CONDICIONAMIENTO DE MIEDO AL CONTEXTO

IMPLICACIONES PARA EL ESTUDIO DE MODELOS ANIMALES DE ESTRÉS POSTRAUMÁTICO

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CAPÍTULO 3

EFFECTS OF SHOCK INTENSITY AND NUMBER OF SHOCKS IN BOTH CONDITIONED AND UNCONDITIONED CONSEQUENCES

INTRODUCTION

Exposure to shock has been traditionally used as a typical stressor and for cue-context fear conditioning. When used as a stressor exposure to shock offers obvious advantages with respect to other stressors if we are interesting in exploring the consequences of unpredictability and uncontrollability. Thus, considerable attention has been paid to characterize short-term and long-term behavioural and physiological effect of exposure to shocks under different conditions and this includes the learned-helplessness paradigm (Maier and Watkins, 2005). One consequence of exposure to relatively severe sessions of shocks is sensitization to other potentially aversive stimuli (Kamprack and Wotjack, 2004). But in addition, exposure to shock can result in the development of cue or context fear conditioning and this is a classical paradigm of learning and emotional memory. Under certain conditions, the CR is not only elicited by the specific cue (i.e. a tone) or context associated to shock, but also by other relatively similar cues (i.e. a tone of different characteristics) or contexts having a certain resemblance with the original one. This phenomenon is known as generalization (Rudy and Pugh, 1996).

Thus, exposure to shock can result in both unconditioned (sensitization) and conditioned (cue/context fear conditioning and generalization). As discussed in the previous chapter, exposure to a brief session of shocks in absence of any additional cue causes contextual fear conditioning, but also hypoactivity in novel environments (Radulovic et al, 1998a; Daviu et al, 2010). Importantly, hypoactivity in novel environments is not associated to shock exposure per se but it is dependent on the development of context fear conditioning, perhaps reflecting fear generalization (Radulovic et al, 1998a; Daviu et al, 2010). Several previous reports have studied how shock number or shock intensity can affect cue/context fear conditioning, sensitization and generalization.

There are some studies showing that both context (Young and Fanselow, 1992; Cordero et al, 1998; Merino et al, 2000) and cue (Quinn et al, 2008) fear conditioning are dependent of the intensity of the shocks used for training. In contrast, some other studies did not observe differences in freezing in function of shock intensity when animals were re-exposed to conditioned cue and/or context (Bevins et al, 1997; Baldi et al, 2004). The lack of effect of shock intensity on cue fear conditioning has also been observed when other

behaviours were assessed during re-exposure to the cue alone (Laxmi et al, 2003). These controversial results could be explained by the shock protocols used. A ceiling effect may appear after intermediate shock intensities, suggesting that freezing behaviour in fear-conditioning protocols might approach to an all-or-none phenomenon (Baldi et al, 2004).

Other authors have obtained data questioning the apparent relationship between the intensity of the US and the degree of the CR (Kamprath and Wotjack, 2004). They suggested that the apparent higher CR response after high shock intensity training could be explained by the contribution of sensitization (a non-associative component). Thus, an enhanced response to the tone was observed both in the cue (tone)-fear conditioning group and in a group only conditioned to the context (therefore, not exposed to the tone). Interestingly, by subtracting the freezing data from the context group to the cue group, differences among prior shock intensities disappeared. That is, in the cue-fear conditioning groups, freezing behaviour specifically elicited by the tone was independent of shock intensity. Additional evidence for the existence of a non-associative component for freezing behaviour is the existence of an incubation period that progressively enhances freezing response to a neutral stimulus (a tone) after context fear conditioning, without a parallel change in the CR to the context (Siegmund and Wotjack, 2007b).

Regarding the influence of the number of CS-US pairings, in a cue-fear conditioning protocol, the animals exposed to a large number of shocks (10 shocks) showed more freezing during training than the animals exposed to fewer shocks (3 shocks) (Quinn et al, 2008). Nevertheless, during retrieval (both after context and tone) levels of freezing were independent of the number of trials, whereas those animals trained with high intensity shocks froze more than those trained with low intensities. This lack of effect of shock number has been confirmed by other authors using freezing and complementary behavioural measures of fear (Laxmi et al, 2003). These results indicates that the number of shocks do not appear to affect the freezing response to fear-conditioned cues.

How shock parameters affect sensitization and generalization phenomena is not well established. Nevertheless, it appears that fear generalization is dependent on shock intensity (Fanselow, 1981; Laxmi et al, 2003; Baldi et al 2004). Pilot results from our lab suggest that shock-induced hypoactivity may

be related to shock intensity rather than numbers of shocks (unpublished data).Thus, the main objective of the present work was to directly study the influence of the number and intensity of shocks on context fear conditioning and fear generalization. To evaluate such influence we measured both behaviour and the response of the HPA axis.
The HPA axis appears to be sensitive to conditioning. Thus, higher corticosterone levels has been observed when animals shocked in a context were re-exposed to the conditioned context without shocks (Goldstein et al, 1994; Campeau et al, 1997; Cordero et al, 1998; Merino et al, 2000). This increase in plasma corticosterone levels was found to be dependent on the intensity of shock administered during training (Cordero et al 1998). Although a sensitized HPA response has been reported long after shock exposure (Van Dijken et al, 1993) and long-term HPA axis sensitization is typical of exposure to high intensity stressors (Johnson et al, 2002; Belda et al, 2008; Gagliano et al, 2008), our results showed that the HPA response to conditioned contexts actually reflects contextual fear conditioning (Daviu et al, 2010). Thus, we included the response of the HPA axis as an additional measure of conditioning.

Finally, to have a more complete picture of the influence of the above parameters we decided to use a context for fear conditioning higher than those typically used for fear conditioning in rat. The rationale for this is that fear conditioning apparatuses in mice are clearly higher than in rat when compared to the respective size of animals. In rats, the relatively restricted space makes it difficult to distinguish between inactivity and freezing and does not allow for the rats to display other behavioural repertoire.

Moreover, considering the associative nature of shock-induced hypoactivity in novel environments, we wanted to study whether hypoactivity is maintained after extinction of fear conditioning. One previous study using context fear conditioning has reported a reduction of generalization of fear (freezing) to other environments after fear extinction (Golub et al, 2009). Interestingly, extinction training in rodents is considered as an analogue of exposure therapy in PTSD patients. This therapy consists of the confrontation (real or virtual) of the patients with the fear-associated stimulus (CS) in absence of the original aversive stimulus (US) (Kearns et al, 2012). Nowadays exposure ther-

apy is considered the most empirically supported PTSD treatment (Taylor et al, 2003; Nemerof et al, 2006; Milad et al, 2006; Foa, 2009).

MATERIAL AND METHODS

ANIMALS

Male Sprague-Dawley rats were used with the same characteristics as previously reported (Material and Methods).

PROCEDURE

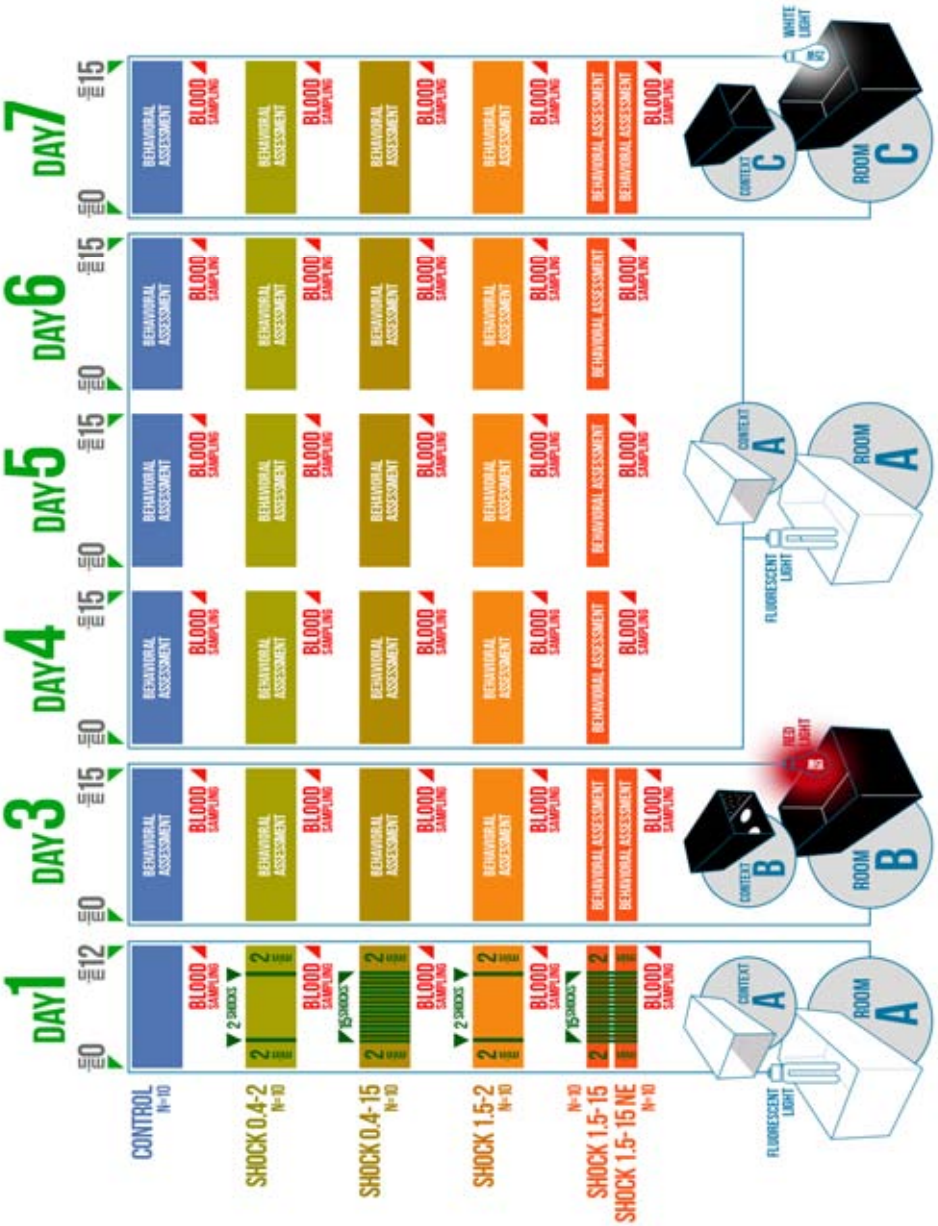
Animals were distributed into one control group and 4 shocked groups (Table 1). All shock groups followed a similar procedure. On day 1, after 2 min of habituation to the chamber –Context A-, the animals received 2 or 15 shocks(intensity 0.4mA or 1.5mA), 3s duration and the inter-trial intervals (ITI)of 7min or 30 s respectively. Animals were removed from the shock chamber 2min after the last shock. The control group was exposed to the shock chamber for 12 min (the same time as shocks groups) without receiving shock.

Table1. Summary of treatments

Group	Intensity	Number	ITI	(n)
CONTROL	-	-	-	10
0,4 2	0,4	2	7 min	10
0,4 - 15	0,4	15	30 s	10
1,5 - 2	1,5	2	7 min	10
1,5 - 15	1,5	15	30 s	20

On day 3 (48h after contextual fear conditioning acquisition in Context A) all the animals were exposed to a novel environment -Context B-. The animals were tested in Context B before being re-exposed to Context A in order not to interfere with the development of shock-induced hypoactivity. On day 4, 5 and 6 all animals (except half of the animals in the group 15-1.5mA which were not exposed to the conditioned context to avoid the development of fear conditioning extinction, 1.5 -15 NE group) were re-exposed to the conditioned context (Context A). On day 7, all groups including the 1.5 - 15 NE

group, were tested in another novel environment - Context C-. Exposure to the context always lasted 15 min. At the end of each test, a blood sample was taken in order to analyse the HPA response. Freezing behaviour, immobility and locomotor activity were assessed.



All contexts were Plexiglas cages of the same size (57 x 41 x 70 cm) with an important number of differential characteristics. In all the environments the frontal wall was made of clear Plexiglas in order to record frontally to analyse freezing behaviour.

- **Context A:** The side and back walls were opaque and white. The floor consisted of a metal removable grid floor of 44 stainless steel rods, 0.4 cm diameter, and spaced 1.5 cm centre to centre (Panlab S.L.U, Barcelona). Animals were transported from the vivarium to the experimental room in a small white plastic box without bedding (29 × 27 × 14 cm) covered with a piece of cloth. The room had white walls and a fluorescent light. The apparatus were cleaned between animals with a tap water solution containing ethanol (5% v/v).
- **Context B:** The side and back walls were black and rough. The floor was black and had 4 holes. Animals were transported to the experimental room in their home-cages. The room had black walls and red light (red bulb 15 W). The apparatus were cleaned between animals with water solution containing soap.
- **Context C:** The side and back walls were black and smooth. The floor was black and smooth. Animals were transported to the experimental room in their home-cages. The room had black walls and white light (white bulb 25 W). The apparatus were cleaned between animals with water solution containing soap.

BEHAVIORAL AND ENDOCRINE MEASURES

An experimenter blind to the treatments measured the behavioural variables. Distanced travelled was assessed by video tracking analysis using the centre of gravity of the animal (Smart, Panlab, S.L.U., Barcelona, Spain). Freezing and immobility were measured manually by stop watch. Time spent immobile was obtaining measuring immobility containing freezing. The latter was further independently re-evaluated and after subtracted to immobility in order to obtain two independent measures. Finally, endocrine analysis was assessed by radioimmunoassay.

STATISTICAL ANALYSIS:

Statistical analysis was done in two stages. First, endocrine data included one between subjects factor: group (5 levels control, 0.4-2, 0.4-15, 1.5-2 and 1.5-15) and behavior was analysed using group as the between subjects factor (5

levels) and block (3 levels, block of time 1, 2 and 3) as the within subject factor. In this analysis only the differences with the control group were studied. In the second stage, additional analysis was done using number (2 levels, 2 and 15 shocks) and intensity of shocks (2 levels, 0.4 and 1.5mA) as the between subjects factors for endocrine data and number, intensity and block (the latter as the within-subject factor) for behavior. In this second analysis the control group was excluded. Finally, in order to study the consequences of extinction, we used a different statistical analysis with group (3 levels, control, 1.5-15 and 1.5-15NE) as the between subjects factor and block (3 levels, block 1, 2 and 3) as the within subjects factor.

RESULTS

The following analysis was done not including the non-extinguished group (1.5-15 NE). There is a specific section (section 3) to analyse this particular issue.

1.EFFECT OF SHOCK PARAMETERS IN THE BEHAVIOURAL RESPONSE TO CONTEXTUAL FEAR CONDITIONING

Freezing behaviour in **Context A** (day 4) is represented in Fig1A. Statistical analysis showed a significant effect for group (Wald X^2 (4) = 32.66; $p<0.001$), block (Wald X^2 (2) = 6.51; $p<0.05$) and the interaction group x block (Wald X^2 (8) = 21.69; $p<0.01$). The decomposition of the interaction revealed that shocked groups froze more than the control in the first two blocks of time ($p<0.001$in all cases). By the third block of time differences with regard to control group disappeared (except for the 0.4-2 $p<0.05$ due to the low variance).The analysis excluding controls revealed no differences related to the number (NS) or intensity of shocks (NS). Activity in the conditioned context followed the same pattern as freezing (Fig1B). The analysis revealed significant effect for group (Wald X^2 (4) = 67.06; $p<0.001$), block(Wald X^2 (2) = 13.60; $p<0.001$) and the interaction group x block (Wald X^2 (8) = 129.56 $p<0.001$). After decomposition of the interaction, hypoactivity with respect to controls was observed in all shocked groups during the first two blocks of time ($p<0.001$). In the third block only the 0.4-2 group ($p<0.01$) was statistically different from control group. Again, no differences were observed related to number (NS) or intensity (NS) of shocks when control group was ex-

cluded from the analysis. Finally, the statistical analysis of immobility (Fig1C) showed a significant effect for group (Wald X^2 (4) = 150.12; $p<0.001$), block (Wald X^2 (2) = 30.45; $p<0.001$) and the interaction group x block (Wald X^2 (8) = 47.84; $p<0.001$). After decomposition of the interaction all shocked groups showed higher levels of immobility during the two first blocks of the test ($p<0.001$ vs control in the first and second block), but differences disappeared in the last block. No differences were observed related to number (NS) or intensity of shocks(NS).

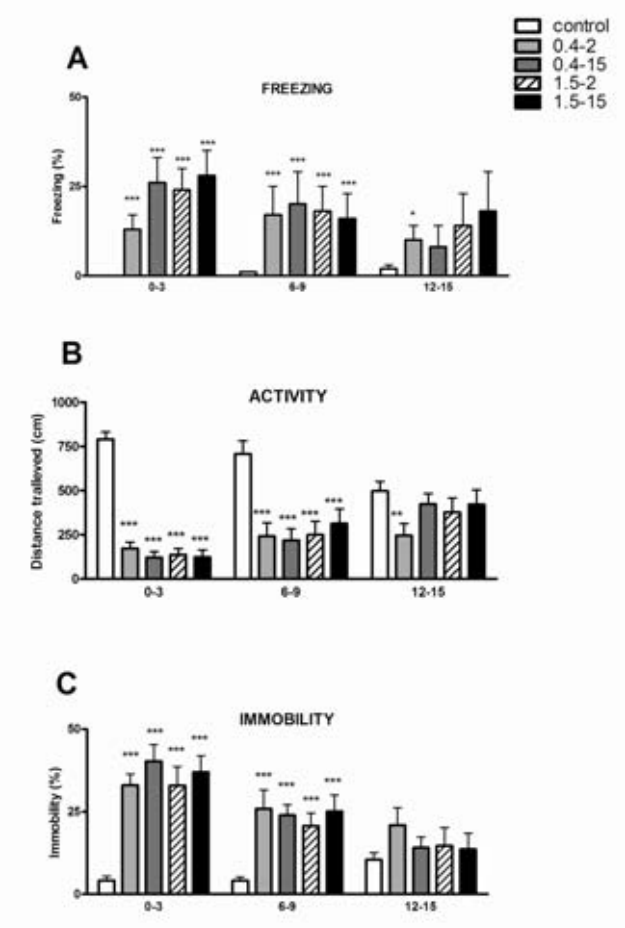


Figure1.Behavioral measures in Context A (conditioned context) on day 4. A) Percent time spent in freezing, B) distanced travelled and C) percent time spent in immobility in a shock chamber during 15 min (divided into 5 min blocks). For control and animals exposed to 2 or 15 shocks of 0.4 or 1.5 mA. * $p<0.05$, ** $p<0.01$ and *** $p<0.001$ vs control group. Means and S.E.M. are shown.

On day 5 all animals were exposed again to **Context A** to induce the extinction. The analysis of freezing behaviour (Fig2A) revealed only a significant effect for group (Wald X^2 (4) = 10.77; $p<0.001$) and the interaction group x block (Wald X^2 (5) = 22.79;$p<0.001$). Decomposition of the interaction indicated that shocked groups showed high levels of freezing in comparison to the control group only in the first block ($p<0.05$ in all cases). Moreover, the analysis excluding controls revealed that the interaction number x block was significant (Wald X^2 (2) = 7.84; $p<0.05$), being all the other factors and interactions NS. No differences between 2 shocks and 15 shocks groups were found at any time. Only the last block of 15 shocks groups was statistically significant compared to respective first block of the test ($p<0.05$).

Activity analysis (Fig2B) showed a significant effect for group (Wald X^2 (4) = 32.96;$p<0.001$) and the interaction group x block (Wald X^2 (4) = 91.96; $p<0.001$). Further decomposition revealed that in the first block, all shocked animals showed hypoactivity ($p<0.001$ vs control). In the second block, only 0.4-2, 0.4-15 ($p<0.01$) and 1.5-2 ($p<0.05$) shocked groups remained hypoactive compared to control group. On the other hand, the analysis excluding controls showed that block (Wald X^2 (2) = 8.59; $p<0.05$), and the interaction number x block (Wald X^2 (2) = 9.53; $p<0.01$) and intensity x block (Wald X^2 (2) = 6.33; $p<0.05$) were statistically significant, being all the other factors and interactions NS. During the first block of the test, shocked animals exposed to 15 shocks protocol showed less activity than 2 shocks animals independently of the shock intensity. Moreover, animals exposed to 1.5mA shocks showed a significant increase of their activity in the second block and last block of the test vs respective first block ($p<0.01$ and $p<0.05$). Also, the same effect was observed in 15 shocks groups that differed from their first block both in the second and last block of the test ($p<0.001$ in all cases).

Regarding immobility (Fig.2C) statistical analysis showed a significant effect for group (Wald X^2 (4) = 50.86; $p<0.001$), block (Wald X^2 (2) = 52.11; $p<0.001$) and the interaction group x block (Wald X^2 (8) = 57.23; $p<0.001$). Further decomposition showed a significant increase of immobility in all shocked groups in the first (0.4-2 $p<0.01$, $p<0.001$ vs control all the others) and in the second block of the test (0.4-2, 0.4-15 and 1.5-15$p<0.01$ and 1.5-2 $p<0.05$ vs control). The analysis excluding controls revealed a significant effect for block (Wald X^2 (2) = 61.24; $p<0.001$) and the interaction number x block (Wald X^2 (5) = 7.17; $p<0.05$), being all the other factors and interactions

NS. Both 2 shocks and 15 shocks conditions showed a decrease of immobility through blocks ($p<0.001$ in all cases vs respective first block), although the decrease was stronger in the 15 shock groups.

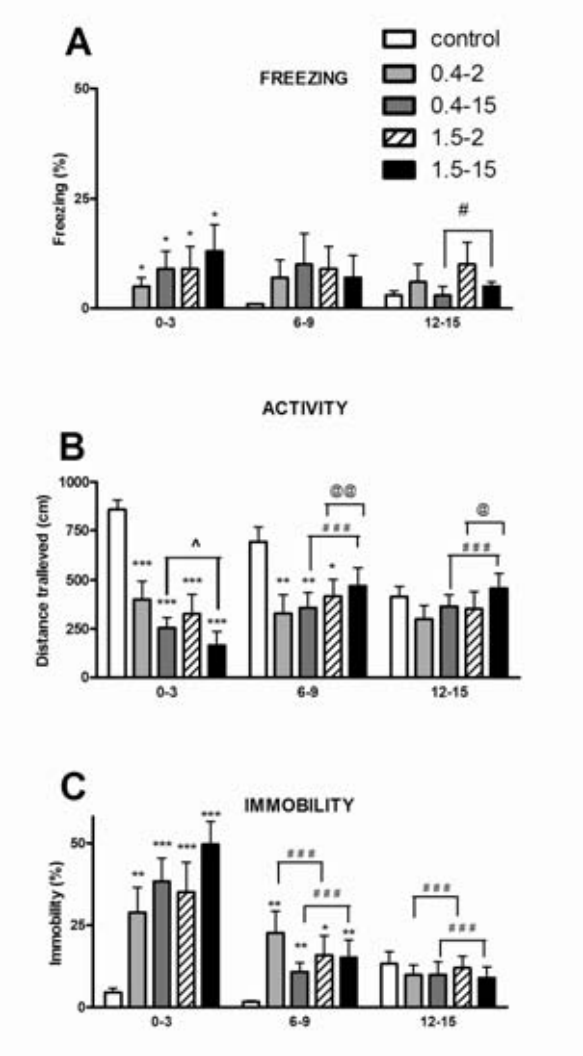


Figure2. Behavioral measures in Context A (conditioned context) during the first day of extinction (day 5). A) Percent time spent in freezing, B) distanced travelled and C) percent time spent in immobility in a shock chamber during 15 min (divided into 5 min blocks). For control and animals exposed to 2 or 15 shocks of 0.4 or 1.5 mA.* $p<0.05$, **$p<0.01$ and ***$p<0.001$ vs control group. # $p<0.05$ and ### $p<0.001$ vs first block by respective number of shocks. @ $p<0.05$ and @@ $p<0.01$ vs first block by respective intensity. Means and S.E.M. are shown.

The second day of extinction in context A is showed in Fig.3A.Freezing behavior analysis revealed no significant differences between groups. However, significant differences were found regarding hypoactivity (Fig3B). Statistical analysis showed a significant effect for group (Wald X^2 (4) = 16.33; $p<0.01$), block (Wald X^2 (2) = 11.58; $p<0.01$) and the interaction group x block (Wald X^2 (8) = 69.52; $p<0.001$). Decomposition of the interaction revealed that in the first block all groups showed hypoactivity ($p<0.001$ in all cases). In the second block only statistically significant differences were found in 0.4-2 animals ($p<0.05$ vs control group). The immobility analysis (Fig3C) revealed a significant effect for group (Wald X^2 (4) = 31.99; $p<0.001$) and the interaction group x block (Wald X^2 (8) = 25.85; $p<0.001$). Decomposition of interaction showed that in the first block of the test all shocked groups presented higher levels of immobility (0.4-2 $p<0.01$, 0.4-15 $p<0.05$, 1.5-2 and 1.5-15 $p<0.001$ vs control). In the last block only 0.4-15 ($p<0.05$) and 1.5-2 ($p<0.01$) groups were found statistically different from controls animals. In all cases, the analysis excluding controls revealed no differences related to the number (NS) or intensity of shock (NS).

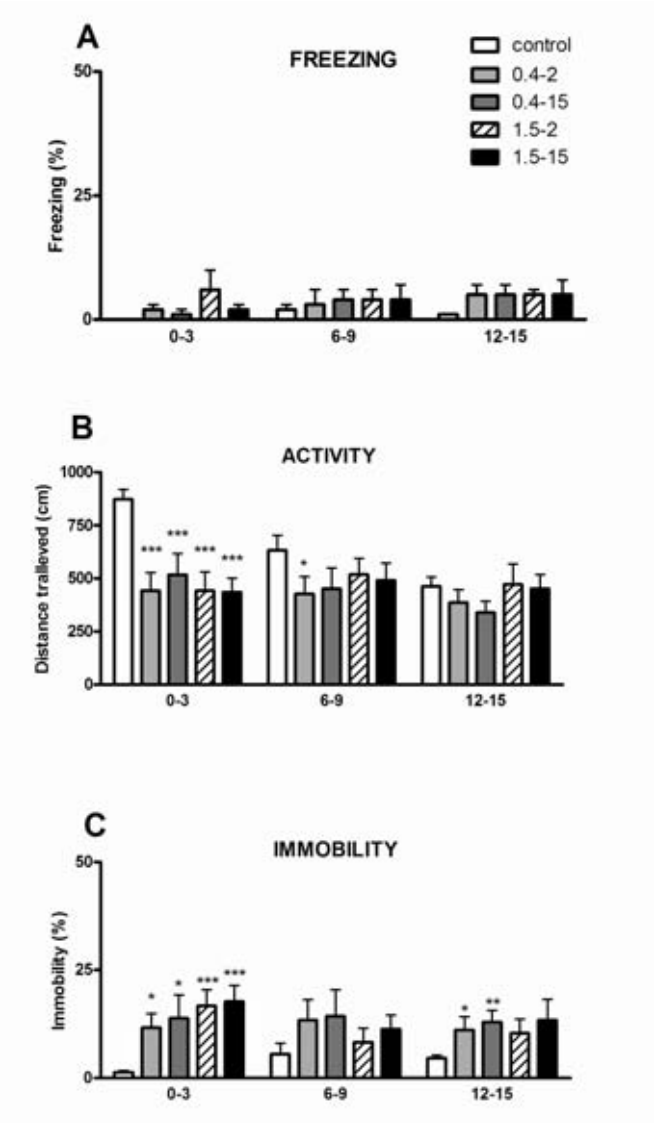


Figure3.Behavioral measures in Context A (conditioned context) during the second day of extinction (day 6). A) Percent time spent in freezing, B) distanced travelled and C) percent time spent in immobility in a shock chamber during 15 min (divided into 5 min blocks). For control and animals exposed to 2 or 15 shocks of 0.4 or 1.5 mA.* $p<0.05$, **$p<0.01$ and ***$p<0.001$ vs control group. Means and S.E.M. are shown.

2.BEHAVIORAL EFFECTS OF SHOCK PARAMETERS IN OTHER ENVIRONMENTS: INFLUENCE OF INTENSITY AND NUMBER

On day 3 all animals were exposed to **Context B**. Freezing analysis (Fig4A) showed a significant effect for group (Wald X^2 (4) = 29.04; $p<0.001$), block (Wald X^2 (2) = 19.47; $p<0.001$) and the interaction group x block (Wald X^2 (8) = 29.33; $p<0.001$). After decomposition of the interaction all shocked groups showed higher levels of freezing, during the first block, as compared to control group (0.4-2, 0.4-15 and 1.5mA-2 $p<0.01$, 1.5-15 $p<0.001$). In the second block only the 1.5-15 showed higher levels of freezing behavior in comparison to controls ($p<0.01$).Further analysis excluding controls revealed a significant effect for block (Wald X^2 (2) = 19.59; $p<0.001$) and intensity (Wald X^2 (1) = 5.46; $p<0.05$), being all the other factors and interactions NS. High intensity shock groups showed more freezing than low intensity shock groups ($p<0.05$) regardless of number of shocks administered.

Analysis of activity (Fig4B) showed a similar pattern than freezing, a significant effect for group (Wald X^2 (4) = 43.85; $p<0.001$), block (Wald X^2 (2) = 15.14; $p<0.001$) and the interaction group x block (Wald X^2 (8) = 19.47; $p<0.001$). Decomposition of the interaction revealed that all shocked animals showed hypoactivity in the first ($p<0.001$ in all cases) and second (0.4-2 $p<0.01$, 0.4-15 $p<0.05$, 1.5-2 and 1.5-15 $p<0.001$ vs control) blocks of the test. In the last block, only the 0.4-2 group was statistically different from controls ($p<0.05$).

The immobility analysis (Fig 4C) showed a significant effect for group (Wald X^2 (4) = 52.21; $p<0.001$), block (Wald X^2 (2) = 47.82; $p<0.001$) and the interaction group x block (Wald X^2 (8) = 46.87; $p<0.001$). Further decomposition revealed that in the first block all shocked groups differed from controls ($p<0.001$ in all cases). In the second block, only 0.4-2 ($p<0.05$), 1.5-2 ($p<0.01$) and 1.5-15 ($p<0.001$) groups were statistically different from controls. In the last block no differences were found. The analysis excluding controls revealed that neither activity nor immobility showed differences related to number (NS) or intensity of shocks (NS).

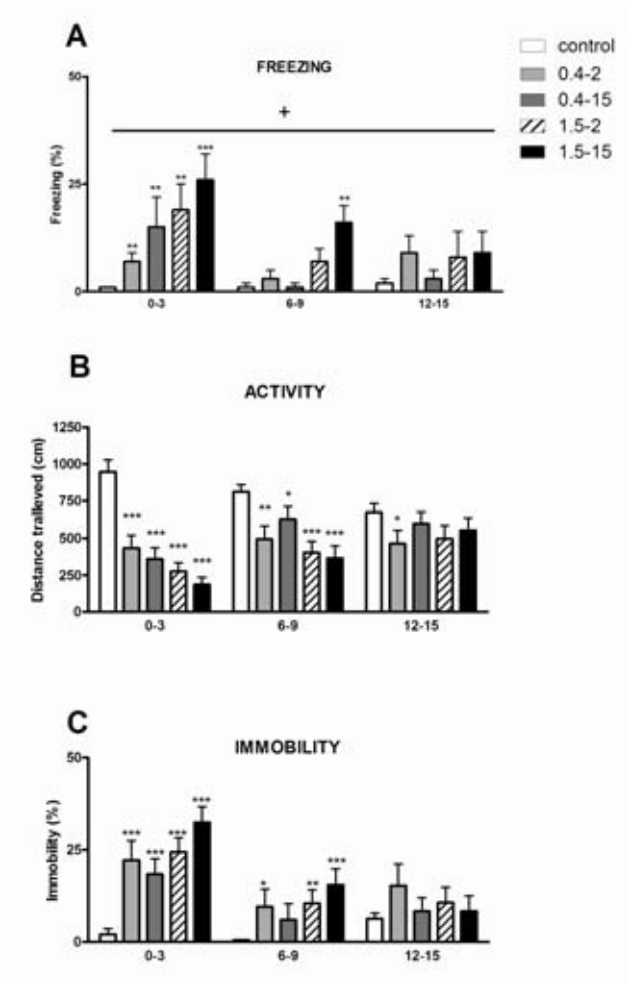


Figure4.Behavioral measures in Context B on day 3. A) Percent time spent in freezing, B) distanced travelled and C) percent time spent in immobility in a Context B during 15 min (divided into 5 min blocks), for control and animals exposed to 2 or 15 shocks of 0.4 or 1.5 mA. * p<0.05, **p<0.01 and ***p<0.001 vs control group. + p<0.05 vs 0.4mA groups. Means and S.E.M. are shown.

On day 7, after the extinction protocol, all animals were exposed to other environment -**Context C**- (Fig 5A). The analysis of freezing behaviour revealed no significant effect forgroup, but a significant effect for block (Wald X^2 (2) = 19.98; p<0.001), reflecting a progressive decline in freezing over the 15 min session. On the other hand, analysis of activity (Fig 5B) showed a significant effect for group (Wald X^2 (4) = 13.06; p<0.05) and block (Wald X^2 (2) = 140.68; p<0.001) but no interaction between them. Further comparisons

showed that shocked: 0.4-2 ($p<0.05$), 1.5-2 ($p<0.01$) and 1.5-15 ($p<0.01$) groups presented a mild hypoactivity as compared to controls (in all blocks).Analysis without controls showed no differences related to number (NS) or intensity (NS) of shocks on freezing, activity or immobility (Fig5C). However, regarding immobility despite the interaction number x intensity (Wald X^2 (1) = 4.99; $p<0.05$) was statistically significant, no significant differences in further comparisons were found.

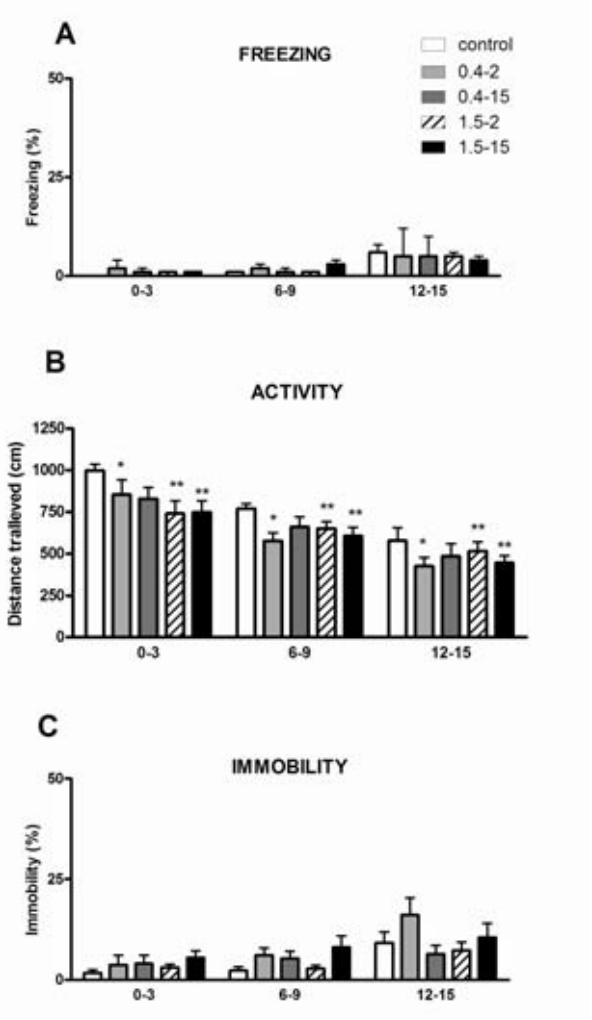


Figure5.Behavioral measures in Context C on day 7. A) Percent time spent in freezing, B) distanced travelled and C) percent time spent in immobility in a Context B during 15 min (divided into 5 min blocks), for control and animals exposed to 2 or 15 shocks of 0.4 or 1.5 mA. * $p<0.05$ and ** $p<0.01$ vs control group. Means and S.E.M. are shown.

3.EXTINCTION PROTOCOL COULD REVERSE THE HYPOACTIVITY CAUSED BY THE FEAR CONDITIONING PROTOCOL:

In this section we address directly the effect of the extinction protocol in the long-lasting effects of fear conditioning. The 1.5-15 NE animals were exposed to the same conditioning protocol than 1.5-15. However, despite these animals were exposed to the novel environments -Context B and Context C- no extinction protocol was followed, in order to determine the specific effects of fear conditioning extinction on shock-induced hypoactivity. In this section the behavioral and endocrine analysis between control, 1.5-15 and 1.5-15 NE group is reported.

Regarding **Context B** (day 3), behavioral analysis (Fig 6 left panel) revealed for freezing (A) a significant effect for group (Wald X^2 (2) = 28.43; $p<0.001$), block (Wald X^2 (2) = 15.28; $p<0.001$) and the interaction group x block (Wald X^2 (4) = 20.54; $p<0.001$). Further decomposition indicated that shocked animals showed higher levels of freezing in the first block compared to controls ($p<0.001$). In the second block, only 1.5-15 animals remained statistically different from controls ($p=0.001$). Regarding activity (B), statistical analysis showed a significant effect for group (Wald X^2 (2) = 39.18; $p<0.001$), block (Wald X^2 (2) = 12.78; $p<0.01$) and the interaction group x block (Wald X^2 (4) = 67.22; $p<0.001$). Decomposition of the interaction revealed that during the first block, all shocked animals showed hypoactivity compared to controls ($p<0.001$). In the second block, both 1.5-15sh and 1.5-15sh NE ($p<0.001$ and $p<0.01$ respectively) remained hypoactive in comparison to controls. Finally, immobility analysis (C) showed a significant effect for group (Wald X^2 (2) = 45.54; $p<0.001$), block (Wald X^2 (2) = 48.86; $p<0.001$) and the interaction group x block (Wald X^2 (4) = 58.51; $p<0.001$). Decomposition of interactions revealed that all shocked animals showed higher immobility in the first ($p<0.001$) and in the second block of the test, both 1.5-15sh ($p<0.001$) and 1.5-15sh NE ($p<0.05$) compared to controls.

On day 7, all animals were exposed to **Context C**(Fig 6 right panel). The freezing analysis (A) revealed a significant effect for group (Wald X^2 (2) = 11.95; $p<0.01$) and the interaction group x block(Wald X^2 (4) = 48.86; $p<0.001$). Further decomposition revealed that in the first block, 1.5-15NE group froze more than both control ($p<0.001$) and 1.5-15 ($p<0.001$) groups. In the second block, the same differences were maintained(as compared to

control: $p<0.001$ and 1.5-15groups: $p<0.05$ groups). In the last block no differences among groups were found.

Regarding the activity analysis (B), results showed a significant effect for group (Wald X^2 (2) = 19.87; $p<0.001$), block (Wald X^2 (2) = 17.36; $p<0.001$ and the interaction group x block (Wald X^2 (4) = 41.30; $p<0.001$). The decomposition of the interaction showed that both 1.5-15 ($p<0.01$ in the first and in the second block) and 1.5-15 NE ($p<0.001$ in the first and $p<0.01$ in the second block) animals were hypoactive as compared to controls, but the 1.5-15 NE animals showed more hypoactivity than 1.5-15 during the first block of the test ($p<0.01$).

The immobility analysis (C) revealed a significant effect for group (Wald X^2 (2) = 14.22 $p<0.001$) and the interaction group x block (Wald X^2 (4) = 16.90 $p<0.001$). Further decomposition showed higher immobility in both 1.5-15 and 1.5-15 NE groups as compared to controls in the first and second block ($p<0.001$ in all cases). However, the 1.5-15 NE animals showed higher immobility as compared to 1.5-15 group in the first ($p<0.01$) and the second ($p<0.05$) blocks.

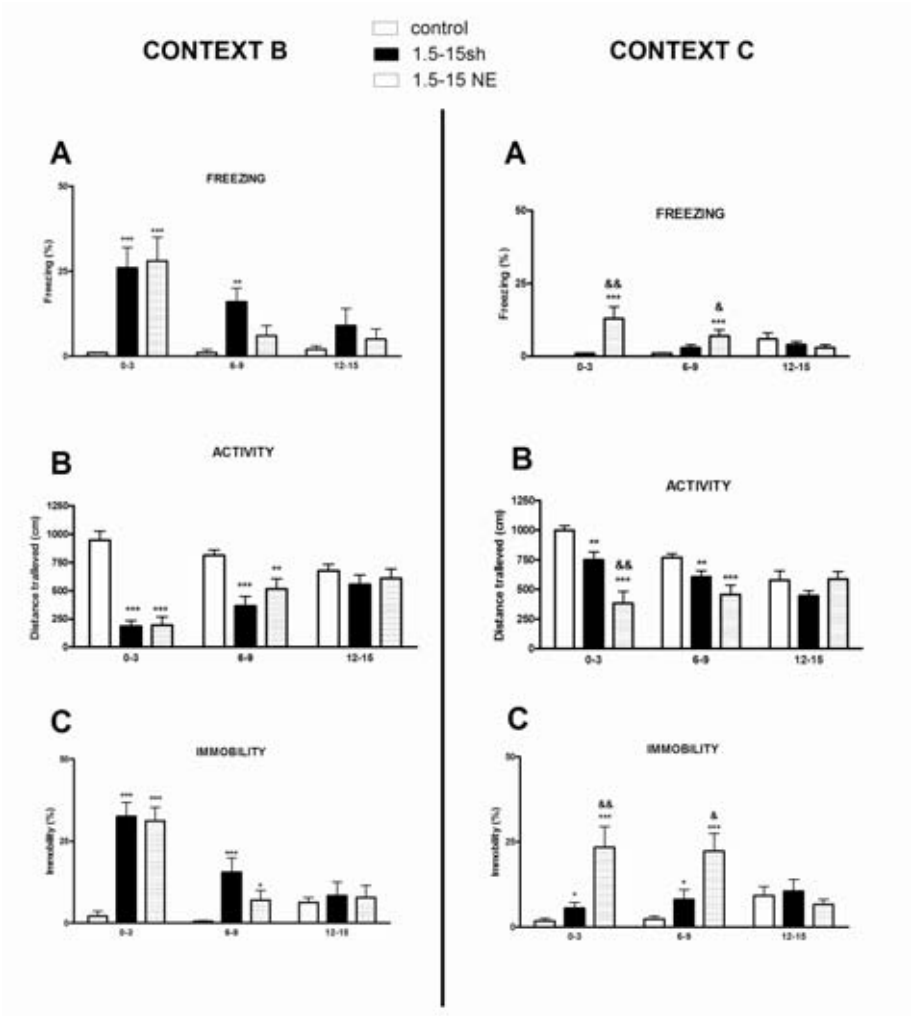


Figure6. Behavioral measures in Context B (left panel) and Context C (right panel) from control, 1.5-15 and 1.5-15 NE groups. The 1.5-15 NE group was exposed to shock but extinction training was not undertaken.A) Percent time spent in freezing, B) distanced travelled and C) percent time spent in immobility during 15 min (divided into 5 min time block). * p<0.05, **p<0.01 and ***p<0.001 vs control group. & p<0.05, && p<0.01 and &&& p<0.001 vs 1.5-15 group.

4. ENDOCRINE RESPONSE TO FEAR CONDITIONING

The analysis of the ACTH response during acquisition on **Context A** (day 1, Fig.7A) revealed a significant effect for group (Wald X^2 (4) = 70.92; p<0.001). In all shocked groups the plasma ACTH levels were higher than controls (0.4-

2 $p<0.01$ and $p<0.001$ vs all others). Further analysis excluding controls revealed that number (Wald X^2 (1) = 16.45; $p<0.001$), intensity (Wald X^2 (1) = 16.86; $p<0.001$) and the interaction number x intensity (Wald X^2 (1) = 4.42; $p<0.05$) were statistically significant. The 1.5-15 group showed higher levels than both 1.5-2 and 0.4-15 groups ($p<0.001$ in all cases).The analysis of plasma corticosterone (Fig 7B) on day 1 showed a significant effect for group (Wald X^2 (4) = 20.65; $p<0.001$). Further comparisons revealed that only the high shock intensity groups, 1.5-2 ($p<0.01$) and 1.5-15 ($p<0.001$), were statistically different from controls. The analysis excluding controls showed a significant effect for intensity (Wald X^2 (1) = 15.80; $p<0.001$) being all other factors and interactions NS. Shocked animals exposed to high intensity showed higher corticosterone levels than those exposed to low intensity ($p<0.001$) regardless the number of shocks administered.

When exposed again to the **Context A** (day 4, Fig.7A), the ACTH analysis showed a significant effect for group (Wald X^2 (4) = 9.538 $p<0.05$). Further decomposition demonstrated that only the high shock intensity groups (1.5-2 and 1.5-15) showed higher levels of ACTH than controls ($p<0.01$). In the two extinction sessions (days 5 and 6), no significant differences in ACTH levels were found among groups. The analysis excluding control revealed no differences on day 4, 5 or 6 related to number (NS) or intensity of shocks (NS).Corticosterone analysis (Fig.7B)revealed a significant effect for group (Wald X^2 (4) = 11.86; $p<0.05$), but only the 1.5-15 group showed higher corticosterone levels than controls ($p<0.01$). The analysis excluding controls revealed that intensity was statistically significant (Wald X^2 (1) = 6.88; $p<0.01$), showing a higher corticosterone levels in 1.5mA than 0.4mA animals ($p<0.01$). Regarding the analysis of successive exposures (days 5 and 6) to the Context A no differences among groups were found. However, further analysis excluding controls showed a significant effect for intensity on day 5 (Wald X^2 (1) = 4.47; $p<0.05$) and on day 6 (Wald X^2 (1) = 5.20; $p<0.05$). Shocked animals exposed to high shock intensity (1.5mA) showed higher corticosterone levels than animals exposed to low shock intensity (0.4mA) regardless the number of shock administered ($p<0.05$ in all cases).

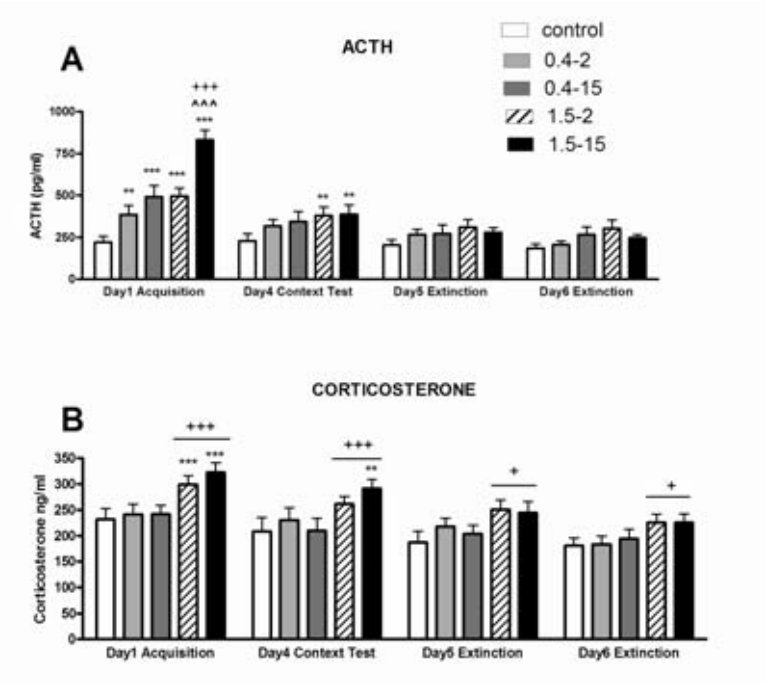


Figure7. Plasma ACTH (A) and Corticosterone (B) levels after acquisition and after 15 min exposure to Context A on days 4, 5 and 6, for control and animals exposed to 2 or 15 shocks of 0.4 or 1.5 mA. **p<0.01 and *** p<0.001 vs control group. ++ p<0.01 and +++p<0.001 vs respective 0.4mA group. ^^^ p<0.001 vs respective 2 shocks group. Means and S.E.M. are shown.

Finally, the analysis of endocrine response to 1.5-15 NE was reported below. Analysis of endocrine response during acquisition on **Context A** (day 1, Fig 10A and 10B) showed a significant effect for group in ACTH (Wald X^2 (2) = 119.89; p<0.001) and corticosterone (Wald X^2 (2) = 11.09; p<0.01). Further comparisons revealed that in the two shocked groups plasma ACTH levels were higher (p<0.001) than in controls. Regarding the corticosterone response, only the 1.5-15 group was different from controls (p<0.01), but 1.5mA-15sh NE did not (p=0.065). In any case, animals from the non-extinguishing group (1.5-15 NE) did not differ from the extinguishing one (1.5-15).

5. ENDOCRINE RESPONSE TO OTHER ENVIRONMENTS: CONTEXT B AND CONTEXT C

The analysis of endocrine response of animals exposed to **Context B** revealed a significant effect for group (Wald X^2 (4) = 21.93; p<0.001). Posterior comparisons revealed that only the 1.5-15 group showed higher ACTH levels as compared to the control group (p<0.001) (Fig 8A). Analysis excluding controls showed significant effect for number (Wald X^2 (1) = 4.50; p<0.05) being all the other factors and interactions NS. Higher ACTH levels were found in 15 compared to 2 shock groups (p<0.05).The corticosterone analysis (Fig 8B) showed a significant effect for group (Wald X^2 (4) = 22.28; p<0.001). Further comparisons revealed higher corticosterone levels in Context B in 1.5-2 and 1.5-15 (p<0.05, p<0.001 vs control group respectively). In addition, analysis without controls revealed that number (Wald X^2 (1) = 3.83; p=0.05) and intensity (Wald X^2 (1) = 4.62; p<0.05) factors were statistically significant. The high shock intensity groups showed higher corticosterone levels than low shock intensity groups (p<0.05), and shocked animals exposed to 15 shocks showed higher corticosterone levels with respect to 2 shocks groups (p<0.05).

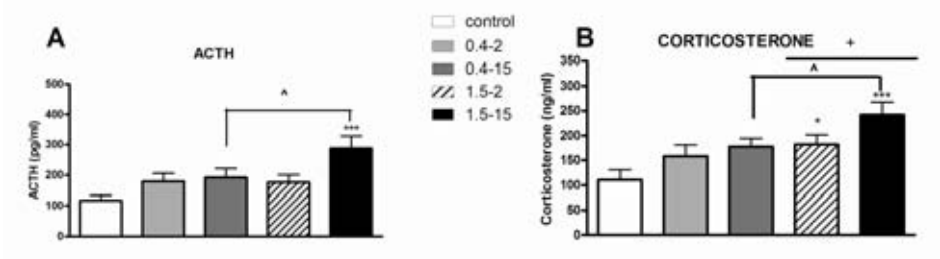


Figure8.Plasma ACTH (A) and Corticosterone (B) levels after 15 min of exposure to Context B, 3 days after shock exposure, for control and animals exposed to 2 or 15 shocks of 0.4 or 1.5 mA. *p<0.05, **p<0.01 and ***p<0.001 vs control group. Means and S.E.M. are shown.

Regarding the endocrine response in **Context C**, neither ACTH nor corticosterone response showed statistically significant differences for group, number or intensity, suggesting a lack of endocrine sensitization (Fig 9A and 9B).

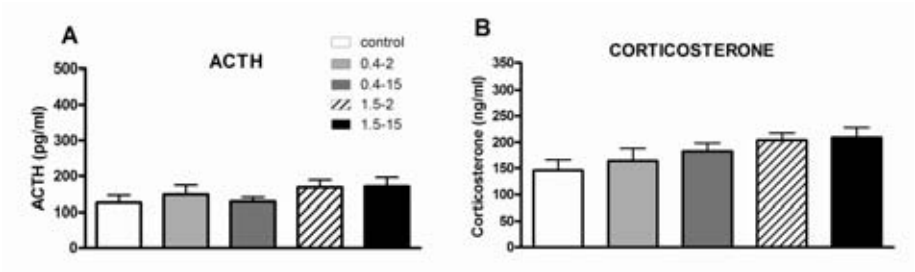


Figure9. Plasma ACTH (A) and Corticosterone (B) levels after 15 min of exposure to Context C, six days after shock exposure, for control and animals exposed to 2 or 15 shocks of 0.4 or 1.5 mA. No significant differences were found. Means and S.E.M. are shown.

Finally, the analysis of the ACTH levels in the 1.5-15 NE group during **Context B** (Fig 10A) revealed a significant effect for group (Wald X^2 (2) = 19.19; $p<0.001$). Later comparisons showed higher ACTH levels in shocked groups as compared to controls ($p<0.001$). The corticosterone response (Fig 10B) followed the same pattern as ACTH, showing a significant effect for group (Wald X^2 (2) = 18.64; $p<0.001$). Both 1.5-15 and 1.5-15 NE presented higher corticosterone levels ($p<0.001$ and $p<0.01$ respectively) than controls. Regarding the endocrine response in **Context C** revealed no differences between groups in ACTH and corticosterone levels after exposure (Fig 10A and 10B respectively).

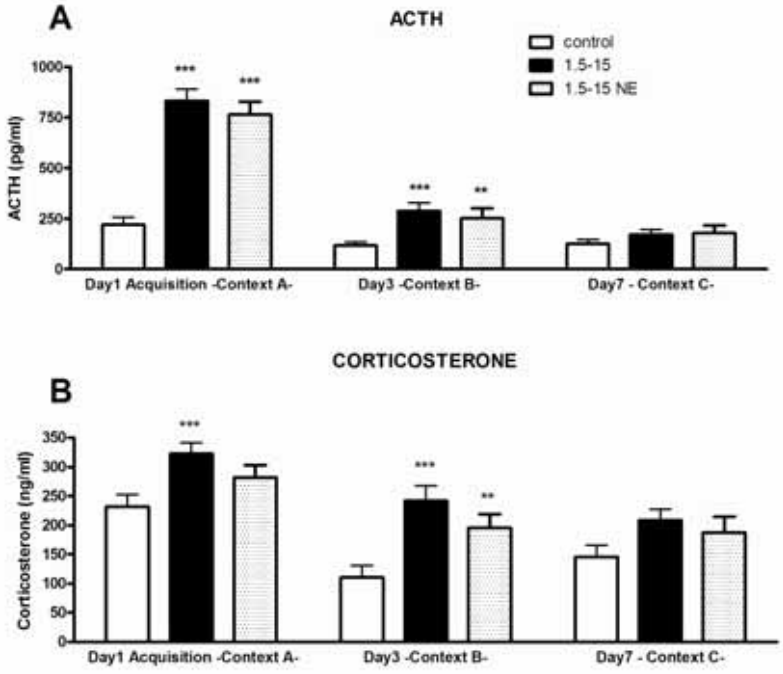


Figure10. Plasma ACTH (A) and Corticosterone (B) levels after acquisition, and after 15 min of exposure to Context B and Context C. Control, 1.5-15 and 1.5-15 NE groups are represented. The 1.5-15 NE group was exposed to shock but extinction training was not undertaken. * $p<0.05$ and ***$p<0.001$ vs control group.Means and S.E.M. are shown.

DISCUSSION

As expected, animals shocked in a specific context (Context A) developed a long-lasting fear conditioning to the context where the shocks were given. Shocked animals re-exposed to the context A (without shock) showed the same level of fear regardless of the intensity or number of shocks. However, the conditioned endocrine response was evident only in the high intensity shock groups. In addition, according to previous results, all animals exposed to the fear conditioning procedure developed hypoactivity in novel environments (Day 3, Context B). Only in the 1.5-15 group hypoactivity was accompanied by a greater endocrine response, suggesting sensitization. Finally, extinction training markedly eliminated hypoactivity in a novel environment (Context C).

SHOCK PARAMETERS AND BEHAVIOURAL RESPONSE TO CONDITIONED CONTEXT (CONTEXT A)

Although freezing behaviour is a rapid and intense response to threatening stimuli and a good measure of conditioned fear in rodents (Blanchard and Blanchard, 1969), some evidence showed that after exposure to mild shock protocols freezing is not the more prominent behaviour (Laxmi et al, 2003). Additionally, there are some difficulties to detect intensity-dependent differences in freezing when high shock intensities are used (Baldi et al, 2004). Taken together, we decided to measure immobility and activity as complementary fear measures. Our results suggest that animals previously exposed to shocks, regardless of their intensity and number, presented the same level of contextual fear conditioning as assessed by freezing, activity and immobility.

The lack of effect of shock intensity on freezing has been previously reported by other authors both in cue (Baldi et al, 2004) and in contextual (Bevins et al, 1997) fear conditioning. Nevertheless, some studies using contextual fear conditioning demonstrated that freezing increased with shock intensity (Young and Fanselow; 1992, Cordero et al, 1998; Merino et al, 2000). These controversies could be explained by the different ranges of shock intensities used among studies. An effect of shock intensity on freezing was usually found when comparing relatively low shock intensities (Young and Fanselow, 1992; Cordero et al, 1998; Merino et al, 2000), but not when comparing in-

termediate-high intensities (Bevins et al, 1997; Baldi et al, 2004), strongly suggesting a ceiling effect on freezing behaviour with high intensity shocks.

Regarding the relevance of number of shocks administered, we failed to found any specific effect on this parameter on the CR. Previous studies using cue-fear conditioning protocols reported a similar absence of effect of shock number on both freezing (Quinn et al, 2008) and activity (Laxmi et al, 2003). Contextual fear conditioning studies observed a positive relationship between the number of CS-US pairings and the intensity of CR, administering only a single shock per day during 6 days (Young and Fanselow, 1992). However, on day 4 the learning curve was asymptotic, reaching already the maximum level of freezing. In our study different ITIs were used in the 2 and the 15 shock groups. It has been observed that the more massed (short ITI) US presentation resulted in lower CR (Fanselow et al, 1993), but it is unlikely that this may have contributed to the present results for two main reasons. First, in the latter study the number of shocks was similar for all ITIs. Second, we have observed similar levels of freezing with 2 and 15 shocks when the ITI was used (unpublished data).

The response patterns of the different behaviours analysed during the 3 days of exposure to the context without shock (extinction) offered interesting results. During the 15 min exposure to the context, within-session extinction was observed in the last 3 min period for all behaviours, but during successive exposure to the context on the next days it was apparent that freezing was the behaviour showing faster extinction whereas immobility and activity extinguished slower, both following a similar pattern. This may suggest that freezing represents a stronger fear state than other behaviours, as suggested previously (Laxmi et al, 2003).

BEHAVIOURAL RESPONSE TO NOVEL ENVIRONMENTS

All shocked groups showed enhanced freezing and immobility and reduced activity when exposed to an unknown environment (Context B) with characteristics markedly different from the shock context. This is in fully accordance with previous results demonstrating that exposure to a single session of footshock causes hypoactivity in unknown environments (Van den Berg et al, 1998; Van Dijken et al, 1992a; Van Dijken et al, 1992c; Van Dijken et al, 1993). Importantly, we and other previously demonstrated that this hypoac-

tivity was associated with the development of contextual fear conditioning and could reflect a certain degree of generalization of fear to any unknown environment (Radulovic et al, 1998a; Daviu et al, 2010).

Although neither the number nor the intensity of shocks significantly affected the magnitude of fear conditioning, shock intensity (regardless of the number) appears to be relevant for the magnitude of generalization. The influence of shock intensity was significant for freezing, but the same trend was observed for the other behavioural measures. The results support the hypothesis that the intensity of the aversive stimulus may be important for generalization of fear to other places (Fanseow, 1981; Laxmi et al, 2003; Baldi et al 2004), which is important regarding PTSD in humans and the development of putative animal models of PTSD.

Finally, we demonstrated that it is possible to partially reverse the hypoactivity caused by fear conditioning through an extinction protocol. Whereas the non-extinguished group showed freezing, immobility and hypoactivity in a novel environment (context C) 8 days after initial shock exposure, the extinguished animals did not show evidence of freezing, and both immobility and hypoactivity were modest as compared to the non-extinguished group. These findings supports previous data suggesting that the hypoactivity is dependent on the learning of context fear conditioning (Radulovic et al, 1998a; Daviu et al, 2010) and agree with results demonstrating that extinction blunted context fear generalization (Golub et al, 2009).

A certain level of hypoactivity was still observed after extinction. This may suggests a modest contribution of non-associative components to hypoactivity. One possibility is a long-lasting shock-induced sensitization of the anxiety response generated by novel environments. In fact, Siegmund and Wotjack (2007b) have reported in mice a sensitization of the ASR after shock that is in great part independent on conditioning.

Extinction training, called exposure therapy, has been used as a cognitive therapy in PTSD patients. It diminishes PTSD symptoms in subjects exposed to trauma (Powers et al, 2010; Foa, 2011). Some studies suggest the capability of fear extinction to disrupt fear memory reconsolidation after recall (Agren et al, 2012). However, controversial results are found about this efficacy (Nor-

rholm et al, 2008, Golkar et al, 2012). Moreover, little is known about the extinction protocol in human fear generalization.

ENDOCRINE RESPONSE TO TRAINING, CONDITIONING AND GENERALIZATION

The endocrine response to shock training (Day 1) revealed a greater ACTH response in all groups exposed to shock as compared to controls merely exposed to the shock chamber. More interestingly, an interaction between shock intensity and number of shocks was observed in that the group exposed to 15 shocks of 1.5mA showed the highest ACTH response as compared with the other shocked groups that did not differ between them. Plasma corticosterone followed a somewhat different pattern in that only the two groups receiving the higher intensity shocks (regardless of the number of shocks) showed higher corticosterone levels than controls. This dissociation is not unlikely considering the delayed different time-course of corticosterone as compared to ACTH and the prompt saturation of adrenocortical synthesis with intermediate levels of ACTH (Armario et al, 2012). These results are in partial agreement with previous data showing a positive relationship between shock intensity and corticosterone response (Cordero et al, 1998), but also introduce the number of shocks as a new parameter to take into account. It appears that shock intensity is the most relevant parameter to activate ACTH release, but the effect is particularly relevant when increasing the number of shocks received.

When the capability of ACTH or corticosterone to reflect contextual fear conditioning was assessed, we observed that a higher response than controls was only observed in the two groups exposed to the higher shock intensity. The following of the ACTH and corticosterone responses over the extinction sessions, showed a similar dependency of shock intensity as that observed during the first testing session and a poor sensitivity to the extinction process. The present data partially support our previous results demonstrating that HPA response is able to reflect conditioning to unique single-shock session (Daviu et al, 2010) or to predator odour (Muñoz-Abellán et al, 2009; 2010). It is possible that the sensitivity of HPA hormones to reflect contextual fear conditioning is not only dependent on the intensity of the US, but also of how stressful is the novel environment itself. If the novel environment is more stressful this may mask conditioning when US are not severe. The present results also suggest that ACTH is likely to be a better index of conditioning

than corticosterone, at least under certain conditions, although simultaneous measurement is recommended.

Previous results showed that the shock-induced hypoactivity in novel environments was not associated with an enhanced HPA response (Daviu et al, 2010). In the present work similar results were observed except for the 1.5-15 condition, which resulted in higher ACTH response to the Context B. As the response to shock exposure (day 1, Context A) followed the same pattern, it appears likely that the results are consequence of stress-induced HPA sensitization that we know is associated with severe stressors, including shock exposure (i.e. Johnson et al, 2002; Belda et al, 2008). On the contrary, this enhanced ACTH response to context B is unlikely to be related to fear generalization as the latter phenomenon was similar in the two high shock intensity groups.

CONCLUSIONS

In conclusion, the manipulation of intensity and number of shocks has different consequences depending on the evaluated response. From a behavioural point of view, shock manipulation does not appear to be very relevant. But, an intensity-dependent endocrine response is observed when animals were exposed to the conditioned context. On the other hand, the endocrine sensitization observed in 1.5-15 group after novel environments exposure is related to the high response showed after shock exposure. Moreover, this sensitization observed in this severe condition disappears across time. Finally, we demonstrate that hypoactivity is partially reversed by an extinction protocol. This last finding provides other therapeutic approaches to the treatment of fear related pathologies as PTSD.

DISCUSIÓN GENERAL

El propósito principal de este trabajo ha sido estudiar la relación entre los efectos a largo plazo de una exposición a un estímulo estresante y la capacidad de éste para inducir miedo condicionado, estableciendo una distinción entre efectos condicionados e incondicionados. Además de la implicación teórica, una finalidad importante ha sido la de aportar información relevante para el desarrollo de modelos relacionados con la patología de ansiedad como es el caso del PTSD.

HIPO-ACTIVIDAD EN AMBIENTES NUEVOS: CONTRIBUCIÓN DE COMPONENTES ASOCIATIVOS

Como se ha observado en diversos estudios, especialmente los realizados en los años 90 por el grupo de Tilder y colaboradores y otros laboratorios holandeses, la exposición a una sesión breve de choques eléctricos es capaz de inducir a largo plazo una disminución de la actividad de los animales expuestos a un ambiente nuevo (Van den Berg et al, 1998; Van Dijken et al, 1992a; 1992c; 1993). La interpretación de esta hipo-actividad y de otros efectos derivados de una sola exposición a estímulos estresantes, siempre ha resultado un tema controvertido, debido a que el hallazgo de determinados efectos a largo plazo divergía en función del modelo de estrés utilizado. Los efectos observados se han relacionado con la aparición de síntomas depresivos (Bruijnzeel et al, 2001a; 2001b), con una sensibilización emocional (Van Dijken et al, 1993) o con un aumento generalizado de la ansiedad (Imanaka et al, 2006; Khan y Liberzon, 2004;Kohda et al, 2007). No obstante, los efectos muy modestos de la administración de ansiolíticos sobre la hipo-actividad causada por la exposición a un choque eléctrico(Van Dijken et al, 1992b) o la ausencia de efectos a largo plazo de la exposición a estímulos estresantes más severos como la IMO (Belda et al, 2008) o la administración de choques eléctricos en la cola característicos del paradigma de la indefensión aprendida (Maier y Watkins, 2005), introduce dudas importantes respecto a las interpretaciones clásicas.

Datos previos obtenidos en otros laboratorios (Radulovic et al, 1998a), junto con trabajos realizados muy recientemente (Sauerhöfer et al, 2012) aportan una nueva perspectiva para esclarecer la falta de consistencia de los efectos a largo plazo. Concretamente, ponen de manifiesto la existencia de componentes asociativos y no asociativos en los efectos a largo plazo de la exposición a un choque eléctrico. Estos datos, junto con datos previos que demostraban la ausencia de efectos a largo plazo de estímulos estresantes más severos que el

choque eléctrico en la cola, nos llevaron a centrar nuestra atención en el establecimiento del miedo condicionado, asumiendo que el establecimiento de dicho condicionamiento sería necesario para el desarrollo de algunos de los efectos a largo plazo. De este modo, la incapacidad de algunos estímulos para generar condicionamiento podría explicar la falta de consistencia en los efectos encontrados cuando comparamos distintos estímulos estresantes. La elección del choque eléctrico como modelo para el estudio del condicionamiento de miedo al contexto está basada en el gran número de trabajos al respecto y la gran reproducibilidad de los resultados.

El primer estudio realizado (capitulo 1, experimento1) mostró, de acuerdo con datos previos (Van den Berg et al, 1998; Van Dijken et al, 1992a; 1992c; 1993), que los animales sometidos a una sesión corta de choques eléctricos mostraban tanto un aumento del freezing (CR)a corto y a largo plazo en el contexto del choque, como hipo-actividad a largo plazo en ambientes nuevos. Más interesante aún, pudimos observar que esta hipo-actividad no desaparecía tras sucesivas exposiciones a diferentes ambientes, observándose efectos hasta 12 días después de la exposición al choque.

Tras los resultados obtenidos con la primera aproximación, seguía siendo necesaria la comprobación directa de la relación entre el establecimiento de miedo al contexto y el desarrollo de hipo-actividad en otros ambientes. Con este objetivo, en el segundo experimento se introdujo un grupo experimental sometido al procedimiento del choque inmediato (Fanselow, 1990). Dado que este procedimiento impide normalmente el establecimiento del condicionamiento del miedo al contexto, su utilización nos permitía separar de manera clara los efectos asociados al condicionamiento de los efectos incondicionados del choque debido a sus propiedades estresantes. Los resultados de este estudio (capitulo 1, experimento 2) mostraron que los animales sometidos al procedimiento de choque inmediato, a diferencia de los animales condicionados, no mostraron ni condicionamiento al contexto ni tampoco presentaron una disminución de la actividad en la exposición posterior a otro ambiente distinto. El hallazgo de que la hipo-actividad a largo plazo en ambientes nuevos era dependiente del desarrollo de un condicionamiento al contexto estaba en consonancia con datos obtenidos por otros autores que ya habían señalado la relación existente entre el establecimiento del condicionamiento y la generalización de la conducta de freezing (Radulovic et al, 1998a). Los autores sugerían también que estos efectos dependientes de la adquisición de un

condicionamiento no estaban relacionados con un aumento de la ansiedad. En consonancia con estos datos previos, nuestro estudio tampoco aportó resultados interpretables como un aumento de la ansiedad. Los animales condicionados no presentaron una disminución ni del tiempo ni del número de las entradas en los brazos abiertos del EPM, ni tampoco mostraron un aumento de la respuesta endocrina delante de los ambientes nuevos, hecho que podría estar indicando un aumento de la respuesta emocional (Armario, 2006).

Hemos confirmamos, por lo tanto, la existencia de efectos a largo plazo dependientes del desarrollo de un condicionamiento de miedo al contexto. Posteriormente a nuestro trabajo, otros autores han demostrado la misma dependencia (Sauerhöfer et al, 2012). La aparición de esta hipo-actividad estaría mediada principalmente por un componente asociativo y no podría interpretarse como una sensibilización frente a cualquier situación estresante. Además, aportamos datos nuevos que revelan la estabilidad de este efecto (12 días), mostrando una ausencia de extinción como consecuencia de la exposición repetida a distintos ambientes nuevos.

¿CUÁL PUEDE SER LA INTERPRETACIÓN DE ESTA HIPO-ACTIVIDAD?

Como se ha comentado anteriormente, después de la exposición a un choque eléctrico, podemos observar sensibilización en algunas de las conductas utilizadas para medir la CR (Kamprack yWotjack, 2004). La exposición a una sesión breve de choques eléctricos puede inducir la aparición de una mayor respuesta de freezing frente a estímulos sensoriales que nunca han sido asociados al choque eléctrico (v.g. tono). Sin embargo, en nuestro estudio, los animales sometidos al procedimiento del choque inmediato no muestran hipo-actividad en ambientes nuevos, por lo que no podemos considerar que dicha hipo-actividad esté desencadenada por un mero proceso de sensibilización frente al estrés que representa un ambiente nuevo. Es necesario considerar explicaciones alternativas que pongan de manifiesto la contribución de un componente asociativo, como en el caso de la generalización del miedo.

El estudio de la generalización del miedo se lleva a cabo, de manera habitual, mediante el análisis en otros contextos de la misma respuesta (CR) que se ha valorado durante la exposición al contexto asociado al choque (en nuestro caso el freezing). Sin embargo, observamos ausencia de freezing tras la exposición a los diferentes ambientes, con la salvedad del cilindro. La aparición de

conductas alternativas al freezing como posibles respuestas de miedo ya ha sido descrita por otros autores (Laxmi et al, 2003), relacionando diferentes intensidades de miedo con distintos repertorios de conductas. Aunque el freezing es la expresión más intensa de miedo, pueden observarse también otras conductas defensivas o de cautela, como la hipo-actividad, con niveles menores de miedo. Así, se observaría hipo-actividad en ambientes que difieren mucho en tamaño y otras características del contexto condicionado original, mientras que en el cilindro, cuyo tamaño es similar al del contexto del choque, aparecería freezing como reflejo de un mayor nivel de miedo. No obstante, el aumento del freezing en el cilindro podría estar relacionado simplemente con su tamaño, dado que la exposición a un ambiente de dimensiones reducidas favorecería la aparición de freezing. En cambio, en ambientes de mayor tamaño se vería favorecida la aparición de otras conductas alternativas.

Existen diferentes teorías acerca de cómo se procesan las señales del contexto, de las que se deriva también la explicación del fenómeno de la generalización. Clásicamente se han barajado dos posibilidades, la teoría elemental (Rescorla y Wagner, 1972) y la configuracional (Pearce, 1994). Sin embargo, en los últimos años, la visión de una tercera posibilidad que englobe a las dos anteriores ha adquirido relevancia. Aunque el contexto se pueda procesar desde un punto de vista elemental, coexiste con un procesamiento del contexto que forma una imagen global que es más que la suma de sus elementos (Rudy et al, 2004).Sin embargo, la interpretación de la hipo-actividad en ambientes nuevos parece ir más allá de la generalización basada en los elementos que configuran el contexto. Los animales estarían generalizando que todos los ambientes nuevos pueden ser potencialmente peligrosos, mostrando así una conducta de cautela frente a estos. Estaríamos por lo tanto frente a un tipo de generalización cognitiva no basada en el procesamiento sensorial del contexto.

ESTUDIO PARAMÉTRICO DEL CHOQUE ELÉCTRICO: EFECTOS CONDICIONADOS E INCONDICIONADOS

Todos los efectos descritos anteriormente utilizando el choque eléctrico como US se han obtenido con sesiones de choques eléctricos breves. Debido a este hecho, no podemos descartar que el aumento de la intensidad del estrés pueda inducir cambios de mayor magnitud de la CR o incluso un aumento de la

ansiedad generalizada. Con este objetivo, estudiamos los efectos de la manipulación de dos parámetros: la intensidad del choque eléctrico y el número de choques. Como se ha comentado extensamente en la Introducción y en el Capítulo III, la relación entre la intensidad del US y la CR puede depender del rango de intensidades de choque utilizado. Así, si nos centramos en el freezing como CR, cuando se trabaja con intensidades relativamente bajas se puede observar una relación positiva entre la intensidad del choque y la de CR (Young y Fanselow 1992; Cordero et al, 1998; Merino et al, 2000); en cambio, se observa un efecto techo cuando las intensidades utilizadas son intermedias o elevadas (Bevins et al, 1997; Baldi et al, 2004).

Teniendo en cuenta las consideraciones mencionadas anteriormente, la realización de un estudio paramétrico del choque eléctrico (Capitulo 3) planteaba la necesidad de introducir algunas modificaciones en el procedimiento experimental. En este estudio se utilizaron cajas de condicionamiento con dimensiones superiores a las utilizadas en los estudios anteriores con el objetivo de valorar si otras variables, como por ejemplo la actividad y la inmovilidad, podrían resultar más sensibles que el freezing a la manipulación de los parámetros del choque. La utilización de cajas estándar para el condicionamiento ofrece pocas posibilidades de medir conductas alternativas al freezing. Además, en el caso de las ratas, la proporción del tamaño de los animales respecto al tamaño de las cajas promueve la inactividad que es fácilmente confundible con el freezing. Los estudios anteriores que habían mostrado la existencia de conductas de miedo alternativas al freezing habían sido llevados a cabo en ratones, los cuales, debido a su tamaño más reducido respecto al contexto, pueden desarrollar conductas alternativas al freezing (Laxmi et al, 2003).

Los resultados del estudio mostraron que ninguna de las variables conductuales analizadas (freezing, actividad e inmovilidad) eran sensibles a los dos parámetros estudiados (intensidad y número). Tampoco se observó efecto del número e intensidad de los choques durante la fase de extinción del condicionamiento. No obstante, el comportamiento a lo largo de los días de las diferentes medidas conductuales fue distinto. El freezing fue la primera medida que mostró extinción. En cambio, la inmovilidad y la actividad mostraron extinción pero aún se observaron diferencias con el grupo control en la última extinción. Este patrón de extinción diferencial del freezing respecto a la inmovilidad y la hipo-actividad parece razonable en términos de intensidad

de respuesta de miedo, siendo la hipo-actividad o la inmovilidad respuestas residuales asociadas a un nivel menor de miedo (Laxmi et al, 2003).

Del mismo modo que en los anteriores estudios, todos los grupos sometidos a choque eléctrico mostraron hipo-actividad cuando fueron expuestos a un ambiente nuevo. En este caso, el grupo 1.5-15parecía mostrar una mayor inhibición de la actividad aunque no llegó a ser significativa respecto a los otros grupos sometidos a choque. En cambio, el freezing se mostró sensible a la intensidad del choque recibido, observándose una mayor generalización de la respuesta en aquellos animales que habían sido condicionados con la intensidad más alta (independientemente del número de choques administrados).Por consiguiente, a diferencia de lo observado en el contexto condicionado, en este caso sí se observa una leve influencia de la manipulación de los parámetros, más concretamente de la intensidad, corroborando datos previos obtenidos en nuestro laboratorio (datos no publicados).

La muy moderada influencia del número e intensidad de choques no es explicable porque el nivel de estrés causado por los diferentes protocolos de choques fuera similar en todos los casos. En respuesta al choque el día de la adquisición, observamos la máxima respuesta de ACTH en el grupo sometido a 15 choques de 1.5mA, el protocolo más intenso. Teniendo en cuenta que este efecto diferencial, del número de choques no se observa en los grupos expuestos a la intensidad de 0.4mA, asumimos que la modificación del número de choques estaría afectando a la respuesta hormonal sólo cunando la intensidad es elevada.

CHOQUE VERSUS INMOVILIZACIÓN: ESTUDIO COMPARATIVO DE LOS EFECTOS CONDICIONADOS

La IMO es considerada un estímulo de alta intensidad teniendo en cuenta los marcadores clásicos de estrés (Armario y Jolín, 1989; Martí et al, 2001; Márquez et al, 2002). La exposición aguda a la IMO es capaz de inducir a largo plazo alteraciones en la memoria declarativa (Andero et al, 2012), y en la extinción de la memoria del miedo (Andero et al, 2011) así como sensibilización emocional puesta de manifiesto por un aumento de los niveles de ansiedad cuando se sobrepone otro estímulo estresante de menor intensidad (Belda et al, 2008). No obstante, a diferencia de lo que ocurre con otros estímulos estresantes de menor intensidad (Armario et al, 2008), no modifica a largo plazo de manera consistente los niveles de ansiedad o de actividad en ambien-

tes nuevos, así como tampoco consigue inducir conductas de evitación relacionadas con el estímulo estresante (efectos condicionados) (datos no publicados).

Considerando la hipótesis de que determinados efectos a largo plazo son dependientes del desarrollo de un condicionamiento de miedo, nos propusimos estudiar el condicionamiento al contexto utilizando la IMO como US y el choque como modelo de referencia. En el segundo bloque de experimentos (capitulo 2, experimentos 1 y 2) se llevó a cabo un estudio comparativo (choque eléctrico o IMO como US) mediante dos experimentos independientes, pero en los dos casos se valoró la CR (conductual y endocrina) en dos momentos distintos: a corto plazo y a largo plazo. La respuesta conductual fue analizada valorando el freezing en el caso del choque y la actividad en el caso de la IMO.

A corto plazo, los animales sometidos a choque eléctrico presentaban un aumento de la conducta de freezing cuando permanecían en el contexto condicionado una vez terminado el choque. Asimismo, los animales sometidos a IMO también mostraron una disminución de la actividad comparada con los controles. Cabe mencionar, que el uso de la actividad como CR inmediatamente después del estrés tiene serias limitaciones, dado que la exposición a estímulos estresantes de elevada intensidad induce una disminución inespecífica de la actividad que puede durar varias horas (Reinstein et al, 1984; Pol et al, 1992).Días más tarde, el estudio de la CR a largo plazo reveló que los animales sometidos a choque eléctrico seguían mostrando CR. Sin embargo, los animales que habían recibido la IMO como US, todo y presentar hipo-actividad a corto plazo, no mostraron una disminución de la actividad como CR cuando fueron expuestos8 días más tarde al contexto condicionado. La hipo-actividad observada el día del condicionamiento no se tradujo en una disminución de la actividad 8 días más tarde, confirmando así el origen inespecífico de la misma el día del condicionamiento.

En los dos primeros estudios ambos estímulos se valoraron por separado. Además, los contextos condicionados, los tiempos utilizados y las medidas conductuales analizadas fueron, por razones técnicas y metodológicas, distintas. En el estudio del condicionamiento del contexto con la IMO como US fue necesario cambiar el contexto condicionado respecto al utilizado en el caso del choque por problemas con el alojamiento de la tabla de IMO por su tamaño. El aumento del tamaño del contexto obligó a cambiar la CR (activi-

dad), debido que el freezing en espacios grandes tiende a disminuir (Bolles y Collier, 1976).No obstante, estas modificaciones no deberían afectar a la capacidad de la IMO para inducir condicionamiento. Se ha demostrado que el olor al depredador, todo y no compartir muchas características metodológicas con el choque eléctrico, promueve sin dificultad el condicionamiento de miedo al contexto (Blanchard et al, 2003c; Muñoz-Abellán et al, 2008). En cualquier caso, se optó por realizar un tercer estudio para garantizar la máxima similitud en el procedimiento, aun teniendo en cuenta que la naturaleza y características de los estímulos son muy dispares. Además, se planteó la valoración del posible papel de la tabla de IMO como clave condicionada específica.

Los resultados de este tercer estudio (capitulo 2, experimento3) mostraron que, a pesar de la homogenización del contexto y del procedimiento experimental, la IMO, a diferencia del choque, se volvió a mostrar incapaz de inducir condicionamiento del miedo a un contexto. De nuevo, sólo el grupo sometido a choque eléctrico mostró CR a largo plazo delante del contexto condicionado, siendo observable por un aumento del freezing, una disminución de la actividad motora y un aumento de la respuesta del eje HPA frente al contexto condicionado (Daviu et al, 2012).

La obtención de manera recurrente de resultados negativos con el uso de la IMO como US, planteó la posibilidad de que algún estímulo más concreto pudiera estar enmascarando el condicionamiento del contexto. Está bien establecido que cuando se lleva a cabo un condicionamiento del miedo a una clave específica, la CR al contexto puede verse reducida (v.g. Baldi et al, 2004). La clave oscurece el condicionamiento del contexto debido a que este queda relegado a un segundo plano (background) y la clave se convierte en el estímulo más relevante, propiciando la aparición del fenómeno denominado ensombrecimineto (overshadowing) (Maren et al, 1994). Existía la posibilidad de que la tabla donde el animal era inmovilizado estuviera actuando como un CS específico, más relevante que el contexto, provocando un ensombrecimiento del último. Con el objetivo de estudiar esta posibilidad, en este tercer estudio, durante la re-exposición al contexto condicionado, la mitad de los animales controles e inmovilizados fueron expuestos al contexto condicionado en el que de forma adicional se había incorporado la tabla de IMO (sin estar el animal sujeto a ella).Los resultados del estudio mostraron que la presencia de la tabla de IMO cuando se valoró el condicionamiento no produjo

una disminución de la actividad en el contexto condicionado. Además, los animales no exhibieron ninguna conducta de evitación hacia la tabla, descartando la aparición de un posible condicionamiento a la tabla de IMO (CS específico) que estuviera debilitando el condicionamiento al contexto.

¿POR QUÉ LA INMOVILIZACIÓN NO ES CAPAZ DE PRODUCIR CONDICIONAMIENTO DE MIEDO AL CONTEXTO?

Podemos considerar varias posibilidades para explicar por qué la IMO no se muestra eficaz como US en un protocolo de condicionamiento de miedo al contexto. La naturaleza tan dispar de los dos estímulos puede ser un factor importante a tener en cuenta En primer lugar, a diferencia del choque eléctrico, el animal sometido a la IMO no pueda explorar el contexto mientras se administra el US. Como se ha comentado en varias ocasiones en este trabajo, la falta de reconocimiento del contexto previo al choque eléctrico origina el déficit del choque inmediato (Fanselow, 1990). La imposibilidad de explorar el contexto durante la IMO podría provocar un déficit similar. Pero, todo y la falta de exploración del contexto durante la exposición a la IMO, que podría estar causando una disminución del potencial de ésta para producir condicionamiento, la exposición previa al contexto (5 min) antes de presentar el US (IMO) debería ser suficiente para inhibir este efecto adverso como ocurre con el choque (Fanselow, 1990; Rudy y O'Really, 1999).

Por otro lado, el choque eléctrico se caracteriza por ser un estímulo discreto y bien delimitado en el tiempo, mientras que la IMO es un estímulo continuo. Al aparecer sólo una vez, aunque sea de forma más prolongada en el tiempo, se puede asumir que el animal no tiene tantas oportunidades para asociar el contexto (CS) con la IMO (US). Sin embargo, aunque esta explicación pueda ser considerada desde el punto de vista teórico, el hecho de que un sólo choque tenga la capacidad de provocar condicionamiento del miedo (Fanselow, 1980; Rudy,1993; Radulovic et al, 1998a), contradice la visión de que esta característica de la IMO pueda resultar un factor crítico para explicar la ausencia de efectos condicionados. Además, la aproximación al estudio del condicionamiento del miedo con la IMO mediante un protocolo intermitente (más parecido al del choque eléctrico) tampoco ha aportado datos positivos. Datos no publicados de nuestro laboratorio muestran como el uso de un procedimiento de IMO irregular, que consiste en inmovilizar y liberar varias veces al animal durante la fase de adquisición, no induce tampoco ningún

efecto condicionado a largo plazo. Cabe mencionar que en este trabajo nos hemos centrado específicamente en el estudio del condicionamiento del miedo al contexto. No obstante, hemos llevado a cabo estudios de condicionamiento de miedo a un CS específico (olor) con la obtención de resultados igualmente negativos. Todos los datos en conjunto ponen en evidencia que la falta de condicionamiento de la IMO es un fenómeno replicable entre experimentos, independientemente de los procedimientos, estímulos y tiempos utilizados.

Como se ha comentado anteriormente, el choque eléctrico es un estímulo que muestra una gran facilidad para el condicionamiento. Esta facilidad estaría relacionada con el concepto de "relevancia asociativa" acuñado por García y Koelling (1966). Estos autores demostraron que determinados emparejamientos de CS y US específicos propiciaban el aprendizaje. Desde este punto de vista, no podemos descartar que la falta de condicionamiento sea debida a que no se ha encontrado el CS adecuado para el condicionamiento de la IMO. Desde la perspectiva ofrecida por este conjunto de datos, la incapacidad de la IMO de inducir condicionamiento podría estar relacionada con el concepto de "preparedness" propuesto por Seligman (1971).Este concepto pone de manifiesto la existencia de asociaciones entre estímulos que están biológicamente preparadas, proporcionándoles una gran rapidez a la hora de realizar aprendizajes. El choque eléctrico podría ser una de ellas, y la IMO por su parte no gozaría de ésta característica. Esta podría ser una posible explicación de la falta de estudios de condicionamiento con otros estímulos estresantes que no sean el choque eléctrico o el olor al depredador.

Existen otras posibles explicaciones alternativas. Muchas evidencias muestran la relación positiva entre la liberación de glucocorticoides y la formación de memorias emocionales, entre las que se encuentra el condicionamiento del miedo (Sandi y Pinelo-Navas, 2007).Sin embargo, tanto la exposición a la IMO (Armario y Jolín, 1989; Martí et al, 2001) como al choque eléctrico inducen una elevada respuesta del eje HPA (Márquez et al, 2002). Por tanto, una baja liberación de glucocorticoides no explicaría la falta de condicionamiento. Contrariamente, debido a la elevada respuesta endocrina que induce la IMO y sobre todo la prolongada activación post-estrés podría interferir en la correcta consolidación de la memoria. Sin embargo, estudios realizados recientemente (Muñoz-Abellán et al, 2011) han mostrado que la exposición a la IMO de forma simultánea a la realización de la adquisición de un condi-

cionamiento utilizando el olor al depredador como US no induce ningún déficit de memoria sobre el condicionamiento del olor al depredador valorado en el contexto condicionado 7 días más tarde. Estos datos descartan cualquier interferencia de la IMO sobre la adquisición de un aprendizaje.

RESPUESTA DEL EJE HPA COMO MEDIDA DE CONDICIONAMIENTO

Se ha demostrado repetidamente que la activación del eje HPA es sensible a la intensidad de los estímulos estresantes, aunque no todas las consecuencias del estrés pueden predecirse a partir de la valoración de la activación de dicho eje (Armario, 2006; Armario et al 2012). Por otro lado, las dos hormonas del eje HPA (ACTH y corticosterona) no siempre cambian en paralelo, básicamente por tres razones: (a) la diferente dinámica temporal de respuesta; (b) la pronta saturación de la secreción adrenocortical con niveles intermedios de ACTH; y (c) la regulación nerviosa de la adrenal, que modula la respuesta a la ACTH circulante. En las condiciones de nuestros experimentos, los datos de ACTH son en general un reflejo más adecuado del procesamiento de la situación en el SNC, razón por la cual prestaremos más atención a esta hormona.

Diversos estudios previos habían puesto de manifiesto la utilidad de la respuesta del eje HPA (usualmente la corticosterona) como reflejo del condicionamiento del miedo al choque eléctrico (Van de Kar et al, 1991; Goldstein et al, 1994; Campeau et al, 1997; Cordero et al, 1998; Merino et al, 2000) y más recientemente al olor a depredador (Muñoz-Abellán et al, 2009; 2010). Sin embargo, pocos estudios han intentado demostrar si la respuesta obtenida frente al contexto condicionado refleja condicionamiento o una posible sensibilización hormonal como consecuencia de la exposición previa al estímulo estresante. Nuestros resultados aportan evidencias de que esta respuesta es debida específicamente a la exposición al contexto condicionado, es decir, respuesta dependiente de procesos asociativos por dos razones básicas. En primer lugar, los animales sometidos a condicionamiento utilizando el choque eléctrico como US sólo muestran una respuesta aumentada del eje HPA delante del contexto condicionado y no delante de otros contextos diferentes, en concordancia con lo obtenido anteriormente con el olor al depredador (Muñoz-Abellán et al, 2009; 2010). Además, aquellos animales que no han podido desarrollar el condicionamiento (animales sometidos al procedimiento de choque inmediato) no muestran un aumento de la respuesta del eje HPA frente al contexto condicionado.

Cuando se baraja la utilización de la respuesta del eje HPA como medida de respuesta condicionada, es preciso realizar un apunte metodológico. En todos los estudios de otros laboratorios en los que se ha observado una respuesta del eje HPA al condicionamiento, la duración de las exposiciones a las diferentes pruebas ha sido de al menos 15 min y ese es el protocolo seguido en nuestro laboratorio. La razón es que el tiempo utilizado de forma habitual para valorar conductualmente el condicionamiento suele ser de 5 min de exposición al contexto condicionado y este tiempo es insuficiente para que la ACTH, de liberación más rápida que la corticosterona, refleje el condicionamiento (Armario et al, 2012). Puesto que 5 min de exposición son suficientes para encontrar un aumento notable de la ACTH en respuesta a un estímulo estresante (Kovacs y Sawchenko, 1996), parece probable que la valoración de los niveles de ACTH a los 5 min de la exposición al contexto no sean suficientes para que el miedo asociado al contexto se refleje en los niveles circulantes de ACTH.

Nuestros datos indican que la dinámica de recuperación de la respuesta del eje HPA el día de la adquisición también proporciona información relevante acerca de los efectos condicionados a corto plazo. Así, se observó una ralentización de la recuperación de la respuesta de eje HPA por la permanencia en el contexto previamente utilizado para administrar el choque respecto a los animales que fueron devueltos a su caja habitual en el animalario. Estos datos, que están en consonancia con los obtenidos anteriormente (Gao et al, 2008), indican que el mantenimiento de los animales en el contexto asociado al choque sostuvo una activación condicionada del eje HPA, lo que puede ser de utilidad para reforzar los datos conductuales. La valoración de la respuesta del eje HPA en el estudio de los efectos de la intensidad y el número de choques mostró que la respuesta endocrina tras los choques eléctricos se veía incrementada en función del número de choques sólo en con la intensidad alta. Los animales del grupo 1.5-15 mostraron unos niveles de ACTH muy superiores al resto de los grupos. Con respecto a la corticosterona, los dos grupos de intensidad elevada mostraron un aumento de sus niveles sin diferencias entre ellos. Por otro lado, el estudio de la respuesta del eje HPA frente al contexto condicionado reveló que la intensidad del choque era el factor más relevante. Tras la exposición al contexto condicionado, los dos grupos de intensidad alta presentaron una mayor respuesta de ACTH delante del contexto comparados con el grupo control, siendo poco relevante el efecto del número de choques. Cabe destacar, que a pesar de que ambos grupos de cho-

que 1.5 mA presentaban un aumento de los niveles de ACTH, sólo el grupo 1.5-15 presentó niveles de corticosterona superiores. Estas disociaciones entre ambas hormonas sugieren que es mejor valorar ambas para obtener una información más adecuada.

La mayor respuesta de ACTH de los grupos 1.5 mA sólo se observó durante la primera exposición al contexto condicionado, sin diferencias posteriores tras las siguientes exposiciones. No obstante, los niveles de corticosterone se mantuvieron elevados en los grupos de alta intensidad, sugiriendo una elevación muy transitoria de la ACTH no observable a los 15 min o una sensibilización de la respuesta de la adrenal. En los primeros estudios con el choque eléctrico no habíamos observado sensibilización de la respuesta hormonal cuando los animales fueron expuestos a un ambiente nuevo. En cambio, en el estudio de los efectos de la intensidad y el número de choques pudimos observar como los animales sometidos al protocolo de choque más severo (15 choques de 1.5 mA) mostraron un aumento de la respuesta de ACTH cuando fueron expuestos a un ambiente nuevo no asociado con el contexto del choque eléctrico. Teniendo en cuenta que la respuesta hormonal del grupo 1.5-15 el día de la adquisición también fue la más elevada, estos resultados sugieren la aparición de sensibilización heterotípica (efecto incondicionado) cuando aumentamos la severidad del estímulo estresante utilizado para la adquisición del miedo condicionado. Estos datos estarían en consonancia con resultados previos que muestran la existencia de una relación positiva entre la intensidad del estímulo estresante y la sensibilización del eje HPA frente a un estímulo heterotípico de baja intensidad (Belda et al, datos no publicados). Por otro lado, los niveles de corticosterona estaban elevados en los dos grupos de 1.5 mA, a pesar de que sólo el grupo 1.5-15 mostraba aumento de los niveles circulantes de ACTH, lo que de nuevo sugiere una sensibilización de la adrenal. Aunque en este experimento la respuesta del eje HPA se mostró aparentemente más sensible a las modificaciones de los parámetros que las variables conductuales analizadas, es muy probable que esto sea debido a sensibilización no específica.

En conjunto, los resultados hormonales confirman que las hormonas del eje HPA son buenos marcadores de intensidad de estrés y son capaces de reflejar el condicionamiento del miedo, pero la conducta es más sensible con niveles bajos de choque en tanto que la respuesta hormonal podría discriminar mejor las intensidades elevadas. No obstante, es difícil descartar en este segundo

caso que los resultados vengan influidos por procesos de sensibilización inespecífica del eje HPA, que además podrían afectar de manera prioritaria a la corticosterona. Considerando las limitaciones de la conducta y la respuesta hormonal, la valoración conjunta de ambas puede ofrecer una más adecuada valoración de los procesos asociados a la exposición a estímulos nocivos.

IMPLICACIONES PARA LOS MODELOS ANIMALES DE PTSD

El PTSD es un trastorno de ansiedad que se caracteriza a grandes rasgos por la presencia de varias tipologías de síntomas (Yehuda y Ledoux, 2007): (i) re-experimentación de la situación traumática en forma de pesadillas o flashbacks, (ii) evitación de estímulos/lugares relacionados con el evento traumático, (iii) generalización del miedo o conductas de evitación a otros estímulos no relacionados con el evento traumático, y por último (iv) la aparición de síntomas relacionados con un aumento del arousal como por ejemplo la hipervigilancia. Recientemente, en la publicación de la versión actualizada del manual diagnóstico DSM-5, se ha incluido otra tipología de síntomas: los pensamientos y estados de ánimo negativos. La introducción de este cluster de síntomas sitúa al PTSD en la intersección entre ansiedad y la depresión (ver anexo DSM 5).

Como se ha comentado extensamente en la Introducción, se han utilizado diferentes modelos animales para el estudio de los efectos a largo plazo provocados por la exposición a un estímulo estresante. En los últimos años se ha extendido ampliamente la utilización del choque eléctrico como modelo para el estudio del PTSD (Stam, 2007; Armario et al, 2008). Su capacidad para producir efectos condicionados y no condicionados a largo plazo con una sola exposición, o la posibilidad de usar el contexto y/o un CS específico como recordatorios o reminders de la situación traumática le aporta validez aparente como modelo de PTSD. Sin embargo, todo y el gran número de cambios conductuales encontrados con el choque eléctrico, el hecho de que los cambios en la conducta producidos por la exposición al choque eléctrico no puedan ser siempre interpretables en términos de ansiedad (Van Dijken et al, 1992b; Radulovic et al, 1998a; Daviu et al, 2010) es un aspecto crítico para la validación de este modelo para el estudio del PTSD.

La falta de efectos del choque eléctrico sobre la ansiedad nos hizo plantear la posibilidad del uso de la IMO como modelo de PTSD. La IMO es un estímulo muy severo, incluso comparado con el choque eléctrico (Márquez et al, 2002).

Es capaz de inducir déficits en la memoria declarativa (Andero et al, 2011) y en la extinción del miedo condicionado (Andero et al, 2010), además de producir efectos sensibilizadores de la ansiedad valorada en el EPM cuando se sobreponen estímulos estresantes de baja intensidad (Belda et al, 2008). No obstante, la falta de efectos a largo plazo sobre la actividad en ambientes nuevos o sobre la ansiedad en condiciones basales siempre han sido aspectos problemáticos para su consideración como modelo de PTSD. En este sentido, el presente trabajo pone de manifiesto una cuestión más importante si cabe que la falta de efectos sobre la actividad y la ansiedad: la ausencia de efectos condicionados de la exposición a la IMO, que sí representa una barrera importante para poder considerar su uso como un modelo de PTSD.

La generalización del miedo a situaciones diferentes de las que originan el trauma es un síntoma clave en pacientes con PTSD y una de las principales causas de malestar de los pacientes (Mahan y Ressler, 2012). La capacidad de identificar la causa de estas conductas puede resultar clave para el desarrollo de modelos y tratamientos. En este trabajo hemos demostrado que la hipo-actividad inducida por la exposición a una sesión breve de choques eléctricos está asociada al desarrollo de un condicionamiento de miedo al contexto y no desaparece con el paso del tiempo. Además, corroborando datos anteriores (Radulovic et al, 1998a), esta hipo-actividad no es interpretable en términos de un aumento general de la ansiedad, sino que reflejaría un fenómeno de generalización del miedo, lo que viene apoyado por los resultados que muestran que la extinción del miedo condicionado (llevado a cabo 72 h después de la adquisición) es capaz de revertir en gran parte la hipo-actividad en otros contextos. Análoga al procedimiento de extinción en roedores, la terapia de exposición en humanos (Foa, 2011) es una de las aproximaciones terapéuticas más eficaces para el tratamiento del PTSD (Taylor et al, 2003; Nemeroff et al, 2006; Foa, 2011). Sin embargo, el mayor reto de este tipo de terapia es poder resolver la dependencia del contexto que tiene la extinción y poder generalizarla a todos los ámbitos de la vida del paciente (Maren et al, 2013). El factor "momento de la intervención" se ha mostrado relevante en el estudio de los efectos asociativos derivados de la exposición a un suceso traumático (choque eléctrico). En este sentido, un estudio de Myers y colaboradores (2006) mostró como la realización de la extinción del miedo iniciada 10 min después de la adquisición (y no 1 h, 24 h o 72h después), era capaz de prevenirdiversos fenómenos de reaparición del miedo tras la extinción como la reanudación (renewal, reaparición de la CR por cambio de contexto de la extinción), la

recuperación espontánea (reaparición de la CR por el paso del tiempo) o la restauración (reinstatement, reaparición de la CR por la administración del US sin señalizar). Además, la extinción del miedo condicionado, tanto temprana (24 h) como tardía (28 días), es capaz de disminuir la generalización del freezing a otros contextos no asociados con el choque (Golub et al, 2009).Los primeros estudios de intervenciones tempranas con terapias de exposición en personas que habían experimentado una situación traumática mostraron que los síntomas relacionados con la depresión y la ansiedad se veían reducidos una semana más tarde en aquellos pacientes que habían sido tratados en el momento de entrar a emergencias (Rothbaum et al, 2008). Estudios posteriores más exhaustivos volvieron a mostrar la eficacia de estos tratamientos tempranos, obteniendo una reducción de la severidad de los síntomas relacionados con el PTSD y una disminución de la comorbilidad con depresión a las 12 semanas (Rothbaum et al, 2012).En consonancia con los resultados obtenidos en animales, estudios de neuroimagen en humanos muestran como la perturbación de la reconsolidación mediante un proceso de extinción realizado 6 h tras la reactivación de la memoria por la presentación del CS, produce una disminución de la activación del complejo BLA y como consecuencia de todo el circuito relacionado con el miedo cuando los sujetos son expuestos 24 h después a una sesión de reanudación (renewal) del miedo condicionado (Agren et al, 2012). Aunque estos hallazgos han reavivado el interés por el posible uso de las intervenciones tempranas (extinción) como prevención para el desarrollo de patologías relacionadas con la exposición a una situación traumática, como el PTSD (Kearns et al, 2012), algunos autores se muestran escépticos (Schiller et al, 2008, Archbold et al, 2009).

También se ha estudiado en modelos animales la relevancia del momento de la intervención en los síntomas no asociativos del PTSD. Los estudios muestran como ni la extinción temprana (24 h) ni la tardía (28 días) consiguieron atenuar de una manera clara un síntoma considerado no-asociativos como es el aumento de la respuesta de sobresalto (Golub et al, 2009). Esta afectación diferencial estaría mostrando que la generalización del miedo (freezing o hipo-actividad) en otros contextos y la sobreactivación son procesos distintos, aportando datos relevantes que apoyan la importancia del momento de la intervención. En muchos casos la terapia de exposición tardía solo estaría afectando a los componentes asociativos, dejando a los no-asociativos intactos.

CONCLUSIONES

CONCLUSIONES

1. La exposición a una sesión breve de choques eléctricos da lugar a un condicionamiento de miedo al contexto a corto plazo y, paralelamente, a una disminución a largo plazo de la actividad en ambientes nuevos no asociados al choque. Esta hipo-actividad no se extingue tras sucesivas exposiciones a diferentes ambientes nuevos, mostrando ser un efecto muy robusto.

2. La exposición a una sesión breve de choques eléctricos no parece tener efecto sobre la ansiedad, valorada en el laberinto elevado en cruz, dos días después de la exposición al choque.

3. La exposición de los animales al choque eléctrico sin la posibilidad de explorar previamente el contexto (choque inmediato) no induce condicionamiento del miedo al contexto ni tampoco hipo-actividad en ambientes nuevos. Estos datos sugirieren un proceso asociativo, quizás relacionado con la generalización del miedo.

4. Las hormonas del eje HPA (ACTH y corticosterona) son capaces de reflejar no sólo el estrés causado por el choque eléctrico sino también el condicionamiento del miedo al contexto, pero no la hipo-actividad en ambientes nuevos, lo que sugiere que la hipo-actividad representaría una conducta cautelosa más que miedo/ansiedad.

5. Aunque el desarrollo de condicionamiento de miedo al contexto es extremadamente fácil cuando se utiliza el choque eléctrico como estímulo nocivo, la inmovilización no (IMO) es incapaz de inducir dicho condicionamiento, sugiriendo que dicha capacidad no sería una propiedad universal de todos los estímulos estresantes. La falta de condicionamiento de la IMO es observable tanto con medidas conductuales como hormonales.

6. La exposición de los animales a la tabla de IMO no produjo ningún tipo de conducta de evitación interpretable como signo de condicionamiento, descartando que la falta de condicionamiento del miedo al contexto sea debida a un

fenómeno de ensombrecimiento (*overshadowing*) por una señal específica dominante.

7. La incapacidad de la IMO de producir condicionamiento de miedo al contexto podría explicar la falta de efectos a largo plazo sobre la actividad en ambientes nuevos y la ansiedad basal, cambios que sí son observables en otros modelos de estrés agudo.

8. Cuando el condicionamiento del miedo tiene lugar en un compartimento de mayor tamaño que el habitual, los animales desarrollan distintas conductas (*freezing*, inmovilidad e hipo-actividad), pero ninguna de ellas parece ser sensibles a los cambios en la intensidad o el número de choques. Sin embargo, durante la extinción presentan dinámicas temporales distintas, sugiriendo que reflejan distintas intensidades de miedo.

9. El nivel de generalización del miedo a otros contextos parece estar moderadamente influenciado por la intensidad del estímulo estresante utilizado durante la adquisición, pero no por el número de choques.

10. La extinción del miedo condicionado (producida por la exposición repetida al contexto sin el choque) es capaz de revertir en gran medida la hipo-actividad observada en ambientes nuevos inducida por la exposición al choque eléctrico, apoyando la hipótesis del origen asociativo de esta hipo-actividad.

11. Las hormonas del eje HPA son capaces de reflejar de forma consistente la intensidad de las situaciones estresantes y en gran medida el condicionamiento del miedo al contexto, pero presentan algunas limitaciones, particularmente en el caso de la corticosterona, y son en general menos sensibles que la conducta para reflejar los efectos condicionados de la exposición al choque eléctrico.

12. Los resultados de la presente tesis confirman que los efectos a largo plazo de la exposición al estrés probablemente implican en gran medida procesos asociativos, pero no descarta la contribución de procesos no asociativos de

sensibilización de la respuesta emocional a nuevas situaciones de estrés. En cualquier caso, la intensidad del estrés, valorada por los índices biológicos clásicos, no necesariamente predice las consecuencias conductuales a largo plazo y este hecho es de gran relevancia respecto a los posibles modelos animales de PTSD.

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