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Short report

Food and water deprivation disrupts latent inhibition with an auditory fear conditioning procedure

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ABSTRACT

Latent inhibition (LI), operationally defined as the reduced conditioned response to a stimulus that has been preexposed before conditioning, seems to be determined by the interaction of different processes that includes attentional, associative, memory, motivational, and emotional factors. In this paper we focused on the role of deprivation level on LI intensity using an auditory fear conditioning procedure with rats. LI was observed when the animals were non-deprived, but it was disrupted when the rats were water- or food-deprived. We propose that deprivation induced an increase in attention to the to-be-CS, and, as a result, LI was disrupted in deprived animals. The implications of the results for the current interpretations of LI are also discussed.

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1. Introduction

When a neutral stimulus is presented without being followed by a relevant consequence, and it is subsequently paired with an unconditioned stimulus (US), the conditioned response to the pre-exposed conditioned stimulus (CS) is weaker than to a CS that was novel at time of conditioning. This phenomenon, termed latent inhibition (LI), has been traditionally related to attentional (e.g., Lubow, 1989), memory (e.g., Bouton, 1993), and/or associative (e.g., Escobar et al., 2002) processes, both from psychological and psychophysiological perspectives (see, for a review, Lubow and Weiner, 2010).

The most common idea in this research domain is that LI involves the same mechanisms, irrespective of the type of stimuli or the conditioning procedure employed (e.g., Schmajuk, 2002). Consequently, every theory that has been proposed to explain LI has considered an unique and general process underlying the effect of CS preexposure (De la Casa and Pineño, 2010). The theoretical debate has been mainly centered on two apparently incompatible hypotheses. The first one considers LI to be the result of an *acquisition failure* of the CS–US association at time of conditioning due to a reduction in attention and/or associability to the CS developed during the stimulus preexposure stage (Lubow, 1989; Pearce and Hall, 1980). The second hypothesis attributes the LI effect to a *retrieval failure*, considering that during the preexposure and acquisition stages of a typical LI experiment two associations are established, CS-nothing, acquired during non-reinforced presentations of the CS, and CS–US, established during the conditioning stage. At time of testing, the two associations compete for behavioral expression, a competition that is absent in the non-preexposed group (Bouton, 1993; Miller et al., 1986).

In spite of the extensive research intended to identify the mechanisms underlying the LI effect, the study of motivational processes has been traditionally neglected (but see, García-Burgos et al., 2013; Killcross and Balleine, 1996). In this paper we analyze whether LI is affected by changes in the deprivation level of the animals. Specifically, we designed an experiment using an auditory fear conditioning procedure that does not require food or water deprivation to induce robust conditioning, thus we avoid possible interactions between the motivational state of the animals and the motivational sign of the US (Killcross and Balleine, 1996). The experimental design included three conditions: One set of animals was food-deprived, the second set was water-deprived, and the last set was non-deprived.

Previous research have shown that food and water deprivation generates an increase in exploration and general activity (e.g., Baumeister et al., 1964), and that locomotor reactivity to novel stimulus increases in deprived animals (e.g., File and Day, 1972). Such increased activity to the stimulus presentations can be interpreted as a behavioral index of stimulus processing (e.g., Bradley, 2009), that would indicate higher level of attention to the novel stimulus in the deprived as compared to the non-deprived animals. Attending to these results, we anticipate that attention to the preexposed novel stimulus will decrease faster in the non-deprived than in the deprived animals, and, as a result, LI will be more intense for the former group.

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2. Materials and methods

2.1.1. Subjects

Forty-eight naïve male Wistar rats participated in this experiment ($n = 8$). Mean weight was 333 g (range 302–382). Animals were housed one to a cage under reversed-cycle lighting, and all experimental procedures took place during the light period of the cycle. Sixteen animals received unrestricted access to water and food during the entire duration of the experiment. For a second set of 16 rats, food was removed 72 h before the experimental treatment, with the animals receiving food access 30 min each day. Finally, for the last set of 16 animals, water bottles were removed 72 h before start the experimental treatment. The animals in this condition received daily water access for 30 min. All procedures were conducted in accordance with the guidelines established by Directive 86/609/CEE of the European Community Council, and the Spanish R.D. 223/1988.

2.2. Apparatus

Four identical Panlab chambers (model LE111) each measuring 26 cm height × 25 cm length × 25 cm width were used for pre-exposure, fear conditioning, and testing. Each chamber was enclosed in a sound-proof module (model LE116). The walls of the experimental chambers were made of white acrylic plastic. The floor in each chamber consisted of stainless steel rods, 2 mm in diameter, spaced 10 mm apart (center to center). The US was a 1-s, 0.5-mA unscrambled AC 50-Hz foot shock from a constant-current generator (Model LE100-26) that was delivered to the floor of each chamber. A loudspeaker was located at the top of each chamber, which produced a 70 dB 2.8-kHz 30 s tone that was used as conditioned stimulus. The chambers' floor rested on a platform that registered and recorded the animal's movements. A percentage score indicating general activity was computed by the experimental software (PANLAB Startfear) for the proportion of the total time that movement was detected.

2.3. Procedure

The experimental treatment was arranged following a 2 × 3 factorial design (Preexposure: Preexposed vs. Non-preexposed × Deprivation: Non-deprived vs. Water-deprived vs. Food-deprived). Half of the animals, in the Preexposed (PE) condition, received 25 tone-alone presentations, while the other half, those in the Non-preexposed (NPE) condition, remained an equivalent period of time in the experimental chambers without any additional stimulation. The second factor, Deprivation, included a group of Non-deprived (ND) animals, a second group Water-deprived (WD), and a Food-deprived (FD) group. The single experimental session programmed to evaluate LI started with a 300 s period without any stimulation, followed by a preexposure stage consisting in 25 preexposures of the 30-s tone, with an ITI of 30 s (±10), or an equivalent time without stimulus exposure for the animals in the NPE condition. A single conditioning trial started 30 s after the last tone presentation (or the equivalent time for the NPE groups), and consisted in one single pairing between the 30-s tone that coterminated with an electric foot-shock (1 s, 0.5 mA). A 180-s intertrial interval separated conditioning and test trial, which consisted in a 180-s tone-alone trial similar for all the animals. The total duration of the session was, approximately, 40 min.

General activity during tone preexposure (or an equivalent period of time for the animals in the NPE condition) was registered. In addition, to obtain an index of conditioning, activity during the tone at testing was transformed into a suppression ratio (SR) using the following formula: (activity during tone)/(activity during a previous period without tone + activity during tone), where 0.5 indicates no differences between activity level between both periods (i.e., no conditioning), and 0.0 indicates complete freezing during the tone (i.e., maximum conditioning). As the baseline period selected to calculate SR immediately follows the US presentation, and it could includes some unconditional responses, we also analyzed separately mean percent activity during CS at testing to obtain a complementary measure of conditioning.

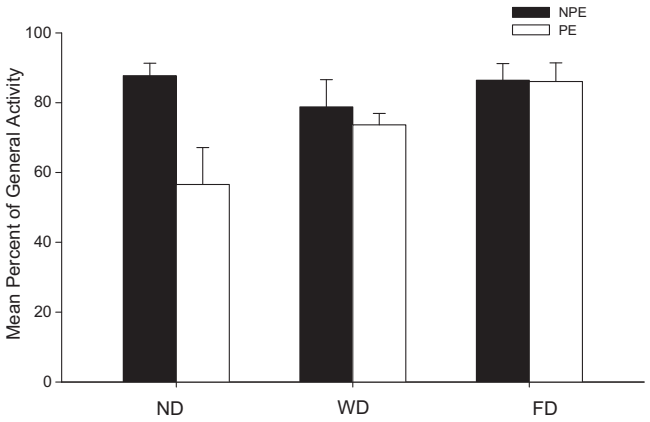

Fig. 1. Mean percentage of general activity collapsed across tone presentations for the Preexposed (PE) groups, or an equivalent period for the Non-preexposed (NPE) groups, as a function of deprivation (ND: Non-deprived, FD: Food deprived, and WD: Water deprived) at preexposure stage. Error bars represent SEMs.

3. Results

Mean percent activity collapsed across preexposure trials, or an equivalent period for the subjects in the NPE condition, as a function of deprivation level are depicted in Fig. 1. As can be seen in the figure, the activity during tone preexposure was lower for the PE/ND group. A mixed 5 × 2 × 3 ANOVA (5-trials blocks × Preexposure × Deprivation) conducted on mean percent general activity during tone presentations (or an equivalent period for the NPE groups) at preexposure stage confirmed this impression. The main effect of 5-trials blocks was significant, $F(4,168) = 15.28$, $p < 0.001$, due to an overall reduction of activity across trials. The main effect of Preexposure was significant, $F(1,42) = 5.44$; $p < 0.05$, due to a higher percent activity for the NPE as compared to the PE condition (Mean = 84.32%, SD = 15.85 and Mean = 72.09%, SD = 22.80, respectively). Finally, the Preexposure × Deprivation interaction was significant, $F(2,42) = 3.33$; $p < 0.05$ (all remaining ps > 0.09). To explore the interaction we conducted post hoc comparisons (Tukey tests, $p < 0.05$) that revealed a significant difference between the PE/ND and the NPE/ND groups, and between the PE/ND and the PE/FD group. No more comparisons were significant.

Fig. 2 (section A) depicts mean SR as a function of Preexposure and Deprivation conditions. As can be seen, the LI effect (reduced conditioning in the PE as compared to the NPE group. was restricted to the ND condition. This impression was confirmed by a 2 × 3 ANOVA (Preexposure × Deprivation) conducted on mean SR. The analysis revealed a significant main effect of Preexposure, $F(1,42) = 7.50$; $p < 0.01$, due to an overall LI effect, with higher level of conditioning for the NPE as compared to the PE condition (Mean = 0.31, SD = 0.09 and Mean = 0.40, SD = 0.15, respectively). The effect of Deprivation was non-significant, $F(2,42) = 1.88$; $p > 0.16$. The Preexposure × Deprivation interaction was significant, $F(2,42) = 3.05$; $p < 0.05$. Post hoc HSD Tukey tests, $p < 0.05$, revealed

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