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Research report

Spaced sessions of avoidance extinction reduce spontaneous recovery and promote infralimbic cortex activation

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ABSTRACT

Extinction-based therapies (EBT) are the psychological treatments of choice for certain anxiety disorders, such as post-traumatic stress disorder. However, some patients relapse and suffer spontaneous recovery (SR) of anxiety symptoms and persistence of avoidance behaviour, which underlines the need for improving EBT. In rats, recent evidence has highlighted the relevance of the temporal distribution of extinction sessions in reducing SR of auditory fear conditioning, although it has seldom been studied in procedures involving proactive avoidance responses, such as two-way active avoidance conditioning (TWAA).

We examined whether the temporal distribution of two extinction sessions separated by 24 h or 7 days (contiguous versus spaced extinction paradigms, respectively), influences SR after 28 days of a TWAA task. c-Fos expression, as a marker of neuronal activation, was also measured by immunohistochemistry 90 min after the SR test in the amygdala and the medial prefrontal cortex.

The temporal distribution of extinction sessions did not affect the degree of extinction learning. However, only the rats that underwent the 7-day spaced extinction paradigm maintained the level of extinction in the long term, showing no SR in TWAA. This behavioural finding was consistent with a greater number of c-Fos-labelled neurons in the infralimbic cortex in the 7-day group, and in the Lateral and Central nuclei of the amygdala in the 24-hour group. These findings show that a time-spaced extinction paradigm reduces the spontaneous recovery of active avoidance behaviour, and that this behavioural advantage appears to be related to the activation of the infralimbic cortex.

1. Introduction

Extinction-based therapies (EBT), such as exposure therapy (ET), are the psychological treatment of choice for certain anxiety disorders, such as post-traumatic stress disorder (PTSD) [1,2]. In ET, patients have to confront the conditioned stimuli (CS) related to a stressful experience. Although this therapy is highly effective in reducing conditioned fear and avoidance responses, some patients relapse and suffer spontaneous recovery (SR) of these symptoms again [3].Thus, the prevention of fear and avoidance recovery has become one of the main priorities in the field of anxiety disorders and PTSD in particular.

The behavioural and neuronal features of expression and extinction of conditioned fear responses have been widely studied in animals using fear conditioning (FC) [1,4,5], a PTSD-like validated model [6]. The use of FC in rodents has revealed the relevance of time variable in extinction protocols regarding the recurrence of the fear response. Specifically, spaced intervals between extinction sessions [4] or between trials [7,8], in both rodents and humans, have been proved to be more effective than short time intervals in reducing the long-term SR. Moreover, FC has revealed a hypothetical neural circuit for PTSD, including defective interactive connections between the amygdala and the medial prefrontal cortex (mPFC) regions [1,2,9]. Accordingly, it has been demonstrated that microstimulation of the infralimbic cortex (IL) enhances fear extinction in rodents, whereas the opposite effect has been described after prelimbic cortex (PL) stimulation [5].

While FC reveals passive defensive responses and usually produces freezing and other conditioned reactions that obstruct avoidance, active avoidance paradigms enables the measurement of active defensive responses through avoidance responses to the CS [10]. In active avoidance conditioning, animals must learn an instrumental response to avoid a foot shock when the CS announces its imminent arrival. Interestingly, behavioural avoidance is considered a core symptom of PTSD

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[11] and constitutes a significant predictor of disease severity in PTSD patients [12]. Thus, avoidance as a fear response is excessive in PTSD patients and highlights the need for understanding the behavioural and neural substrates of conditioned avoidance responses to improve EBT benefits.

Pioneer studies in rats about the effects of spacing trials on resistance to the extinction in active avoidance paradigms were already performed in the 1950s [13] and [14], but there are seldom studies using the more complex two-way active avoidance (TWAA) procedure and evaluating the effect of the temporal distribution of the sessions on the resistance to the extinction and SR.

In this study, we aimed to analyse comparative effectiveness of a different temporary distribution of two extinction sessions (24 h vs 7 days between extinction sessions) on the long-term retention of extinction (28 days after the last extinction session) of the conditioned response evaluated in a SR test, by using a TWAA paradigm. Furthermore, we attempted to elucidate the neuronal circuits underlying the effectiveness in the long term of the two distribution conditions of TWAA extinction sessions. This was done by mapping the changes in c-Fos protein expression, a well-known marker of neuron activation [15], in specific areas of the amygdala and the mPFC, two brain regions related to fear and avoidance extinction systems.

2. Methods and materials

2.1. Subjects

A total of 32 male Wistar rats were used (90–100 days old and with a mean weight of 438.27 ± 41.63 g), obtained from the breeding stock of the laboratory of Psychobiology (registry number B99-00029, Autonomous University of Barcelona, UAB). All the rats were individually housed and kept on a 12hr light/dark cycle, with all TWAA sessions being conducted during the light period. All procedures were carried out in compliance with the European Community Council Directive for care and use of laboratory animals and approved by the Ethics Committee at the UAB (order number 2022).

The study was carried out in different batches of 6 rats maximum per week (up to 2 per group) to ensure that the different groups were evaluated in parallel, and only differing in terms of the independent variable “time between extinction sessions”. After handling (1 daily 10-min session for 5 consecutive days), up to 2 rats per week were randomly chosen to form the Naïve group (n = 8; see Fig. 1), which served as a negative control of TWAA training effects on c-Fos expression, while the remaining animals underwent the procedures described below.

2.2. Two-way active avoidance (TWAA) conditioning

TWAA was conducted in two 50 × 24 × 23 cm identical automated two-way shuttle-boxes (AccuScan Instruments Inc. Columbus, Ohio, USA), enclosed in two separate sound-attenuating boxes ventilated by an extractor fan and controlled by Fusion software. Rats underwent two 5-min habituation sessions (Hab1 and Hab2) of free ambulation in the shuttle-box in order to become familiarized with the learning environment. Two days later, the rats received three 50-trial TWAA acquisition sessions, once daily (Ac1 to Ac3), consisting of the introduction of an 80 dB and 1 kHz lasting 3 s (CS) followed by a 0.6 mA and maximum 10 s duration electrical foot shock (US). Both stimuli terminated simultaneously when the rat crossed, or after 13 s in the case that the rat did not respond, following a delay procedure. The inter-trial interval (ITI) varied randomly from 50 to 70s. The ITI was set to be shorter than those used in other studies [10] in order to make the task more demanding, based on results of previous experiments in our laboratory. The total number of avoidance responses (considered as conditioned responses, CRs) and the mean latency (i.e. the time taken to move from one compartment to another from the CS presentation) were recorded for each session.

One day after the Ac3, rats were tested in two 50-trial TWAA extinction sessions (Ext1 and Ext2) at two different time intervals between sessions (24 h or 7 day, see Fig. 1). Thus, after Ex1 rats were randomly assigned to the 24-hour (*contiguous*) or to the 7-day (*spaced*) groups. Extinction sessions were similar to the acquisition but without US presentation. Following a rest period of 28 days from the Ex2, the effects of the two extinction paradigms were tested in a single 50-trial TWAA SR test (SR-t), which replicated the characteristics of the extinction sessions. The number of CRs, here considered as the responses made during the total duration of the CS, as well as mean latency per session were also recorded.

The locomotor activity of the animals was also recorded. In the habituation sessions as the number of crossings performed during the 5-min session duration (Hab1 and Hab2), and in the acquisition, extinction or SR sessions as the number of crossings performed in the ITI. Since individual differences in the locomotor response to a novel environment can affect fear conditioning and extinction in rats [16], the number of crossings in the habituation sessions was used as covariate in the variance analyses.

2.3. c-Fos immunochemistry

For c-Fos immunolocalization, rats were euthanized 90 min after the SR-t (24-hour and 7-day groups) or after the last handling session (Naïve group). They were anesthetized with a lethal dose of pentobarbital (200 mg/kg body weight, i.p.) and perfused transcardially with a solution of 0.1 M phosphate buffer saline (PBS), pH 7.4, followed by a solution of 4% paraformaldehyde in PBS. Brains were post-fixed in 4% paraformaldehyde in PBS solution for 2 h and then placed in 15% sucrose in PBS for 3 days and 30% sucrose in PBS at 4 °C until they sank. Serial coronal sections of cryopreserved brains (30-μm thick) were

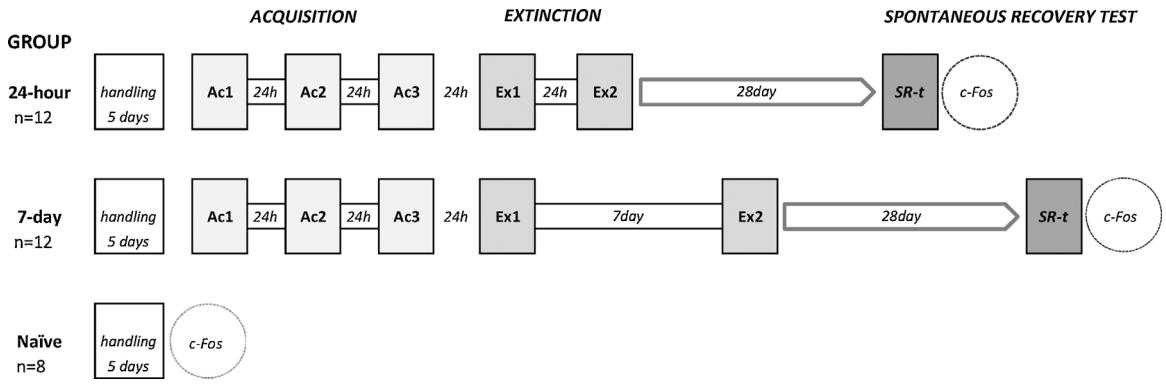

Fig. 1. Scheme, experimental design of the study, in which the main procedural characteristics of each experimental group are specified (Ac: *acquisition sessions*; Ex: *extinction sessions*; SR-t: *spontaneous recovery test*). Sacrifice for c-Fos immunodetection was performed at 90 min.