Neuroscience Letters 474 (2010) 32–36

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

One-way avoidance learning in female inbred Roman high- and low-avoidance rats: Effects of bilateral electrolytic central amygdala lesions

Ignacio Morón[a], Mª José Gómez[b], Mª Dolores Escarabajal[b], Lourdes de la Torre[b], Antonio Cándido[a], Antonio Maldonado[a], Adolf Tobeña[c], Alberto Fernández-Teruel[c], Carmen Torres[b,*]

[a] Faculty of Psychology, University of Granada, 18071, Granada, Spain
[b] Department of Psychology, Faculty of Humanities, University of Jaén, Campus de Las Lagunillas s/n, 23071, Jaén, Spain
[c] Medical Psychology Unit, Department of Psychiatry and Forensic Medicine, Institute of Neurosciences, School of Medicine, Autonomous University of Barcelona, Campus Bellaterra, 08193, Barcelona, Spain

ARTICLE INFO

Article history:
Received 30 November 2009
Received in revised form 16 February 2010
Accepted 1 March 2010

Keywords:
Anxiety
Avoidance
Central amygdala
Electrolytic lesion
Roman high- and low-avoidance rats
Safety

ABSTRACT

Female inbred Roman high- (RHA-I) and low- (RLA-I) avoidance rats show differences in one-way avoidance learning only when the task implies a highly aversive situation (1 s in the "non-shock"-associated safe compartment, as opposed to 30 s). These between-strain differences seem to depend on strain differences in emotionality, given that: (i) they are abolished by IP administration of the GABAergic anxiolytic diazepam (Torres et al. [32]) and (ii) avoidance responding appears to correlate with cellular density in the basolateral amygdala (Gómez et al. [9]). In the present study we further analyzed whether the implication of the amygdala in one-way avoidance depends on the experimental situation aversiveness (30 s vs. 1 s in safety). After bilateral electrolytic lesions (1 mA; 20 s) of the central amygdala (CeA), RHA-I and RLA-I rats were exposed to a danger compartment (where they received a warning signal – 88 dB tone – followed by a 1 mA electric foot-shock), and a safe compartment, where these stimuli were not presented. The number of trials needed to reach 5 consecutive avoidance responses was used as dependent variable. Sham lesioned RLA-I rats showed poorer performance than sham lesioned RHA-I rats only under the 1 s condition. The CeA lesion disrupted the avoidance response only in 1 s groups, abolishing the between-strain performance differences observed under this condition. These results indicate the implication of CeA in one-way avoidance performance, and suggest a reciprocal modulation of fear and reinforcement (i.e. fear relief) in this form of aversive learning.

The Swiss sublines of Roman high-avoidance (RHA/Verh) and Roman low-avoidance (RLA/Verh) rats were initially selected and bred for their very good (RHA/Verh) vs. extremely poor (RLA/Verh) acquisition of the two-way active avoidance response [7]. Two inbred strains (RHA-I and RLA-I), derived from those outbred rat lines in 1993, are maintained at the Barcelona Laboratory since 1997 [8]. This psychogenetic selection procedure led to stable between-strain divergences related to (among others) anxiety/fearfulness and behavioural inhibition/activation (i.e. coping style) traits. Thus, when exposed to a broad variety of emotional situations, such as fear conditioning of startle/freezing responses, Vogel's punishment test, frustrative nonreward, novel environments, elevated "zero" maze and light–dark testing procedures, RHA rats (outbred or inbred) consistently exhibit decreased stress-related behavioural and endocrine responses [4,19,21,23]. Recent studies conducted in our lab indicate that RHA-I and RLA-I rats also show performance differences in one-way avoidance learning [9,32]. In this task, subjects are exposed to two different compartments. In the "danger compartment" they receive a warning signal followed by an electric foot-shock. In the "safe compartment" the warning signal or the shock never appear [25]. Given that animals can easily associate one compartment with danger and the other one with safety, avoidance response has been considered as a mixture of flight from fear and approach to safety, the weight of each component (aversive vs. appetitive) being a function of the relative time spent in the danger and safe compartments, respectively [3]. We found RHA-I/RLA-I performance differences when the task implied a highly aversive situation mainly dependent on flight from fear (1 s of stay in the safe compartment), whereas no strain differences were observed when animals remained 30 s in that compartment [32]. These behavioural differences seemed to depend on the repeatedly observed between-strain divergences in emotional reactivity, given that: (i) they were abolished by IP administration of the GABAergic anxiolytic diazepam [32] and (ii)

* Corresponding author at: Department of Psychology, University of Jaén, Campus Las Lagunillas s/n, Building C-5, Office number 120, 23071 Jaén, Spain.
Tel.: +34 953 21 22 92; fax: +34 953 21 18 81.
E-mail address: mctorres@ujaen.es (C. Torres).

0304-3940/$ – see front matter © 2010 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2010.03.001

they correlated to differences in cellular density in the basolateral amygdala (BLA), a brain structure related to fear conditioning and anxiety [9]. The present experiment was conducted with the goal of further analyzing the implication of amygdala in the one-way avoidance performance differences characterizing the RHA-I and RLA-I rat strains, focusing our attention on central amygdala (CeA). Serial processing based theories suggest that BLA is involved in the formation of associations between neutral and fear stimuli [24], whereas CeA is believed to be a region that regulates the expression of autonomic and somatomotor emotional responses, including those involved in one-way avoidance learning [16]. Given that RHA-I and RLA-I significantly differ in fearfulness, it could be expected a more interfering effect of the CeA lesion when animals were exposed to the more fearful/aversive 1 s condition, as opposed to 30 s.

Thirty-four female RHA-I and 34 female RLA-I rats from the colony of inbred Roman rats maintained at the Autonomous University of Barcelona were used. Animals used in the present study were about 120-day old at the beginning of the experiment (weights between 220 and 286 g) and they were individually housed with food and tapwater ad lib. Room temperature was kept at about 20 °C with a 12-h light/dark cycle. Training took place during the light phase. The day and the time of testing were counterbalanced across groups in order to avoid the oestrus cycle influence. The experiment was conducted following European Union (EU) guidelines on the use of animals for research (86/609/EEC).

A Letica one-way avoidance chamber of two equal compartments, 27 cm long × 25 cm wide × 28 cm high, made of Plexiglas was used. The compartments were separated by a 0.5 cm thick partition, 25 cm wide × 28 cm high, with a square 9 × 9 cm hole and a removable gate to allow movement between compartments. The floor in both compartments was hinged to operate a micro-switch when depressed controlled by a PC-XT microcomputer. The warning signal was a 2000 Hz tone of 88 dB. The danger compartment was fitted with a grid floor of 19 stainless steel rods 4 mm in diameter and spaced 2 cm apart center to center, connected in series to a Letica LI-2900 module capable of delivering a continuous scrambled shock. The roof of the danger compartment consisted of a black glass panel, which was removed only to put the rat into the chamber. A rigid, non-transparent white plastic carrying box 24 cm long × 14 cm wide × 19 cm high was placed in the safe compartment and used as the safe compartment and to move the rat when the safe time was completed. An air extractor installed outside the avoidance chamber produced a background noise of 70 dB.

Surgery was performed under general anaesthesia with sodium thiobarbital (50 mg/kg, Lab. Abbot, Spain). Electrolytic lesions of the CeA were made introducing a 200 μm stainless steel electrode, insulated except the distal cross-section, at four insertion points (two on each hemisphere). After the animals were placed in the head holder and the bilateral trephine holes (1.0 mm diameter) were made, the stainless steel electrode was introduced at AP −2.3, L ±4.4, V −8.2 and AP −3, L ±4.8, V −8 to bregma [26]. In the CeA lesion groups, a 1 mA direct negative current was passed through the electrode, over the CeA, for 20 s. In the control groups, in turn, the electrode was introduced up to the surface of the CeA, but no current was administered (sham lesion). After the stereotaxic surgical procedure of both, lesioned and sham groups, the electrode was removed and the wound sutured.

After a week of recovery from surgery period, rats were removed from their homecages and put into the avoidance box, placed in an adjacent quiet room, where they were allowed to freely explore both compartments for 5 min. Thereafter, the communication gate was closed shutting the rat in the danger compartment, and the trials began. Each trial consisted of a warning signal (conditioned stimulus, CS) followed after 5 s by an electric shock of 1 mA (unconditioned stimulus, US). Both the warning signal and the shock were continued until the animal moved into the safe compartment, or until 30 s had elapsed. The gate between the two compartments was opened in the presence of the warning signal and closed when the rat entered the safe compartment. Time in the danger compartment before the onset of the warning signal was the same for all experimental conditions (15 s). Once the 30 s vs. 1 s safe time elapsed, the transportation box was lifted over the apparatus and the rat was returned to the danger compartment (the roof of this compartment was opened briefly for about 2 s, and then closed). The box was then replaced in the safe compartment of the avoidance chamber. The rats were randomly assigned to one of the eight groups (see Table 1).

Table 1
Experimental design (CeA: central amygdala).

Group	*n*	Strain	Time in safe compartment (s)	Lesion
30/RHA/CeA	8	RHA-I	30	CeA
30/RHA/SHAM	8	RHA-I	30	SHAM
30/RLA/CeA	9	RLA-I	30	CeA
30/RLA/SHAM	8	RLA-I	30	SHAM
1/RHA/CeA	9	RHA-I	1	CeA
1/RHA/SHAM	9	RHA-I	1	SHAM
1/RLA/CeA	9	RLA-I	1	CeA
1/RLA/SHAM	8	RLA-I	1	SHAM

At the end of the experiment, the subjects were deeply anaesthetized with an overdose of sodium thiobarbital and intracardially perfused with isotonic saline and 10% formaldehyde solution. The brains were removed and stored in the same fixative solution for at least 48 h. After that, the brains were cut into 50 μm coronal sections prepared with a freezing microtome (752 Vibroscile Tissue Cutre, Campeen Instruments Limited, UK). The location and extension of the electrolytic lesions was verified with cresyl violet staining under a light microscope. The images were captured using a TV Olympus (U.PMTVS; 7M 03796, Japan) camera. The histological analysis showed that the bilateral electrolytic lesions of the amygdala affected principally the dorsal part of CeA and, partially, the lateral amygdala (see Fig. 1).

The number of trials to reach five consecutive avoidance responses (5CARS) was used as dependent variable. When an animal did not reach this criterion after 100 trials had elapsed, it was removed from the avoidance box and a value of 100 was assigned for that criterion. The criterion was considered to be met at the first of the sequence of consecutive responses.

The mean number of trials required to carry out 5CARS in each experimental condition was subjected to a three factor analysis of variance, with Strain (RLA-I vs. RHA-I), Safety (30 s vs. 1 s in safe compartment) and Lesion (CeA vs. Sham) as factors. For all statistical analyses, alpha was set at 0.05.

Fig. 2 presents the mean numbers of trials needed to reach five consecutive avoidance responses (5CARS) in CeA lesioned and control RHA-I and RLA-I rats exposed for 30 vs. 1 s in the safe compartment.

A 2 (Strain) × 2 (Safety) × 2 (Lesion) ANOVA showed a significant main effect of Safety, $F(1, 60) = 64{,}683$, $p < 0.0005$ and Lesion, $F(1, 60) = 15{,}243$, $p < 0.0005$. The main effect of Strain was marginally significant, $F(1, 60) = 3.941$, $p < 0.052$. The interaction of Safety × Lesion was also significant, $F(1, 60) = 10{,}488$, $p < 0.002$. Subsequent analyses conducted to explore the source of this interaction showed that the simple effect of Lesion was significant in the 1 s safety condition $F(1, 33) = 14{,}457$, $p < 0.0001$, but not in the 30 s safety condition, $F(1, 31) = 1736$, n.s.

In this study, sham- and CeA-lesioned animals, psychogenetically selected on the basis of their two-way avoidance acquisition response (RHA-I and RLA-I rats), were trained in a one-way avoidance task with the goal of exploring whether the implication of CeA