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Behavioural Brain Research 169 (2006) 231–238

BEHAVIOURAL BRAIN RESEARCH

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Research report

Environmental enrichment increases responding to contextual cues but decreases overall conditioned fear in the rat

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Received 7 November 2005; received in revised form 5 January 2006; accepted 12 January 2006
Available online 13 February 2006

Abstract

This study aimed at investigating the effects of environmental enrichment on various aspects of contextual processing in adult female rats. In experiment 1, simple conditioning was studied using either a training procedure allowing overshadowing of the contextual cues by signalling footshock with a discrete tone or a training procedure allowing a reduction of this overshadowing by explicitly unpairing the footshock and the tone. In experiment 2, contextual discrimination and contextual occasion-setting were assessed. Rats were daily exposed to two different contexts. In one context, a footshock was delivered 30 s after the offset of a tone, whereas in the other context the same tone was presented alone. Experiment 3 examined familiarization to a new context. Experiment 1 showed that environmental enrichment reduced the overshadowing of contextual cues by the tone and also reduced freezing to the more predictive cue according to the training procedure used. Experiment 2 showed that environmental enrichment increased the ability of rats to discriminate two contexts. Experiment 3 showed that enriched rats familiarized faster to a new context than standard rats. Taken together, these results suggest that environmental enrichment in adult rats enhances learning about contextual cues and reduces overall fear associated with aversive events.

Keywords: Conditioned freezing; Context; Housing conditions; Tone CS

1. Introduction

In laboratory, an enriched environment refers to housing conditions providing animals with enhanced possibilities of physical activity, sensory stimulations, social interactions and various learning opportunities as compared to standard housing conditions. Exposure to an enriched environment during adulthood was shown to modify many aspects of rodents' behaviour. For example, environmental enrichment ameliorates sensory-motor performances in a beam-walking task [5], decreases anxiety-related behaviours in the elevated plus-maze [2], accelerates habituation of exploratory activity in response to novelty [25,31], and improves learning and memory in a variety of hippocampal-dependent tasks (for a review see Ref. [24]) including spatial learning [11,14,20,25,29], object recognition [4,11,12,26], and contextual conditioning [8,22,26]. Evaluation of the effects of environmental enrichment on hippocampal-dependent learning and memory tasks has proved to be especially relevant, as cellular and molecular evidence for plasticity has been described in this brain area following exposure to enriched environment (for reviews see Refs. [17,18,27]). For instance, it has been recently shown that newly generated neurons in the dentate gyrus were involved in the enhancing effect of environmental enrichment on object recognition in the long term [4].

In rodents, contextual learning is frequently investigated using a signalled Pavlovian fear conditioning paradigm. Typically, this paradigm consists of exposing subjects to a single or a series of footshocks (unconditioned stimulus, US) signalled by a salient discrete stimulus (the conditioned stimulus, CS) in a given context. When reexposed to either the training context or the CS, rodents exhibit species-specific conditioned responses (CRs) of fear to both the CS and the context (e.g. Ref. [3]). Among those responses, freezing, that is defined as the suppression of all visible movements except those needed for respiration [2,9], is the most commonly measured response.

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doi:10.1016/j.bbr.2006.01.012

Contextual conditioning after tone-shock pairing was reported to be improved after exposure to an enriched environment in mice [8,22,26]. However, two of the abovementioned studies also reported improved cued-fear conditioning performances [22,26], whereas in the study by Duffy et al. [8], environmental enrichment increased only contextual fear conditioning.

The aim of the present study was to assess the effects of environmental enrichment on different aspects of contextual information processing in adult rats. In a first experiment, we investigated the effects of enrichment on conditioning to the context and to a tone CS. We used a signalled or paired training procedure that enabled overshadowing of the context by the CS in rats housed in standard conditions [23]. Additional groups of rats were similarly tested after training with an unpaired procedure using the same parameters, except that tone and footshock deliveries were explicitly unpaired, thus reducing contextual overshadowing. In a second experiment, we investigated the effects of environmental enrichment on other aspects of contextual processing such as contextual discrimination and contextual occasion-setting. Finally, in a third experiment, we investigated the effects of environmental enrichment on familiarization to a new context.

2. Material and methods

2.1. Subjects and housing conditions

A total of 96 female Long-Evans rats (Centre d'Elevage René Janvier, France) aged 11–12 weeks on arrival in the laboratory were used. They were individually housed in standard plastic cages (42 cm × 29 cm × 15 cm) with food and water provided ad libitum, in a temperature (22 ± 1 °C)- and humidity (55 ± 5%)-controlled room under a 12–12 h light–dark cycle (lights on at 8:00 a.m.). One week later, rats were randomly assigned to one of two different standardized housing conditions [28]. For the standard housing condition, rats were grouped by three in standard laboratory cages. For the enriched housing condition, rats were grouped by 12 in larger cages consisting of two wire-mesh cages (112 cm × 40 cm × 40 cm) connected by two openings in the contiguous walls. Various objects (tunnels, running wheels, toys, ladders, chains, etc.) were placed in the cage and changed daily. Moreover, food and water were placed at various locations from day to day. After 4 weeks of differential housing conditions, rats were isolated in standard cages and gently handled for 1 min on each of three consecutive days. Behavioural assessments began the next day and were carried out during the light phase of the cycle.

All procedures involving animals and their care were conducted in conformity with the institutional guidelines, which comply with national (council directive 87848, 19 October 1987, Ministère de l'Agriculture et de la Forêt, Service Vétérinaire de la Santé et de la Protection Animale; permission 7294 to M.M.) and international (directive 86-609, 24 November 1986, European Community) laws and policies.

2.2. Apparatus

Eight identical conditioning chambers (25 cm × 27 cm × 18 cm) were used. Chambers were made of transparent plastic with a transparent ceiling and placed in ventilated (background noise between 65.7 and 70.2 dB) light- and sound-attenuated boxes (57 cm × 38 cm × 38 cm, Campden Instruments Ltd.). An illumination of 6 lx was maintained by a bulb through a frosted plastic plate. Each chamber was equipped on one wall with a 1-cm-diameter hole, light indicators (always off) and a loudspeaker. A camera (MCT-210 MS, OptoVision, Toulouse, France) was fitted inside each box, above the center of the chamber, and monitored the entire chamber from the top through a 2.45 mm wide angle lens. The grid floor of each chamber consisted of parallel 0.3 cm diameter stainless-steel bars, 0.8 cm apart. A sawdust tray was placed under the grid floor. As described, the chambers referred to the conditioning context. A second context, referred to as the neutral context, consisted of the same chambers with other tactile, visual and olfactory cues. A white plastic plate was placed on the grid floor. A gray plastic plate with holes (to enable unaltered tone diffusion) was positioned with an angle of 110° with the floor in front of the loudspeaker. Black and striped patterns were fitted on the outside of the transparent walls and no sawdust was placed under the floor chamber. Tone and shock delivery were controlled by a computerized interface (Med-PC, Med Associates Inc., St. Albans, VT, USA). Automatic freezing measurements were performed as described in detail by Marchand and collaborators [16]. Briefly, video signals of two sets of four cameras were sent to a PC type microcomputer (Pentium type, 660 MHz, 512 Mo RAM) equipped with a Scion LG3 video capture card (Scion Corporation, Frederick, Maryland, USA) via two Quad-type multiplexers (Computar QSMX-II). Data acquisition was carried out by a script written under the "Scion Image" software that allowed the monitoring of all eight chambers at a sampling rate of 1 Hz. The analysis of freezing behaviour was conducted with a set of procedures written under Excel® Visual Basic® that allowed the computation of the standard deviation of each set of three contrast measurements, one before and one after each instant being analyzed, and its comparison with a fixed threshold; when at least two adjacent values were below threshold, they were classified as freezing [16]. The percentage of time spent freezing over blocks of selected duration was then computed. The system was also calibrated to monitor activity by adjusting the threshold and a similar analysis could be conducted on this variable. The thresholds used to monitor freezing and activity behaviours were those previously selected to obtain the best linear correspondence with human-based values of freezing and walking [16].

2.3. Experiment 1: effects of environmental enrichment on conditioning

2.3.1. Procedure

Twenty-four rats of each housing condition were randomly assigned to one of two training procedures conducted in the conditioning context. For the paired training procedure, four footshocks US (0.6 mA, 0.8 s, scrambled) were delivered pseudo-randomly (mean time interval between successive footshocks = 351 s) immediately after the tone CS (15 s, 4000 Hz, 10 dB above background) offset. The first footshock occurred 195 s after rat placement in the chamber. For the unpaired training procedure, the four footshocks were delivered at the same time as in the paired training procedure, but the tone was provided after (for the two initial presentations) or before (for the two last presentations) the USs (mean interval between these two stimuli = 202 s). The total duration of both training procedures was 27 min. On the following day (context test session), conditioned freezing to the conditioning context was assessed for a 40 min extinction session. The next day (tone test session), conditioned freezing to the tone was assessed by placing rats in the neutral context. For this session (27 min), four tones were delivered at variable time intervals (mean = 351 s), the first tone presentation occurring 3 min after the placement of the rat in the chamber. Conditioning and testing sessions were always conducted between 8:30 a.m. and 1:00 p.m.

2.3.2. Data analysis

For each training condition, freezing scores were computed during the following periods: the first 3 min of the conditioning session (baseline freezing), the four 1-min periods following each footshock delivery (post-shock freezing; the duration of the period analyzed was chosen because during training, the CS was absent during this period in both paired and unpaired groups), the first 15 min of re-exposure to the conditioning context (contextual freezing) and the 15 s of tone presentations during the tone test (conditioned freezing to the tone). Freezing scores were analyzed using Student *t*-test except for post-shock freezing scores, which were analyzed using an analysis of variance (ANOVA) with housing condition as between-subject factor and the number of footshocks delivered as within-subject factor. ANOVA was complemented by post-hoc Student–Newmann–Keuls (SNK) tests when appropriate. In all cases, the threshold for rejecting the null hypothesis was set at $\alpha = 0.05$.