Effects of Environmental Enrichment on Rate of Contextual Processing and Discriminative Ability in Adult Rats

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The effect of environmental enrichment on conditioned freezing to contextual cues in adult Sprague-Dawley rats was examined. The freezing of both enriched- and standard-reared rats increased with the time spent in the chamber prior to shock. Both groups of rats showed equally low levels of contextual conditioning following a preshock period of 4 s and equally high levels following a 120-s preshock period. However, following a preshock period of 16 s enriched rats displayed more contextual conditioning than standard rats. That is, enriched rats appeared to process contextual information faster than their standard-reared counterparts. Enriched-reared rats also showed a greater ability to discriminate between the conditioning context and a similar but distinctive context. Hence, in addition to forming a representation of the context in memory more rapidly than standard-reared rats, enriched-reared rats also appear to form a more complex representation.

Key Words: environmental enrichment; contextual conditioning; immediate shock freezing deficit; contextual discrimination.

The rearing environment has been found to have a significant impact on behavior and learning ability. Experimental procedures that have been employed to investigate this environmental effect include rearing animals under conditions of social or sensory deprivation, or within an “enriched” environment. In the enriched-environment procedure animals are exposed to a greater range and degree of sensory experiences and learning opportunities than would normally be received. Animals reared within an enriched environment differ in terms of exploratory activity, object exploration, emotionality, and sleep patterns compared to animals raised in a standard laboratory setting or under impoverished conditions. In addition, enriched-reared animals generally display superior memory and learning ability on difficult problem-solving tasks such as mazes and visual discrimination reversal (for a review of environmentally mediated effects on behavior, see Renner & Rosenzweig, 1987).

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We recently extended the research on the behavioral effects of enriched environments in two ways (Woodcock & Richardson, submitted for publication). First, a contextual conditioning procedure was used to assess the effects of enriched environments on learning and memory, and secondly, animals were tested in the preweaning stage of development. In our study, enriched rats shocked after 120 s displayed higher levels of long-term contextual conditioning than standard rats at 18, but not 26, days of age. These findings indicate that environmental enrichment accelerates the development of the neural system that mediates contextual conditioning. Further, the results suggest that once this neural system is mature in standard-reared rats (i.e., by 26 days of age) differences between the two groups in their ability to learn about a context disappear. Nevertheless, we hypothesized that enriched rats were still superior to standard rats in processing contextual cues at 26 days but that the test we used had not been sensitive enough to detect this. This issue was explored in the present study by taking advantage of two procedures that provide more sensitive measures of contextual conditioning (i.e., the immediate shock freezing deficit and contextual discrimination).

In the immediate shock freezing deficit procedure the time that the animal spends in the conditioning chamber prior to shock is varied. Freezing during reexposure to the shock environment 24-h later has been shown to be dependent on the exposure time in the chamber prior to shock (Fanselow, 1986). That is, minimal exposure times such as 4 s lead to low rates of freezing, and exposure times between 60 and 120 s produce high levels of freezing. Fanselow (1986) proposed that longer preshock periods lead to higher levels of freezing at test since they permit formation of a more complex representation of the context in memory. Hence, limiting the time available to form this representation may reveal differences between enriched and standard rats in contextual conditioning during adulthood. Specifically, we predicted that enriched rats would exhibit greater contextual conditioning than standard rats following a short preshock period (16 s), but that no differences between the two groups would be observed following either an extremely short (4 s) or a lengthy (120 s) preshock period.

A discrimination procedure may also provide a more sensitive measure of contextual conditioning. In this procedure, rats are shocked in one environment and later placed either in the same environment or in a similar, but distinctive, environment. Any decrement in freezing in the distinctive environment, compared to the training environment, is indicative of discriminative ability. It is believed that greater discriminative ability is a result of the formation of a more complex representation in memory of the cues within the training environment and stronger connections between these cues (e.g., Westbrook, Good, & Kiernan, 1994).

In the present study, the degree to which the freezing response generalized to a second, similar but distinctive environment was measured. A pilot study showed that standard rats did not discriminate between these two environments. However, it was hypothesized that enriched rats would show greater discriminative ability than standard rats due to the formation of a more complex representation of the training environment in memory. It was therefore expected that enriched rats would show significantly lower levels of freezing in the alternative environment compared to the training environment and that such a difference would not occur for standard rats.
METHOD

Experimental Design

Each litter was assigned to one of two rearing conditions. The enriched-rearing condition included both preweaning and postweaning enrichment procedures. The preweaning enrichment procedure involved exposing the pups to an intensive sensory stimulation program for 1 h each day, from Postnatal Day (PND) 2 until weaning at PND 21. Animals under the standard-rearing condition remained with their mother and littermates during this daily 1-h period. At 21 days of age the enriched rats were placed into a postweaning enriched environment and the standard rats were placed into standard laboratory containers. Each rearing group remained in their respective environments for a period of 34–40 days until testing at 55–61 days of age. Standard rats were handled on each of the 3 days preceding testing for 5 min in order to reduce behavioral reactivity (Smith, 1972). Enriched rats were handled daily during the preweaning enrichment procedure, and periodically during the postweaning enrichment period.

Subjects

Fifty-eight male Sprague-Dawley rats from 16 litters were used in the study. Each litter was culled to 10 pups at PND 1, and housed in a plastic maternity cage (30 × 45 × 16 cm) until 21 days of age. The cages were kept in a colony room with a 12-h light/dark cycle, and paper pellet bedding was changed once a week for all cages. Food and water were continuously available. At 21 days of age the enriched rats were placed in the postweaning enriched environment (see below), and the standard rats were placed in groups of eight in standard plastic laboratory containers (65 × 40 × 22 cm). All animals were treated in accord with the ethical guidelines of the American Psychological Association and all procedures were approved by the Animal Care and Ethics Committee at the University of New South Wales.

Enriched-Rearing Procedures

Preweaning stimulation. The preweaning enrichment procedure was modified from Schapiro and Vukovich (1970) and has been described in detail elsewhere (Woodcock & Richardson, submitted for publication). In brief, this procedure involved removing the pups from the nest and exposing them to a wide range of sensory stimuli specifically chosen to stimulate auditory, visual, tactile, taste, and olfactory modalities. The stimulation sessions involved placing the pups on cold and warm surfaces, and other surfaces of varying textures such as sandpaper, brushes, and marbles in order to stimulate their tactile sense. The olfactory and chemosensory senses were stimulated by exposing the pups to various odorants and artificial tastes. Each day the animals were rolled slowly in a cardboard tube for 1 min for vestibular stimulation. For additional vestibular and thermod tactile stimulation from PND 10 onward, the animals swam for 10 s in a bowl of warm water. From PND 12, visual stimulation was provided by exposing the pups to a flashing light for 15 s. All of the sensory stimulation occurred in a 1-h period each day. At all other times, rats under the enriched environment condition were kept with their mother and littermates in the standard plastic maternity cage.
**Postweaning enrichment procedure.** At 21 days of age the enriched-reared rats were moved into the postweaning enriched environment which provided constant sensory and physical stimulation. This environment was a large wooden cage (60 × 60 × 120 cm) modeled on the “superenriched” environment of Kuenzle and Knüsel (1974) which was designed to increase the number of learning situations in the animal’s environment. A wooden wall partitioned the cage into two equally sized compartments, restricting access between the compartments to either of two square tunnels supported on a shelf 15 cm below the top of the cage. The two compartments were symmetrical. The following description of the apparatus refers to one side only: A standard plastic laboratory cage with paper pellet bedding rested on an exterior shelf on the side of the cage, allowing access to a small interior shelf through a short plastic tube. A removable walkway connected the interior side shelf to the center shelf where the tunnels were fixed. A steep detachable ramp connected the center shelf to the wire floor. The primary route from the floor of one compartment to the next was up the ramp, through the tunnels, and down the ramp in the adjacent compartment. Permanent objects in the cage included a running wheel and a wooden climbing frame. The position of these items in the cage was changed daily. Furthermore, 10 to 15 stimulus items, aimed at stimulating various sensory modalities and increasing physical activity, were placed on the floor of the cage and in the plastic laboratory cages. These items were replaced every 2 days with new items taken from a general pool of around 50 items which included a branch, balls, and plastic objects of various sizes, shapes, and textures. The environment was located in a room with a 12-h light/dark cycle. Food and water were continuously available but were moved to a new position in the cage each day.

The physical conditions of the cage remained stable over a period of 6 days during which time the litters were weaned and placed into the cage. In the ensuing days, until the completion of the enrichment procedure, the conditions in the cage were changed every second day to make movement around the cage progressively more difficult. That is, the various ramps and connecting platforms were gradually removed and replaced by ropes or hanging chains of wooden rings or other objects. Thus, movement around the cage became physically demanding since it was necessary for the rats to climb new objects and traverse ropes, and cognitively demanding since access to food, water, and the laboratory boxes was dependent upon working out new methods of moving around the cage.

**Apparatus**

Training was conducted in a rectangular chamber (9 × 9 × 13 cm) with a Perspex ceiling and metal back panel, a hinged Perspex door at the front, stainless-steel rods on the floor, and wire mesh sides (referred to as E1 from here on). The stainless-steel rods were 3 mm in diameter, placed 1 cm apart center to center. The chamber was suspended in the center of a sound-attenuating box, above a metal tray containing paper pellets. Illumination was provided by a 15-W white light bulb, and a ventilation fan provided a background noise of 60 dB. A window in the door of the sound-attenuating box enabled video recording of the subject. Unscrambled shock could be delivered to the grid floor of the test chamber via a custom-built constant current shock generator. The floor and
walls of the chamber were wiped with 5% almond essence between each trial, and fresh paper pellets were placed in the tray.

Testing occurred either in E1 or in a second environment (E2) aimed at being similar but distinctive to E1. This environment was similar to E1 but its rear and side walls were constructed of stainless-steel rods. Also, attached to the outside of the walls were sheets of cardboard with 2 cm wide black and white vertical stripes. The chamber was located in the same sound-attenuating box as E1 above a tray of paper pellets. A solution of 5% acetic acid was used to wipe the walls and floor of the E2 chamber between trials. The chamber was illuminated by the same 15-W light bulb as E1, and a ventilation fan provided a background noise of 60 dB.

**Procedure**

On the day of training each rat was placed in the conditioning chamber and given one footshock (1 s, 0.5 mA) at 4, 16, or 120 s later. There were eight subjects in the 4- and 120-s enriched groups, and seven in each of the remaining groups. Animals were removed from the chamber 120 s after the shock. Twenty-four hours later each animal was returned to the chamber and the time spent freezing during a 5-min period was recorded using a stopwatch (from the videotape). Fourteen additional rats, seven from each rearing group, that were shocked 120 s after being in the chamber were placed into E2 at test instead of E1.

**Scoring and Statistics**

The percentage of time that each rat was observed to be freezing was used as the dependent variable. A $2 \times 3$ factorial ANOVA with two groups (enriched and standard rearing) and three preshock periods (4, 16, or 120 s) was performed to examine the effects of rearing condition and preshock period on freezing. A $2 \times 2$ factorial ANOVA with two groups (enriched and standard rearing) and two testing environments (E1 and E2) was also conducted to examine the effects of rearing environment on the ability of subjects to discriminate between the two test environments. This analysis used the two groups of subjects from each rearing condition that were shocked after 120 s. A second rater who was naive to each rat’s experimental condition scored a random selection of 12 rats from the video recordings. The interrater reliability between the two raters on these 12 samples was very high ($r = .995$).

**RESULTS**

The mean percentage of time spent freezing during the 5-min reexposure to the conditioning chamber by the subjects under each rearing condition and each of the three preshock conditions is presented in Fig. 1. A linear increase in freezing was observed as the preshock period increased [$F(2, 40) = 10.62; p < .01$]. Enriched and standard rats froze equally overall [$F < 1$], and there was no interaction between rearing condition and preshock period [$F(2, 40) = 1.15; p > .1$]. However, an ANOVA of the freezing scores in the 16-second condition revealed that the enriched rats in this group froze significantly more than standard rats [$F(1, 14) = 4.45; p = .053$].
FIG. 1. Mean percentage freezing scores for enriched and standard rats after preshock periods of 4, 16, and 120 s.

The mean percentage of time freezing during the 5-min reexposure to E1 and E2 by the subjects under each rearing condition shocked after 120 s is presented in Fig. 2. There were no differences between enriched and standard rats overall \[F(1, 25) = 1.07; p > .1\] or between the two test environments \[F(1, 25) = 3.61; p > .05\]. There was also no interaction between rearing condition and testing environment \[F < 1\]. Because of a priori predictions that enriched-reared rats would discriminate between E1 and E2 but standard rats would not, one-way ANOVAs were conducted separately for each rearing condition. These analyses revealed a significant decrement in freezing from E1 to E2 in the enriched group \[F(1, 13) = 4.84; p < .05\] but not in the standard group \[F(1, 12) < 1\].

DISCUSSION

As expected, both enriched- and standard-reared rats displayed equally low levels of freezing when shocked after 4 s, and equally high levels of freezing when shocked after 120 s. A preshock period of 16 s produced intermediate rates of freezing with enriched rats freezing significantly more than standard rats. This increase in freezing with increasing preshock periods may reflect the ability of the rat to form a more sophisticated representation of contextual cues in memory over time (e.g., Fanselow, 1986). The finding that enriched rats froze more than standard rats following the 16-s preshock period supports our predictions and indicates that during this limited preshock period, enriched rats formed a more complex representation of the context in memory than standard rats. Furthermore, since enriched rats did not display higher levels of contextual conditioning than standard rats with a 120-s preshock period (which presumably provided ample time to encode the contextual cues), these findings suggest that these rats processed the contextual cues more rapidly than the standard rats. The finding that differences between enriched- and standard-reared rats in contextual conditioning were revealed with a 16-s preshock period but not a 120-s preshock period is in line with the common trend within the literature that a more difficult task (in this case, one that severely restricts the time available to form a representation of the context) is more likely to discriminate between the learning abilities of enriched-and standard-reared rats.
The difference in contextual conditioning between enriched- and standard-reared rats shocked after 16 s was attributed to faster processing of contextual cues by the enriched rats during the preshock period. However, this effect might have been the result of differences between the two rearing groups in exploratory activity. That is, some researchers have reported that enriched rats show higher levels of exploratory activity than standard rats when they are first placed in an open field (Studelska & Kemble, 1979; Weldon & Smith, 1979; Woodcock, 1994). Increased exploratory activity within the conditioning chamber prior to shock would expose the rats to more environmental cues, which would enable a more complex representation to be formed and subsequently increase freezing during test. This issue is of particular relevance for the groups shocked after 16 s which presumably have limited time available to form a representation of cues in memory. To test this possibility the exploratory activity in the chamber prior to shock of enriched and standard rats from the 16-s condition was examined. The conditioning chamber was mapped by identifying 12 “focus points.” These focus points included the eight corners of the chamber, as well as the points midway between the corners along the four longest edges of the chamber. Using the video recordings of the session in which shock was delivered, the number of different focus points visited by each subject in the chamber during the 16 s prior to shock were recorded. There were no differences between enriched and standard rats in the number of different focus points visited \([F < 1]\), nor in the total number of focus points visited \([F < 1]\). These findings indicate that enriched-reared rats were not exposed to more contextual cues than the standard rats in the conditioning chamber prior to shock. Hence, if the increased freezing in enriched rats is not attributable to access to more environmental cues prior to shock, it is more likely that, as originally suggested, the processing of these cues occurs more rapidly in enriched rats.

The small decrease in freezing from E1 to E2 seen in the standard group represents high generalization or low discriminative ability. This was expected due to the physical similarity of the two contexts. Also as predicted, enriched rats showed a greater decrement in freezing from E1 to E2 which represents less generalization, or higher discriminative ability. These results may be explained in terms of our working hypothesis that during training, enriched rats formed a more complex integrated network of contextual cues in
memory and a strong link between this network and the representation of the shock. Subsequently, the distinctive cues in E2 were more apparent and discriminable, which accounted for the greater decrease in freezing within E2.

**GENERAL DISCUSSION**

Previous investigations into the effects of environmental enrichment on learning and memory have generally concluded that the memory of enriched-reared rats is superior to that of standard-reared rats in a number of situations (e.g., Renner & Rosenzweig, 1987). Recently, we investigated the effects of environmental enrichment on long-term contextual memory and concluded that this develops earlier in enriched rats (Woodcock & Richardson, submitted for publication). The present experiment has added to this research by indicating that enriched-reared rats process contextual information more rapidly than their standard-reared counterparts. In other words, a cognitive process more fundamental than memory appears to be influenced by environmental enrichment—that is, information-processing speed. Furthermore, when sufficient time is available to form a representation of the context, enriched-reared rats discriminate between this and a second context better than standard-reared rats. Thus, not only do enriched rats appear to form a representation of contextual cues in memory more rapidly, but this representation also appears to be more complex or integrated. This increased complexity of the memory trace, however, could well be a consequence of faster processing. That is, if enriched rats process contextual information more rapidly, more time would be available for additional processing which should enable formation of an enhanced representation of the context in memory.

The present study is not the first to suggest an effect of enriched-rearing on information-processing speed. For example, it has been previously reported that the evoked potential latencies of enriched-reared rats are shorter than those of standard-reared rats (Edwards, Barry, & Wyspianski, 1969; Mailloux, Edwards, Barry, Rowsell, & Achorn, 1974). These findings suggest that enriched rats process information more rapidly than standard rats. In addition, Woodcock (1994) reported that the activity level of enriched-reared rats in an open field declines more rapidly than that of standard-reared rats. One interpretation of this finding is that enriched rats learn faster about cues within the environment, which may also reflect faster processing speed.

The account of the present findings in terms of faster processing or the formation of more complex representations of contextual cues in memory can be challenged by the fear sensitization hypothesis recently proposed by Rosen and Schulkin (1998). According to this hypothesis exposure to stressful stimuli sensitizes the neural fear circuit and as a result this circuit becomes more active in subsequent fear-inducing situations. Thus, from this perspective the stressful preweaning enrichment procedure in the present experiment would have sensitized the fear circuit of these animals; consequently, when they were tested in the aversive contextual conditioning paradigm their fear circuit would be more active and this would account for the higher rates of freezing. However, although sensitization of the fear circuit could account for the greater freezing of enriched rats under the 16-s condition in the present study, it would not predict the equivalent freezing rates of enriched- and standard-reared rats shocked after 4 and 120 s. Furthermore, the fear sensitization hypothesis does not predict the greater discrimination that was observed in enriched rats, since the lower rates of freezing displayed by these rats in the alternative
environment are indicative of less fear. Finally, although the fear sensitization hypothesis would predict better performance of enriched animals on some aversive learning paradigms, it does not account for the superior performance of enriched-reared animals on appetitive learning tests (e.g., Denenberg, Woodcock, & Rosenberg, 1968; Venable et al., 1988).

Since the effects of environmental enrichment on contextual conditioning do not appear to be due to the effects of “stress,” it is possible that they reflect an increase in information-processing speed as originally suggested. However, the mechanisms by which enriched-rearing increases information-processing speed have not been considered. At a neural level, faster information-processing speed translates to faster nerve conduction velocity. The two primary factors that determine nerve conduction velocity are myelin thickness and axon diameter. If the heightened sensory stimulation within the enriched environment increases neuronal activity which in turn increases myelin thickness and/or axon diameter, then nerve conduction velocity and speed of information processing may in turn be increased.

Any neural modifications such as these following rearing within an enriched environment are unlikely to occur throughout the brain since research indicates that the neural areas that are modified are directly related to the sensory modality that is stimulated. For example, stimulation of the olfactory, visual, and tactile senses has led to modifications of the olfactory bulb, visual cortex, and somatosensory cortex, respectively (Godde, Spengler, & Dinse, 1996; Mailloux et al., 1974; Rosselli-Austin & Williams, 1990). Thus, the current enrichment procedure would be expected to lead to neural modifications only in those regions corresponding to the types of stimulation provided by the enriched environment (i.e., in the auditory, visual, somatosensory cortices, etc.). However, due to the hypothesized role of the hippocampus in contextual processing (e.g., Phillips & LeDoux, 1992), the faster contextual processing and more complex representations formed by enriched-reared rats in the present study suggest that the efficiency of hippocampal cells had increased. The most likely feature of the enriched environment that would cause such an increase in the efficiency of hippocampal cells is the complexity of, and constant changes in, the spatial environment. However, processing of contextual information may also have been facilitated by increases in the efficiency of processing in other neural areas (e.g., visual and olfactory) resulting from the stimulation of corresponding senses. The notion of modality-specific activity-mediated neural changes has implications for the importance and the relevance of specific types of environmental stimulation used to enhance cognitive functioning. That is, improvements in learning and memory may be maximized following an environmental enrichment procedure that stimulates multiple sensory systems, exposes the animal to complex spatial environments, and provides numerous problem-solving opportunities.

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