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Competing response decrement as a measure of escape learning and memory in young mice: Effect of learned inhibition, maturation, or age-dependent shock sensitivity?

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In order to further assess the validity of the competing response measure as an index of learning and memory abilities in young mice, three groups of mice under different response-reinforcement contingencies received consecutive daily 25-trial sessions on a straight-alley shock-escape task beginning at 7 days of age. In the first group, shock offset was contingent upon reaching the goal end of the alley on each trial. Each mouse in the second group received identical amounts of shock and handling on each trial as a littermate in the first group, but shock offset was not contingent upon reaching the goal for members of this group. Mice in a third group also began test sessions yoked to littermates in the first group, but were unyoked on the day following that on which their littermate achieved a criterion performance of a 50% reduction in competing responses from one session to the next. On all following days, shock offset was contingent upon reaching the goal on each trial. The results support earlier research suggesting that 24-h memory of escape training develops at about 9 days of age for this task, and demonstrate that changes in competing responses due to maturation and/or age-dependent differences in shock sensitivity can be separated from those due to learning/memory functions.

In a recent paper reviewing literature on the ontogeny of learning and memory in lower animals, Campbell and Coulter (1976) point out that remarkably few experiments have been related to the acquisition by very young organisms of basic response-reinforcement contingencies characteristic of simple approach or escape tasks. They state further that this paucity of studies may be due to the fact that learning by adult subjects on these tasks is typically assessed in terms of response speed, a measure which may place the immature animal at a disadvantage.

Over the past 7 years, research from our laboratory, as well as that from Misanin's lab at Susquehanna University, has examined the development of acquisition and memory processes by neonatal and infant mice and rats using a simple straight-alley shock-escape task. As suggested by Campbell and Coulter (1976), our research has clearly indicated that the running speed measure is, at best, inconsistent in reflecting improved escape behavior over a training session for rats (e.g., Misanin, Haigh, Hinderliter, & Nagy, 1973; Misanin, Nagy, Keiser, & Bowen, 1971) and mice (e.g., Nagy, Misanin, & Newman, 1970; Nagy, Misanin, Newman, Olsen, & Hinderliter, 1972) younger than 15 days of age. However, other indices suggest that escape performance improves for both species over a training session as early as 5 days of age. In addition to the increasing number of subjects which reach the goal end of the alley over the training session within the maximum trial latency (e.g., Nagy et al., 1972; Nagy & Misanin, 1973), responses which interfere with reaching the goal, such as competing responses (180-deg turns away from the goal), wall climbs, rolls (all four paws off the grids), and pivots (turns of less than 180 deg away from goal), all demonstrate significant decreases with continued training (Misanin et al., 1973). Based upon comparisons with maturation-control and yoked groups, our interpretation of these data has been that they reflect learned inhibition by these young animals. In spite of additional reports from our lab which demonstrated that variables which affect learning in adult animals also affect competing responses in a manner consistent with a learned inhibition interpretation (for review, see Nagy, 1976; Nagy, Anderson, & Mazzaferri, 1976), Campbell and Coulter (1976) have suggested that the differences in competing responses may simply reflect age-related changes in reactivity to shock.

More recent research, however, has supported our earlier conclusions that acquisition is possible at very early ages on simple tasks. For example, 7-day-old rats (Misanin et al., 1973) and 9-day-old mice (Nagy & Murphy, 1974) are capable of learning in a discriminated shock-escape T-maze task, while rats are capable of learning in an appetitive-approach task by 10 days of

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age (Amsel, Burdette, & Letz, 1976). Although alternative tasks are now available with which to study acquisition and retention functions at early ages in rats and mice, we decided to conduct one additional study employing the competing response measure in an attempt to further clarify its usefulness as an index of acquisition, especially in light of the fact that so much prior research has utilized this dependent measure. Therefore, the design employed in the present study was such that changes in competing responses due to maturation and/or age-dependent differences in shock sensitivity could be distinguished from those changes due to response-reinforcement contingencies in the straight-alley shock-escape task.

METHOD

Subjects

The subjects were 42 Swiss-Webster albino mice (*Mus musculus*), half of each sex, born and reared in 30.4 x 18 x 12 cm polyethylene cages with wire-grid tops and wood chips on the floor. Subjects were selected from seven different litters containing at least three mice of each sex. From each of the seven litters, one male and one female were arbitrarily assigned to each of three training groups. In this manner, each group was represented by a like-sex littermate. Mothers remained with the pups at all times, except during testing sessions. Nesting material was provided and ad-lib food and water were available. The colony room was on a normal 12-h light-dark cycle beginning at 0800 h, and both the colony and testing rooms were maintained at 24 ± 1°C.

Apparatus

The apparatus was a 22.8 x 3.2 x 6.4 cm Plexiglas straight alley with a grid floor of 1-mm-diam stainless steel rods spaced 3 mm center to center and parallel with the length of the alley. A 60-Hz scrambled ac shock source (Harvard Instrument Company, Model 3121) delivered .1-mA constant current to the grid floor. A removable Plexiglas door, placed 5 cm from one end of the alley, formed the startbox.

Procedure

Beginning at 7 days of age, each of the training groups was exposed to shock in the straight alley under one of three conditions. In the first, or Trained, group the mice received 25 escape training trials in which shock offset was contingent upon reaching the goal end of the alley on each trial. A trial began with the mouse pup being placed into the startbox, facing the goal end of the alley. After 3-5 sec, the door was removed, shock was initiated, and a running time meter (.1 sec) was started. When the mouse reached within 6 mm of the goal end of the alley, the shock and timer were turned off, and the mouse was removed from the alley and held in the experimenter's closed hand for a 45-sec intertrial interval. Subjects that did not reach the goal within 300 sec on any trial were gently prodded to the goal and the shock terminated. A maximum latency score of 300 sec was assigned for such trials. Care was exercised to insure that on all trials the subject was actively crawling or running forward at shock offset. Upon completion of the 25 trials, the pup was toe-clipped for identification and returned to the home cage. Each mouse pup in the Trained group received a similar training session at consecutive 24-h intervals until criterion performance was attained. When the number of competing responses (180-deg turns away from the goal) made within a training session by each pup decreased by at least 50% from one test session to the next, criterion was considered to have been achieved. However, training continued for at least 2 additional days following criterion or at least through 12 days of age. In this manner, the number of consecutive days that each mouse pup received training trials varied as a function of its own performance, but all pups received training from at least 7 through 12 days of age.

Each mouse in the second, or Yoked 2, group received identical amounts of handling and shock on each trial as did its littermate in the Trained group. The general training procedure was similar to that of the Trained group, with the exception that shock offset was not contingent upon reaching the goal for members in the Yoked 2 group, but rather upon the time required for its Trained littermate to reach the goal on each trial. The yoking procedure continued for each consecutive training day for the Trained group.

Mice in the third, or Yoked 1, group were generally treated in a similar fashion as their Yoked 2 littermates. However, on the day that each of their Trained group littermates achieved criterion performance, mice in the Yoked 1 group were unyoked on that day and on each following day so that shock offset for each pup was dependent upon reaching the goal on each trial.

RESULTS AND DISCUSSION

Because mice in the Trained group achieved criterion on different training days (8-12 days of age) and, consequently, mice in the Yoked 1 group were unyoked at different ages, conventional analysis of the data with age as a variable was not appropriate. Instead, performance scores were examined on 4 consecutive days, beginning with the day preceding criterion for each mouse in the Trained group. For example, if a particular mouse in the Trained group demonstrated greater than a 50% reduction in competing responses made during training sessions between 9 and 10 days of age, that subject's scores, as well as those of both littermates in the two yoked groups, would be derived from Days 9, 10, 11, and 12.

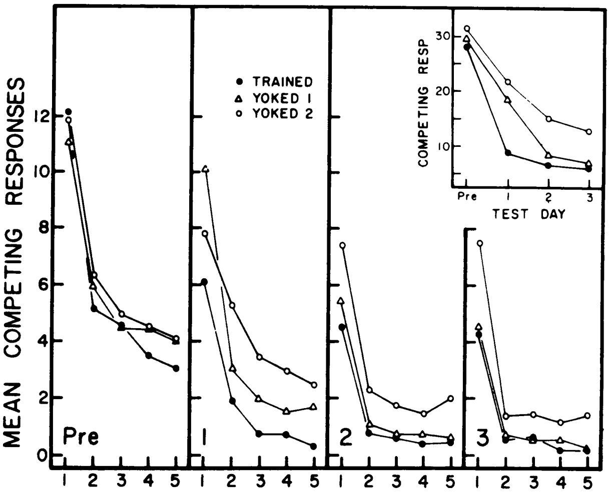

Figure 1. Mean number of competing responses made on the Pre criterion and 3 subsequent test days (based upon Trained group's performance) for all groups as a function of response-reinforcement contingencies and trial blocks. The figure inset depicts the mean total competing responses for all groups over the 4 test days.

While this procedure has the disadvantage that mice within a group are not of equivalent age on a particular test day, age or maturation level may be considered equivalent across groups since littermates were assigned to each group. Thus, any differences in performance across groups on a particular day cannot be attributed to maturation, but are most likely reflecting differences in training contingencies.

Figure 1 presents the mean number of competing responses made over trial blocks by all groups on 4 consecutive test days, commencing with the precriterion day for each Trained subject. The figure inset depicts the mean number of competing responses made during the entire test session for all groups on these days. A four-way analysis of variance was conducted on these data, with the factors being group, test day, gender, and trial block. Overall, competing responses differed among groups $[F(2,36) = 5.47, p < .05]$ and decreased over test days $[F(3,108) = 96.99, p < .0005]$ and trial blocks $[F(4,144) = 132.68, p < .0005]$. In addition, the three-way interaction among these factors was reliable $[F(24,432) = 1.53, p < .05]$.

To examine further the effect of the various training conditions upon the numbers of competing responses, t tests (two-tailed) were conducted among the scores depicted in the figure inset on each of the 4 days. On the day preceding the criterion day (Pre) for the Trained group, all groups exhibited an equivalent number of competing responses [all $ts(26) < .92$]. On Test Days 1-3, however, the Yoked 2 group, which received identical amounts of shock and times in the apparatus on each trial as their Trained group littermates, made significantly greater numbers of competing responses on each day than did the Trained group [all $ts(26) > 2.56$, all $ps < .02$]. This finding clearly demonstrates that neither time in the apparatus on each trial nor maturation can account for the differences in competing responses found between the Trained and Yoked 2 groups on Days 1-3. It further suggests that mice in the Trained group were sufficiently mature on the Pre test day to learn to inhibit competing responses and retain that inhibition for at least a 24-h interval, as indicated by the relatively few competing responses made on Test Day 1.

If, in fact, the large decrease in the number of competing responses exhibited by the Trained group between the Pre and first test days represents a memory function, it follows that all groups were sufficiently mature on the Pre test day to exhibit 24-h memory of prior training. However, the Yoked 1 group, which was first unyoked on Test Day 1, made significantly more competing responses than did the Trained group on that day $[t(26) = 2.83, p < .01]$, but did not differ from the Yoked 2 group $[t(26) = .91]$. On the day following the one on which shock offset was contingent upon reaching the goal for the Yoked 1 group (Test Day 2), that group made reliably fewer competing responses than did the Yoked 2 group $[t(26) = 2.54, p < .02]$, but did not differ from the Trained group $[t(26) = .53]$. This finding clearly indicates that, while maturation may account for part of the decrement in competing responses made over test days, the major component of the decrement exhibited by the Trained group from Pre to Test Day 1 and by the Yoked 1 group from Test Day 1 to Day 2 represents the memory of prior training conditions in which shock offset was contingent upon reaching the goal end of the alley. The mean age of the Trained group on the day preceding the large decrement in competing responses was 9.07. This result is consistent with previous findings that rats (Misanin et al., 1971) and mice (Nagy & Mueller, 1973; Nagy et al., 1972) are first capable of exhibiting 24-h retention of prior shock escape training in a straight alley by 9 days of age.

Although the Yoked 2 group made about twice the number of competing responses on each trial block over Days 1-3 than was made by the Trained group, the Yoked 2 group also exhibited a decrease in competing responses over trial blocks on each day. However, at least part of this decrement is due to the fact that the Trained group was reaching the goal more rapidly over trial blocks with continued training. On Test Day 1, the Trained group displayed a reliable increase in mean running speed, from .0755 on Trial Block 1 to .2069 by Trial Block 5. Increments on Test Day 2 were from .0963 to .2731, while on Day 3 speed increased from .1481 to .3225. Thus, there was less time available on later trial blocks on each test day for the Yoked 2 group to make competing responses. In addition, part of the decrement over trial blocks was probably due to the fact that competing responses did not lead to escape for the Yoked 2 group and their frequency of occurrence subsequently decreased. Perhaps the most striking difference in behavior between the Trained and Yoked 2 groups was in running behavior. As was previously reported (Nagy et al., 1972), the number of instances in which Trained mice reached the goalbox increased markedly over training trials, while the Yoked 2 mice increased the frequency with which they stopped traversing the alley and remained in the startbox for the trial duration.

Finally, the mean running speeds of the Trained group were compared to those of the Yoked 1 group on Test Days 1-3. While there were no differences found on Days 2 and 3 $[ts(26) < .26]$, the Yoked 1 group required significantly more time over trials to reach the goal on the first test day than did the Trained group $[t(26) = 2.09, p < .05]$. Although this finding might suggest a direct relationship between time in the alley and number of competing responses emitted, it must be recalled that the Yoked 1 group made about the same number of competing responses on Day 1 as did the Yoked 2 group, which, in turn, was in the alley the same amount of time as the Trained group on each trial. Thus, while there is some relationship between time in the alley and number of competing responses made, this simple relationship cannot account for the present results.

In conclusion, the results of this experiment provide further evidence that the competing response measure is a reliable and valid index of learning and memory functions at the neonatal and infantile stage of development in the mouse and rat. Although maturation clearly affects the rate of occurrence of this response, appropriate control groups can be readily utilized to separate those changes in competing responses due to maturation from those due to prior training or experience.

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