group, with the 10.0- and 20.0-mg/kg groups displaying lower overall levels. There is also some evidence that the latency of the onset of the drug effect varied differentially with dose level, as the 1.0-mg/kg group showed the earliest increase and the 10.0-mg/kg group showed the latest. The planned comparisons between the saline and drug groups revealed significantly higher activity levels only at the 13th–17th and 19th intervals for the 2.0-mg/kg group and only at the 17th and 19th intervals for the 20.0-mg/kg group (all $ps < .05$). All other comparisons were not significant.

On the basis of the comparisons between *d*- and *l*-amphetamine at comparable low doses, it appears that the isomers are roughly equipotent in altering shuttle activity. Thus, a case can be made for the idea that the drug effects discussed so far are mediated by an NA mechanism (Bunney et al., 1975). The fact that higher doses of *l*-amphetamine were generally ineffective might have been attributable to toxic effects of these drugs at the age under study.

The temporal pattern of drug effects also favors the interpretation that peak activity levels during development are a result of the maturation of an NA excitatory system. Although significant effects of *d*-amphetamine were observed a little over 1 h before those of *l*-amphetamine, there is no reason to believe the delayed effects of the *l*-isomer were more protracted. By the last 30 min of testing, few differences between the isomers were detected. This finding detracts from the suggestion that locomotion is mediated by a DA system (Segal, 1975). These results, taken with the finding that the NA blocker FLA-63 attenuates activity increases in weanling mice (Murphy et al., 1979), support the contention that NA excitatory processes play a major part in the heightened activity increases in rats and mice just prior to the emergence of inhibitory mechanisms.

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(Manuscript received February 24, 1992.)