Age-related differences in rats' spontaneous alternation*

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Fifteen-day-old rats made random choices in a T-maze, while adult levels of alternation (approximately 70%) were noted among 30-day-olds. Also, younger pups were insensitive to a lengthening of the intertrial interval, unlike older animals whose rate of alternation decreased when a 1-h delay was interposed between arm entries. These findings are consistent with the hypothesis that hippocampal development is a factor in the ontogeny of postweaning locomotion. The unsystematic pattern of goal-arm selections among 15-day-olds suggests that ontogenetic variation in the utilization of stimuli may underlie developmental shifts in spontaneous alternation.

Altman, Brunner, and Bayer (1973) have suggested that the 2- or 3-week-old domestic rat is behaviorally similar to the hippocampectomized adult of the species. Further, maturation rates of cells at the dentate gyrus and the development of cholinergic control over behavior are consistent with this brain-development hypothesis (Campbell, Lytle, & Fibiger, 1969; Douglas, 1972).

This particular behavioral syndrome, typical of the young rat, includes: (a) elevated and persistent motor activity (Bronstein, Neiman, Wolkoff, & Levine, 1974; Campbell et al, 1969; Feigley, Parsons, Hamilton, & Spear, 1972), (b) considerable difficulty in the acquisition of a passive-avoidance task to a footshock US (e.g., Ricco & Marazzo, 1972), (c) impaired position-habit reversal (Harley & Moody, 1973), and (d) low levels of spontaneous alternation (Douglas, Peterson, & Douglas, 1973; Kirkby, 1967). The present paper describes attempts to replicate and to further understand the age-related differences in spontaneous alternation.

EXPERIMENT I

The first experiment is a longitudinal investigation of alternation in the rat.

Method

Subjects. Twenty-three male and 14 female rat pups served as Ss. These were derived from four Sprague-Dawley albino litters (9 or 10 pups/dam) born in the Brooklyn College laboratories. The animals were individually marked and housed with their mothers in plastic litter cages. Twenty-two days after parturition, the maternal females were removed from their offspring with all pups left behind in the litter boxes. When the young were 31 days old, they were rehoused, 2 to a cage (same-sex pairs), except for a few individually caged Ss. Purina Lab Chow and water were always available, and the colony was

heated to about 74°F. The vivarium was brightly lighted from 8 a.m. to 2 a.m. daily, and Ss were run during the light part of the cycle, always at about the same time of day.

Apparatus. One bottomless plywood T-maze, painted flat black, was the apparatus. The maze was 6.40 cm high and initially had a width of 6.35 cm, but was widened to 10.80 cm throughout after the Ss were 31 days old. This enlargement was needed in order to accommodate the pups' growth. The apparatus was covered with clear Plexiglas, and three guillotine doors of white plastic divided the maze stem into a startbox and runway (each 20.32 cm long) and goal arms (each 28.85 cm long). A 15-W incandescent bulb, shaded upwards and suspended approximately 76 cm above the choicepoint, provided the only lighting in the experimental chamber.

Procedure. Beginning when the animals were 15 days old (all Ss had their eyes open), they were given one opportunity to alternate every 24 h. This procedure continued for 17 consecutive days, during which time entire litters were taken to the running room en masse. The pups were transported in a litter cage lacking food, water, and a maternal female. These initial trials were analyzed as three 4-day, and a final 5-day week. After 9 days without testing, the Ss, now 41 days old, were run as before for two 5-day weeks (Monday-Friday only). A last week of alternation trials (5 consecutive days) was initiated when the rats were 72 days old. In the interval between the 2 final weeks of alternation trials, Ss were handled by E and periodically given T-maze exposures. Rats were individually hand carried to the running room during the 3 final weeks of testing.

All trials began with an S being placed in the startbox facing the choicepoint. After 5 sec, all doors were lifted. The animal then had 60 sec in which to leave the startbox and a total of 3 min in which to turn into one of the arms. If one of these criteria was not met, that trial was terminated and the S was not included for that day. Retracing was prevented by lowering the startbox door following an animal's exit.

A turn was defined as the movement into one of the arms for a distance sufficient to permit the closing of the guillotine door without touching the rat, except for its tail. Each response was followed by 15 sec of goal-arm confinement. A 20-sec intertrial interval (ITI) followed, and, during this period, the S was placed in a small plastic bucket while the area under each maze was wiped with dilute acetic acid (2%-3%). This cleaning procedure was undertaken to mask any possible odor cues.

Results

For all studies reported herein, the statistical methods of Douglas (1966) were employed. Hence, all references to alternation represent "true" alternation, i.e., the percentage of within-day turns into opposite arms

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Fig. 1. The results of Experiment I are depicted in the left-hand panel, while the interaction of age and ITI (Experiment II) is noted in the right panel.

following a correction for any Trial 1 turn bias exhibited by the group. Two-tailed probabilities are used throughout.

The data are presented in the left panel of Fig. 1, and it is apparent that higher levels of alternation accompany increased age. Alternation exceeded the .001 level of significance during Weeks 3, 5, 6, and 7; the .05 criterion was attained during the second week. Levels of spontaneous alternation approached significance on Week 4 ($p < .16$), but behavior did not exceed chance ($p < .40$) during the initial 4 days of trials.

The first study is consistent with other claims that the rate of alternation in rats is a positive function of age during the first 2 or 3 months of life. The drop in spontaneous alternation among 29-day-olds (Week 4) does not seem to be a reliable inflection. Rats of this age typically show about 70% alternation in our laboratory (Dworkin & Bronstein, 1974).

EXPERIMENT II

Since the initiation of the present studies, Egger (1973) has also confirmed these ontogenetic aspects of alternation. His data are rather unique in that his 15- to 16-day-olds showed reliable perseveration (approximately 25% alternation). Juveniles in our laboratory typically choose maze arms randomly (see Experiment I). Kirkby (1967) and Douglas et al (1973) also report the nonsystematic turning among nonalternating and immature Ss. This uncertainty concerning the appropriate estimate of the young rat's behavior was the basis for Experiment II.

If 2-week-old rats do tend to perseverate, the near-50% alternation of juveniles at the start of Experiment I might not represent "chance" behavior. Rather, these seemingly insignificant rates of alternation could be conceptualized as reliable alternation superimposed upon a low, say 25%, baseline. Such a result might be obtained, for instance, if the stimuli controlling T-maze alternation varied with age.

To establish whether the age groups are each associated with a different baseline rate of alternation, ITI was applied orthogonally to the age variable. The aftereffects of a maze-arm choice are known to be degraded with a lengthened ITI (e.g., Egger, 1973). Older Ss, therefore, should exhibit a reduction in alternation with long ITIs. Among the younger and nonalternating Ss, however, it is uncertain whether an increased latency between turns will be of any consequence. If 15-day-olds were to reduce their alternation below 50% as ITI increased, two conclusions would seem reasonable. First, this "below zero" alternation would indicate that the usual 50% level represented some finite quantity of the behavior added to an underlying perseverative disposition. Second, it would be clear that some information was stored by 15-day-olds following the initial arm entry.

Method

There were 119 Ss, including male and female rat pups¹ (18 litters) taken from the previously noted colony stock and maintained in plastic litter cages with lactating dams. The mothers of nine litters remained with their offspring until the young were 30 days old; the remaining dams were removed from their pups between 24 and 26 days postpartum. (In one instance, the mother was found dead 20 days after parturition.) Food and water were available ad lib, and all other colony specifications remained as noted above.

Each rat was given four unreinforced trials in a T-maze. This was the smaller apparatus (6.35 cm wide) used in Experiment I. Two trials occurred when a pup was 15 or 16 days old (the first day when all littermates' eyes were fully open), with the retesting undertaken on Days 30-31. Counterbalancing for litter identity, half of the pups were run with a 1-min ITI on both testing days, the others received a 60-min ITI.

The animals were carried, in a fresh nesting box, half a litter at a time (between three and five Ss), to the experimental room. The transport cage did not contain food, water, or the maternal female. Using a 30-sec maze-arm confinement following all turns, the procedure of Experiment I was employed. The only exception to that methodology was that rats were returned to the carrying cage (containing littermates) for the appropriate ITI. Pelage markings identified the pups.

Results

As depicted in the right-hand panel of Fig. 1, the effects on alternation of increasing the intertrial latency depend upon S's age. At the 1-min ITI, the younger pups alternated at 47.4% ($p < .66$), while the 75.4% alternation of the 30-day-olds was highly reliable ($p < .001$). This age-related difference was determined to be significant by a McNemar test ($\chi^2 = 5.63$; $p < .02$, cf Siegel, 1956); however, the developmental trend was abolished at the 60-min ITI. Among 30-day-olds there was reliably more alternation at the shorter ITI ($p < .003$). Yet, the longer ITI did not significantly depress the alternation of the 15-day-olds.

GENERAL DISCUSSION

Lowered alternation among 15-day-olds was not induced by the long-delay condition of Experiment II.

It appears, then, that the 50% alternation of the younger pups is not some finite alternation superimposed upon a baseline of perseveration. Also, 15-day-olds are relatively impervious to the effects of ITI, regardless of their rate of alternation. The 50% alternators of the present paper are less sensitive to lengthened intertrial latency than are older Ss. The same relationship holds for Egger's infants, which displayed a 25% rate of alternation. Furthermore, the age-related change in T-maze choices is obtained even with very brief ITIs (Dworkin & Bronstein, 1974; Egger, 1973).

If one views the alternation situation as a learning paradigm, it might be concluded that there is no reliable acquisition among 15- to 20-day-olds. For our animals, at least, consecutive turns appear independent of each other. The randomness of T-maze choices among 15-day-olds suggests that these pups might be insensitive to the consequences of their responses. Hippocampectomized adults also seem unresponsive to environmental change in some situations (Altman et al, 1973). The proposed analogy between immature Ss and these surgically treated adults makes this stimulus-reactivity hypothesis more attractive.

The evidence concerning the ontogenesis of rodents' use of cues is not adequate for a thorough discussion of this hypothesis. Hence, our suggestion that 2-week-old rats may be insensitive to some T-maze cues is plausible but not firmly supported at this time. Since pups do not alternate and yet have difficulty in the acquisition of an appetitively motivated T-maze position habit (Bronstein & Spear, 1972), an analysis of age-related differences in the stimulus control of these responses seems appropriate. The perseveration of infant rats, however, appears to oppose the notion of juveniles' insensitivity. Since Egger's Ss were not behaving randomly, they must have either been utilizing some information or been turning in a stereotyped fashion.

Egger (1973) investigated these two interpretations. Reasoning from Douglas's (1966) model, alternation was attributed to the use of two stimuli. These are: (a) the avoidance of Trial 1 odor trails, and (b) the use of extramaze spatial cues to avoid the direction of a recently chosen path. After the removal of both factors, 15- to 16-day-olds showed a reliable elevation in their rates of alternation. It was concluded that 15-day-olds exploit the same stimuli in perseverating as those employed in alternation of adults. However, extramaze directional factors were eliminated by giving the second trial in a maze with a stem perpendicular to the Trial-1 start alley. This procedure is ambiguous. The intertrial rotation of a T-maze about the choicepoint confounds the, say, 90-deg shift in extramaze orientation, with the changed relationship of the Trial 1 stem and the goal arms on Trial 2. Dember and his associates (Sherrick & Dember, 1966) have shown rats to alternate relative to their direction of movement in a startstem or alleyway

during a prior run. Therefore, the present criticism of Egger's procedures is of some importance. It is not really clear whether nonalternating juveniles are responding to the same cues as older conspecifics. Also, the salience of other extramaze stimuli (e.g., brightness) cannot be excluded in Egger's design.

The present paper confirms the relationship between chronological age and the rate of spontaneous alternation in the rat. This behavior does not occur reliably prior to the age of about 25 or 30 days. This pattern of maturation more or less parallels the ontogenesis of cholinergic and serotonergic control over the rat's behavior (Egger, Livesey, & Dawson, 1973; Mabry & Campbell, 1974). The development of this behavior may, in part, reflect the growth of central nervous system control of responses as mediated by these two enzyme systems.

We have noted the need for a careful analysis of behavioral development from the point of view of stimulus control. Young rats are known to be hyperactive (e.g., Campbell et al, 1969), and such locomotion might represent exploratory activity (Bolles, 1967, p. 289). Since T-maze alternation has often been regarded as indicative of exploratory motivation (e.g., Fiske & Maddi, 1961), juveniles might be expected to show elevated levels of alternation. All of the evidence is against this expectation.

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NOTE

1. Thirty-seven additional Ss were discarded because they failed to meet the latency criteria on at least one trial at 15-16 days of age.

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