

Effects of Environmental, Social, and Auditory Enrichment on Maze Learning in Rats: Implications for Arousal

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Introduction

Rats raised in enriched conditions show neurobiological and behavioral plasticity ranging from neurogenesis to improved learning (Kempermann, Kuhn, & Gage, 1997; Rosenzweig & Bennett, 1996). Effects have been found following environmental enrichment (i.e., rats raised with the opportunity to interact with stimulus objects), social enrichment (i.e., rats raised in groups of 4 or more), and physical exercise (i.e., rats raised with access to a running wheel), all conditions that encourage motor activity. It has therefore been suggested that a voluntary increase in motor activity may be the factor most responsible for the neural and cognitive changes observed (van Praag, Kempermann, & Gage, 2000). Similarly, the "arousal hypothesis" suggests that arousal, rather than motor activity, may be the mechanism common to all forms of enrichment (Walsh & Cummins, 1975). This theory is supported by a study demonstrating improved maze learning in rats following auditory exposure (Rauscher, Robinson, & Jens, 1998).

The data exploring what happens to maze learning after enrichment ceases are not convincing. For example, one study found that one year of enriched living had lasting benefits on spatial learning in rats (Pham et al., 1999), whereas another study found that mice exposed to enrichment for 68 days scored the same as controls when tested 3 months after enrichment ended (Kempermann & Gage, 1999). Further research is therefore needed to determine the long-term effects of enrichment on spatial cognition.

We hypothesized that arousal without increased motor activity may mediate the effects of enrichment on learning. To test this we manipulated motor activity while keeping arousal constant by comparing the maze learning of rats raised in three enrichment conditions, two involving arousal and motor activity (environmental, social) and one involving arousal with minimal motor activity (auditory) to rats raised under typical laboratory conditions (standard). Because we were also interested in determining the duration of enrichment after a return to typical laboratory conditions, we re-tested the animals after 30 days of standard living.

Methods

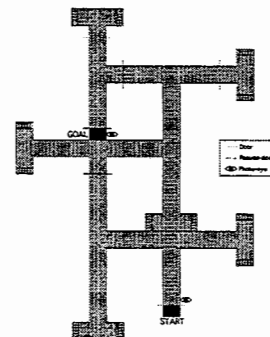
Thirty-two Long-Evans rats (*Rattus norvegicus*) were separated at weaning into one of four groups (4 males, 4 females per group) such that no littermates were assigned to the same group:

- Environmental: rats were kept in same-sex pairs in 46 cm x 46 cm x 21 cm Plexiglas cages supplied with a variety of "toys," including golf balls, rawhide nibble bars, wooden tubes, and wooden dowels.
- Social: rats were housed in 46 cm x 46 cm x 21 cm empty Plexiglas cages in groups of four same-sex cagemates.
- Auditory: rats were kept in same-sex pairs in standard metal laboratory cages (28 cm x 21 cm x 19 cm) in a room containing a cassette player and two 6-inch (12 cm) speakers placed 21 inches (54 cm) apart at a distance of 3 feet (91 cm) from their cages. The first movement (8 min 24 s) of Mozart's Sonata for Two Pianos in D Major, K. 448, was played for 12 hrs per day (8 p.m. to 8 a.m.) at 70 db.
- Standard: rats were kept in same-sex pairs in 28 cm x 21 cm x 19 cm empty metal cages.

On the 37th day of exposure, rats were weighed and put on a 23-hour food deprivation schedule to increase motivation. Water was available ad lib in home cages.

On day 39 (at age 60 days) rats were tested in a 6-unit T-maze (Figure 1). Testing was performed blind. Animals were tested for five days, three trials per

Figure 1. Floor plan of the T-maze.



day. A trial ended after the subject reached a Froot Loop™ in the goal box or after 240 s. A 10-min rest period was given between each trial. All subjects were videotaped.

The maze was constructed of 1/4-inch gray plywood, with walls 15 cm high and alleys 15 cm wide. Pathways measured 50 cm. A Plexiglas top was fitted to the form of the alleys. Doors were suspended from strings attached to pulleys on the room's ceiling. Pseudo doors were included to make all pathways containing doors appear alike. Actual doors were closed behind the animal to prevent excessive retracing. A timer was activated by two laser photoeyes attached to the maze wall 5 cm outside the start box (timer start) and 5 cm inside the goal box (timer stop). A Froot Loop™ was placed in the goal box and in vented enclosures at the end of each blind alley to prohibit the animal from scenting to the goal. The room was lit by red light.

After the last day of testing all rats were housed in same-sex pairs in empty standard laboratory cages with free access to food and water. At age 91 days we randomly selected five animals and again placed them on a 23-hr food deprivation schedule. At 94 days (following 30 days of standard living conditions) we re-tested these rats in the maze using the same procedures as before.

Dependent measures were seconds to complete the maze and number of errors. Errors were operationally defined as entries of 10 cm or more into a blind alley, or retracings of the correct alley. Time was recorded electronically (see Figure 1); errors were tabulated from the videotapes by coders blind to experimental conditions. Inter-rater reliability was $r = 0.99$.

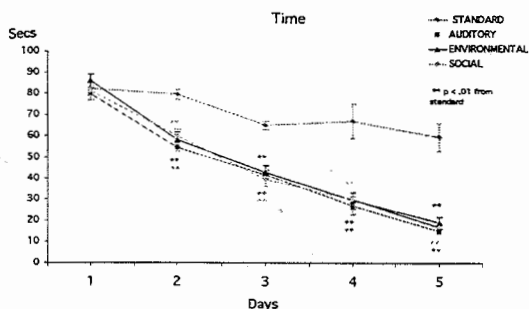
Results

Analyses of variance (Anova) and Tukey HSD tests were performed on time and errors as warranted. An alpha level of .05 was used for all statistical tests. No litter or sex effects were found.

Initial Testing

Time. We performed a two-factor Anova with condition (environmental, social, auditory, standard) as the between-subject factor and day (1–5) as the within-subject factor on the median time it took rats to complete the maze (Figure 2).

Figure 2. Median time to complete the maze for rats in enriched and standard living conditions on each day of testing. Error bars show standard errors of the mean.

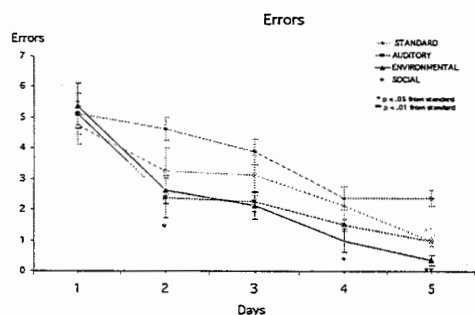


We found main effects for condition ($F(3,28) = 31.90, p = .000$) and day ($F(3,112) = 193.00, p = .000$) and an interaction between condition and day ($F(12,112) = 7.61, p = .000$). Individual one-factor (condition) Anovas performed on each day of testing found no group differences on Day 1 ($F(3,28) = 1.11, p = .361$). All enrichment groups completed the maze faster than the standard group on Days 2 through 5 ($p = .000$ all). No differences were found between enrichment groups on any day of testing.

Tukey HSD tests found no significant differences between consecutive days for the standard group, although its performance on Day 1 was significantly better than its performance on both Days 3 ($p = .02$) and 5 ($p = .000$). In contrast, the three enrichment groups ran the maze significantly faster on Day 2 than on Day 1 ($p = .001$, all). The social group also completed the maze faster on Day 3 than on Day 2 ($p = .001$). All enrichment groups ran the maze significantly faster on Day 4 than on Day 2, and on Day 5 than on Day 3 ($p = .001$ all).

Errors. A two-factor (condition, day) mixed Anova performed on median number of errors found main effects for both condition ($F(3, 28) = 6.97, p = .001$) and day ($F(4, 112) = 44.53, p = .000$). Condition and Day did not interact significantly (Figure 3).

Figure 3. Median number of errors made by rats in enriched and standard living conditions on each day of testing. Error bars show standard errors of the mean.



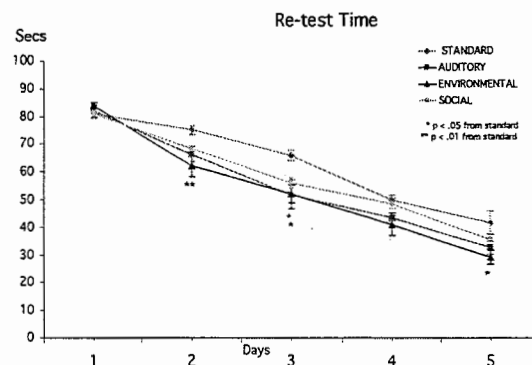
Groups did not differ on the first day of testing. The auditory group completed the maze with fewer errors than the standard group on Day 2 ($p = .048$), and the environmental group made fewer errors than the standard group on Day 4 ($p = .042$). By Day 5 all enrichment groups differed from the standard ($p = .000$, all). The error scores of the enrichment groups did not differ significantly on any day of testing.

The three enrichment groups made significantly fewer errors on Days 4 and 5 than on Day 1 ($p = .000$ all). Both the environmental and auditory groups performed better on Day 2 than on Day 1 ($p = .006$, both), whereas the social group did not improve significantly on consecutive days. The standard group showed improvement only between Days 1 and 4 and Days 1 and 5 ($p = .006$ both).

Delayed Testing

Time. To determine the duration of the effects of enrichment, we performed identical analyses on the data collected after 30 days of standard living conditions ($n=5$ rats per group). The data are presented in Figure 4.

Figure 4. Median time to complete the maze following 30 days of standard living conditions. Error bars show standard errors of the mean.



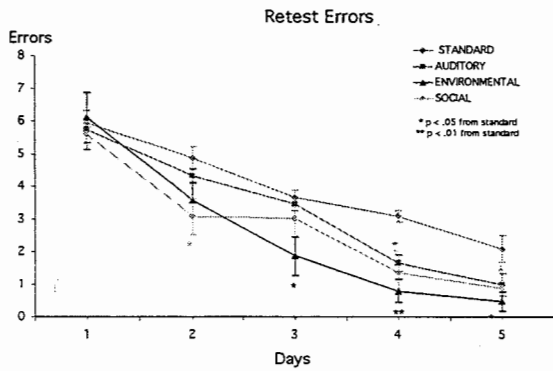
A two-factor (condition, day) mixed Anova found main effects for condition ($F(3,16) = 8.69, p = .001$) and day ($F(4, 64) = 212.56, p = .000$). The interaction was not significant ($F(12,64) = 1.56, p = .127$). Individual Anovas performed on each day of testing revealed no group differences on Day 1. The social group did not differ from the standard group on any day of testing. On Day 2, however, the environmental group completed the maze faster than the standard group ($p = .010$), and on Day 3 both the environmental and auditory groups differed significantly from the standard group ($p = .045$ and $p = .040$, respectively). The Anova performed on Day 4 was not significant ($F(3,16) = 3.01, p = .061$). On Day 5, only the environmental group differed from the standard ($p = .032$). No differences between enrichment groups were found for any day of testing.

All groups completed the maze faster on Day 5 than on Day 1. The environmental and auditory groups performed better on Day 2 than on Day 1 ($p = .001$ and $p = .006$, respectively). The auditory group continued this trend, performing better on Day 3 than on Day 2 ($p = .019$). No other improvements on consecutive days were found for the enrichment groups. The standard group, however, improved significantly from Day 3 to Day 4 ($p = .007$).

Errors. The trend found for errors was similar to that of time (Figure 5). A two-factor (condition, day) mixed Anova again found main effects for condition ($F(3,16) = 5.17, p = .011$) and day ($F(4,64) = 107.15, p = .000$). The interaction between condition and day did not reach significance ($F(12,64) = 1.76, p = .074$). Groups did not differ on the first day of testing. On Day 2, the social group made fewer errors than the standard group, and on Day 3 the environmental group differed from the standard ($p = .052$ and $p = .050$ respectively). All enrichment groups performed significantly better than the standard group on Days 4 and 5. Enrichment groups did not differ from each other on any day.

All groups made fewer errors on Days 4 and 5 than on Day 1 ($p = .000$ all). The environmental and social groups made fewer errors on Day 2 than on Day 1 ($p = .001$ both). No other consecutive day differences were found.

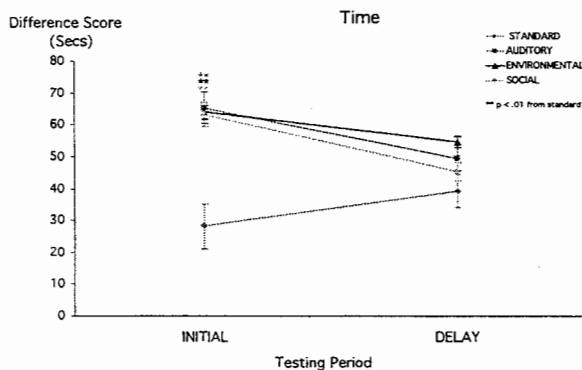
Figure 5. Median number of errors following 30 days of standard living conditions. Error bars show standard errors of the mean.



Comparison of Initial and Delayed Testing

Time. To compare the learning that occurred immediately following enrichment (initial testing) to that which occurred after 30 days of standard living conditions (delayed testing), we subtracted the median time to complete the maze on Day 5 from that of Day 1 for both testing periods. These data are presented in Figure 6.

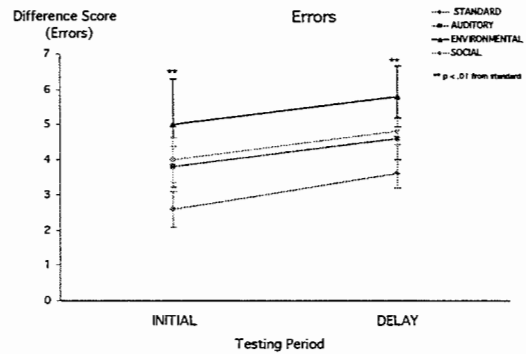
Figure 6. Day 5 median time minus Day 1 median time for initial and delayed testing periods. The higher the score, the greater the difference in maze time between Days 1 and 5. Error bars show standard error of the mean.



A two-factor (condition, testing period) mixed Anova was performed on the initial and delayed testing periods. We found main effects for both condition ($F(3,16) = 15.10, p = .000$) and testing period ($F(3,16) = 7.12, p = .017$), and a significant interaction between condition and testing ($F(3,16) = 4.90, p = .013$). During the initial testing period, all enrichment groups learned more from Day 1 to Day 5 than the standard group ($p = .001$ all). However, when tested after 30 days of standard living the learning from Day 1 to Day 5 of the enriched rats did not differ significantly from that of the standard group, although the difference between the initial and delayed testing periods for the enriched rats did not differ significantly. The rats raised in the standard condition learned more from Days 1 to 5 during the delayed period than during the initial testing period, however this difference was not significant ($p = .594$).

Errors. Identical computations and analyses were performed on the error data (see Figure 7).

Figure 7. Day 5 median errors minus Day 1 median errors for initial and delayed testing periods. The higher the score, the greater the difference in errors between Days 1 and 5. Error bars show standard error of the mean



The two-factor (condition, testing period) Anova performed on the initial and delayed testing periods found a main effect for testing period only ($F(1,16) = 11.33, p = .004$). The standard group made fewer errors overall than the environmental group in both testing periods. However, no other differences between the standard and enrichment groups were found for either period. The environmental group improved more over Days 1 to 5 during the delayed testing period than during the initial period ($p = .019$). No other group differences across testing periods were significant.

Discussion

A summary of the major findings is presented below.

Initial Testing

- Rats raised in enriched conditions completed a spatial maze faster and with fewer errors than rats raised under standard laboratory conditions.
- No differences between the enrichment and standard groups were found on the first day of testing.
- Differences between enrichment and standard groups emerged on Day 2 of testing.
- No significant differences between the enrichment groups were found for either time or errors.
- Learning occurred in all groups, although rats in the standard group learned at a slower rate than rats in the enriched groups

Delayed Testing

- No differences between the enrichment and standard groups were found on the first day of testing.
- The social group did not differ from the standard group on any day of testing; the environmental and auditory groups differed from the standard group on Day 2 and Day 3 respectively.
- The enrichment groups did not differ from the standard group on Day 4; only the environmental and standard groups differed on Day 5.
- No significant differences between enrichment groups were found for either time or errors.
- The enrichment groups learned at a slower rate after 30 days of standard living than when tested immediately after enrichment.

Comparison of Initial and Delayed Testing

- When the difference in performance between Days 1 and 5 was examined, no difference between the enrichment and standard groups for time was found when rats were tested 30 days after enrichment ended.
- The environmental group made fewer errors overall than the standard group in both testing periods.
- Enrichment groups did not differ from each other for either time or errors in either testing period.

These data strongly favor arousal rather than voluntary motor activity as a mediator for the behavioral effects of environmental enrichment. The maze performance of the auditory group, which was not provided with the opportunity to engage in additional motor activity, did not differ significantly from that of the other two enrichment conditions, both of which were provided the opportunity for motor activity. In fact, the only difference between the auditory and standard laboratory conditions was the presence of music in the former. Furthermore, informal observations of the animals' arousal behaviors (e.g., sniffing, exploring, rearing, interacting with another rat, and grooming) for 5 min each day before testing revealed no differences in arousal behaviors between the auditory and other enrichment groups in either the initial or delayed testing periods. Rats in the standard group, however, displayed fewer arousal behaviors overall in both testing periods. This, coupled with the finding that the maze performance of the auditory and other enrichment groups did not differ from each other for either dependent measure, suggests that arousal rather than motor activity may underlie some proportion of the behavioral effects of enrichment.

When tested 30 days after enrichment ceased, animals in the enrichment conditions continued to perform better in the maze than animals in the standard condition, although the difference between the enrichment and standard groups was weaker. In fact, when we analyzed the Day 1 – Day 5 difference scores for the delayed testing period, we found no difference between the standard and enrichment groups for time; for errors, only the environmental group outperformed the standard group. Again, however, no difference between enrichment conditions was found for either time or errors, lending further support to the “arousal hypothesis.”

The finding that the effects of enrichment, as mediated by arousal, decreased substantially after enrichment ended may be due to several factors, including insufficient duration of exposure to the enriched conditions. A complementary study performed with kindergarten children found improved spatial abilities following piano instruction, effects which decreased significantly when the instruction ended (Rauscher, in press). It seems that a minimum of two years of instruction is necessary to induce lasting effects. Further research is needed to clarify the role of exposure duration in rats, a task made difficult due to the risk of habituation with repeated presentation of the stimulus. However, the presence of some, albeit weaker, differences in maze performance between the enriched and standard groups following cessation of enrichment suggests the existence of some sort of neural mechanism. Walsh and Cummins (1975) suggested that arousal increases electrocortical activity which may in turn increase neuronal and glial metabolism. However, a direct test of this theory is beyond the scope of present scientific inquiry.

It is important to note that the standard condition, in which animals were raised without access to any external stimulation other than that which was necessary to maintain them, was actually an impoverished condition compared to the experience of feral rats. These data should therefore be interpreted with caution, as the effects of isolation, rather than enrichment, may be a critical factor. Nevertheless, it seems clear that animals raised in arousing environments perform better in a spatial maze than animals raised under standard living conditions. Motor activity does not appear to be necessary for the behavioral effects observed in this study.

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