5
Study 3

Dissociation between contextual X cued auditory fear conditioning in rats selective bred for high and low conditioned freezing

5.1
Objectives

The objective of the present study was to evaluate the CHF and CLF lines in both contextual and discrete fear-conditioned paradigms.

5.2
Subjects

The experiment was performed using adult female CHF (n=23) and CLF (n=26) rats, from S_{12}/F_2 generation, aged 15-19 weeks and weighing 350-450 g at the time of experiment. Animals were born and maintained in the colony room of the Psychology Department at PUC-Rio with controlled room temperature (24 ± 1°C) and a 12 h/12 h light/dark cycle (07:00-19:00 h). Animals were housed in groups of three to five, according to their respective lines, in polycarbonate cages (18 × 31 × 38 cm) with food and water available *ad libitum*. Fear conditioning occurred during the light phase of the cycle. For 5 days before the fear conditioning experiment, the animals were handled once daily for a period of 2 min.

5.3
Equipments

Both contextual and auditory cued fear conditioning procedures occurred in four observation chambers (25 × 20 × 20 cm), each placed inside a sound-attenuating box. A video camera was mounted in the back of the observation chamber so the animal's behavior could be observed on a monitor outside the experimental chamber. Freezing behavior, defined as the absence of all non-respiratory movements, was used as a measure of fear. The floor of each chamber consisted of 15 stainless steel rods (4 mm diameter) spaced 1.5 cm apart (center-to-center), which were wired to a shock generator and scrambler (Insight, São Paulo, Brazil). An interface with eight channels (Insight) connected the shock generator to a computer allowed the analyst to apply an electric footshock. A speaker mounted outside a grating in one wall of the chamber was used for the delivery of acoustic CS (conditioned stimulus; pure tone, 90 dB, 2 mHz, 30 s). An ammonium hydroxide solution (5%) and a peppermint scent solution (10%) were used to clean the chamber before and after each subject (odors from these solutions were also used to establish unique olfactory contexts).

Although the same equipments described above were used for all procedures, we create two distinct contexts (A and B), in order to avoid generalizations among experimental days. Table 7 shows the specific adjustments for each experimental set. For the first context (Context A), a 15 w red house-light mounted above the conditioning chamber was turned on. The chambers were cleaned with a 5 % ammonium solution. To provide a distinct odor, stainless steel pans containing a thin layer of this solution were placed underneath the grid floors before the rats were placed inside. Rats were transported from their home cages to this context in white plastic boxes.

For the second context (Context B), 15 w red fluorescent lights were turned on providing illumination, ventilation fans were kept off and the chambers were cleaned with a 10% peppermint scent solution. Also, stainless steel pans containing a thin layer of this same solution were placed underneath the grid floors before the rats were placed inside to provide a distinct odor. In order to create a distinct context configuration, two opposite wooden plaques, in a 65^{o} angle were placed in the chamber. Rats were transported from their home cages to this context in black plastic boxes.

	Context A	Context B
15 w red house-light	On	On
Ventilation Fans	On	Off
Cleaning Solution	5% ammonium	10% peppermint scent
Rats Transportation	White plastic box	Black plastic box
Extra Walls	-----------	Two opposite wooden plaques

Table 7: Context configuration adjustment for Contexts A and B.

5.4
Procedures

Day 1

For the acquisition of cued fear conditioning, rats were transferred from the animal facilities in white plastic boxes, placed in Context A and habituated in the observation chamber for 4 min. After this period, fear acquisition was elicited by presenting audible cues (CS) that terminated with electric footshocks (US, 0.6 mA, 1 s, four times). A random stimulus-free period (2-5 min) separated and followed the shocks. After the electric shocks, rats were left in the acquisition chamber for a period of 2 min.

Day 2

For testing of the contextual fear conditioning, 24 h later rats were transferred from the animal facilities in white plastic boxes and placed in Context A for a period of 12 minutes. No footshock or other stimulation occurred during this period.

Day 3

For testing of the cued auditory fear conditioning, animals were transferred from the animal facilities in black plastic boxes and placed in Context B. After a period of 4 minutes of habituation, a CS audible cue (pure tone, 90 dB, 2 kHZ) was presented for 8 minutes. Rats were then returned to their home cages. Figure 18 shows the behavioral paradigm of the experiment.

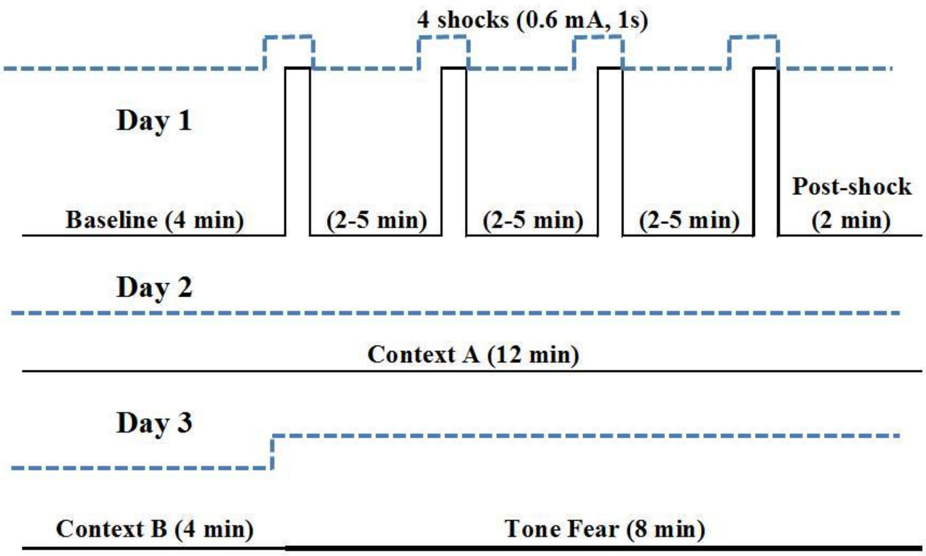

Figure 26: Contextual fear conditioning procedure used for cued auditory fear conditioning; ----- represents auditory conditioned stimulus (CS); —— represents the footshock unconditioned stimulus (US).

5.5
Results and discussion

Figure 27 shows the means (± SEM) percentage of conditioned freezing observed in the acquisition session (Day 1). The Student's t-test showed no significant differences between CHF and CLF animals in the baseline acquisition period (t_{47}=0.89; p=0.38), but analyses indicate significant differences in post-shock freezing (t_{47}=3.63; p<0.001).

Figure 28 shows the means (+ SEM) percentage of conditioned freezing registered in Context A on the second day of experiments. Significant differences between CHF and CLF animals were observed in the levels of conditioned freezing behavior (t_{47}=2.57; p<0.05). These results replicate the behavioral pattern observed in CHF and CLF rats in Studies 1 and 2 of the present thesis, and also of previous report for females (Gomes & Landeira-Fernandez, 2008).

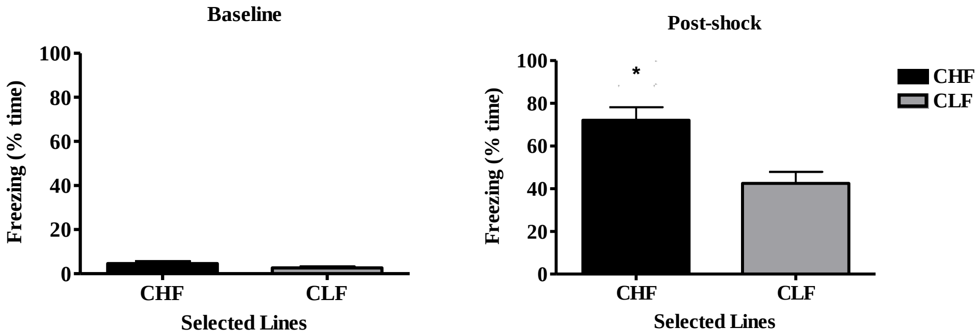

Figure 27: Means + SEM of conditioned freezing observed on Day 1 in baseline and post-shock acquisition periods of CHF and CLF female rats; * denotes significant differences between CHF and CLF rats (P<0.001).

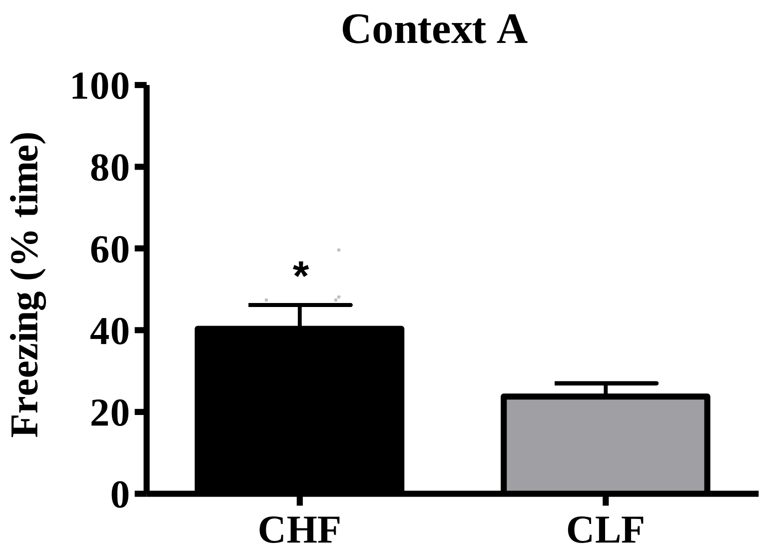

Figure 28: Means + SEM of conditioned freezing along the 12 minutes of Context A retrieval on Day 2 for CHF and CLF female rats; * denotes significant difference (p<0.001).

Figure 29 indicates the means (± SEM) percentage of conditioned freezing in the altered context as well as freezing associated to auditory CS registered on the third day of experiments. Very low levels of freezing in Context B on the third day of experiments were observed. The Student's t-test showed no significant differences between CHF and CLF animals ($t_{47}=1.68$; $p>0.05$). Importantly, these results indicate the absence of generalization to the changed environment of Context B.

The purpose of this study was to evaluate the dissociation between contextual and cued auditory fear conditioning. In this sense, results showed non-significant differences between CHF and CLF rats ($t_{47}=0.33$; $p=0.74$) in tone fear.

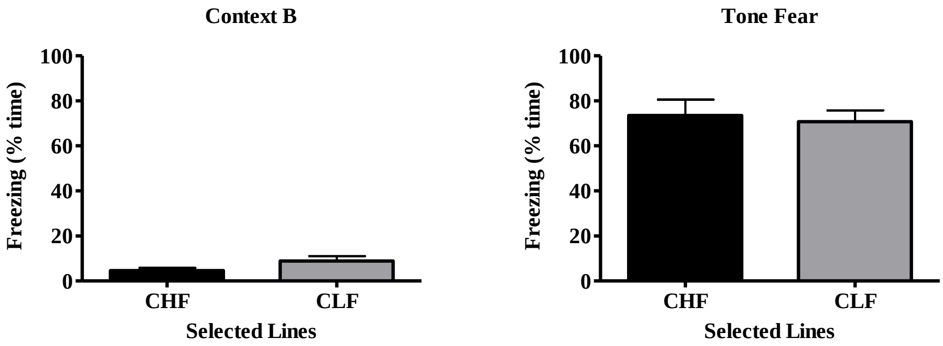

Figure 29: Means ± SEM of conditioned freezing observed on Day 3 in Context B and Tone fear periods of CHF and CLF female rats (P>0.001).

By using a classical cued fear conditioned paradigm, we found that CHF rats, with an innate predisposition to present higher levels of contextual fear conditioning measured as freezing behavior, demonstrated the same levels of freezing in response to a cued auditory stimulus in comparison to CLF rats, for females. Low levels of freezing observed in both lines in the altered context (Context B) prior the occurrence of the tone indicate no generalization. These results suggest that the continuous selective breeding for freezing in response to contextual cues in CHF and CLF rats may not be influencing the neural circuitry underlying freezing behavior in response to discrete/phasic stimuli in these lines of animals. This finding is in agreement with several reports which indicate that fear conditioning in response to a discrete CS and contextual cues is mediated by different neural circuitries (Indovina et al., 2011; Ferreira et al., 2003; Kim &

Fanselow, 1992; LeDoux, 2000; Pohlack et al., 2011), and supports the hypothesis of at least two dimensions of fear conditioning, each related to clinically distinct anxiety disorders. Specific phobias, characterized by cue-specific or phasic fear reactivity, might be modeled by aversive conditioning in response to a discrete CS (Grillon, 2002; Grillon and Davis, 1997). GAD, on the other hand, is characterized by persistent and diffuse or non-cue-specific anxiety and can be modeled by contextual fear conditioning (Brandão et al., 2008; Grillon and Davis, 1997)

Nonetheless, this lack of significant differences in the freezing behavior in response to a discrete CS may be related to strength of the fear conditioning procedure itself. For example, Muigg et al (2008) tested rat lines selectively bred for high (HAB) and low (LAB) anxiety-related behavior in a classical cued fear conditioning task utilizing freezing responses as a measure of fear. In the same manner as our results, they found that cued fear acquisition was similar in both lines. However, they intended to produce similar levels of freezing in both lines in order to study the fear extinction. In this sense, they employed a relatively strong conditioning paradigm, including five CS/US pairings, and a stronger shock intensity (0.7 mA) than we used in the present experiment. Thus, it is possible, through a massive conditioning procedure, to produce similar levels of freezing in two lines selectively bred for high an low anxiety related responses. Moreover, LAB rats show an enhanced (baseline and fear-potentiated) startle response, as compared to HAB rats (Yilmazer-Hanke, Wigger, Faber-Zuschratter, Linke, & Schwegler, 2004).

A divergent result was reported by López-Aumatell et al (2009). In this study, they employed inbred strains (RLA-I and RHA-I) derived from the swiss sublines of the Roman High- (RHA-Verh) and Roman Low- (RLA-Verh) Avoidance, in a fear conditioning procedure. The results indicate that, compared to RHA-I rats, RLA-I animals display higher levels of conditioned fear to contextual and to a visual CS. However, they did not employ an altered context to present the cued visual CS. The fear of a visual CS was measured in the same context where the rats were trained in the previous day. In this sense, it is possible that the behavioral divergence in response to a visual CS may lead to the rise of generalization, and not particularly to the visual CS.

In addition, a possible criticism of these results is related to the intensity of the tone used in the present experiment. Although we used tone intensity (90 dB) in the range of most reported studies, it is not clear whether this intensity is influencing the conditioned freezing response. Indeed, the amount of tone fear observed is much higher than the contextual fear, for both lines. In this sense, further studies employing different tone intensities are necessary to investigate the auditory fear conditioned among CHF and CLF rat lines. Moreover, it is important to employ different CS (visual, olfactory) to dissect possible differences between contextual and discrete fear conditioning in the Carioca lines.