



## Involvement of the prelimbic cortex in contextual fear conditioning with temporal and spatial discontinuity



Thays Brenner Santos, Juliana Carlota Kramer-Soares, Vanessa Manchim Favaro, Maria Gabriela Menezes Oliveira\*

Departamento de Psicobiologia, Universidade Federal de São Paulo, São Paulo, Brazil

### ARTICLE INFO

#### Article history:

Received 15 November 2016

Revised 2 May 2017

Accepted 4 May 2017

Available online 31 May 2017

#### Keywords:

Memory

Contextual fear conditioning

Trace conditioning

Medial prefrontal cortex

Prelimbic cortex

### ABSTRACT

Time plays an important role in conditioning, it is not only possible to associate stimuli with events that overlap, as in delay fear conditioning, but it is also possible to associate stimuli that are discontinuous in time, as shown in trace conditioning for a discrete stimuli. The environment itself can be a powerful conditioned stimulus (CS) and be associated to unconditioned stimulus (US). Thus, the aim of the present study was to determine the parameters in which contextual fear conditioning occurs by the maintenance of a contextual representation over short and long time intervals. The results showed that a contextual representation can be maintained and associated after 5 s, even in the absence of a 15 s re-exposure to the training context before US delivery. The same effect was not observed with a 24 h interval of discontinuity. Furthermore, optimal conditioned response with a 5 s interval is produced only when the contexts (of pre-exposure and shock) match. As the pre-limbic cortex (PL) is necessary for the maintenance of a continuous representation of a stimulus, the involvement of the PL in this temporal and contextual processing was investigated. The reversible inactivation of the PL by muscimol infusion impaired the acquisition of contextual fear conditioning with a 5 s interval, but not with a 24 h interval, and did not impair delay fear conditioning. The data provided evidence that short and long intervals of discontinuity have different mechanisms, thus contributing to a better understanding of PL involvement in contextual fear conditioning and providing a model that considers both temporal and contextual factors in fear conditioning.

© 2017 Elsevier Inc. All rights reserved.

### 1. Introduction

Fear conditioning is a form of associative learning in which an initially neutral stimulus, termed the conditioned stimulus (CS), acquires aversive properties by its paired presentation with an aversive unconditioned stimulus (US). This association results in defensive conditioned responses in the face of the presentation of the CS alone (Blanchard & Blanchard, 1988; Iwata & LeDoux, 1998). Humans and other animal species are able to associate stimuli discontinuous in time with a wide range of time intervals between them. The length of time interval varies according to the stimuli to be associated. A flavor and a noxious drug effect may be optimally associated when separated by hours (Garcia, Ervin, & Koelling, 1966; Welzl, D'Adamo, & Lipp, 2001), a discrete stimulus and a footshock by seconds (Raybuck & Lattal, 2014),

and a discrete stimulus and an air puff or periorbital shock by milliseconds (Woodruff-Pak & Disterhoft, 2008). The last two examples are forms of so-called trace conditioning, in which a discrete CS precedes and ends before the US onset. A tone and a footshock are examples of stimuli used in trace fear conditioning. The term trace comes from the idea that a mnemonic trace of the discrete CS should be maintained during the time interval in order for conditioning to occur (Pavlov, 1927). Trace fear conditioning differs from delay fear conditioning because the stimuli to be associated are continuous in time.

The environment where conditioning takes place is also a powerful CS that elicits conditioned responses. Usually, context is continuously present, and so, it does not have a phasic presentation in relation to the US with a well-defined beginning and end (Phillips & LeDoux, 1992). Unlike a discrete stimulus, context is formed by the unified organization of stable stimuli from multiple sensory modalities. A minimum time of exposure to the context is necessary so that these individual elements may be organized into a single representation (Bevins & Ayres, 1995; Fanselow, 1986).

\* Corresponding author at: Rua Botucatu, 862, 04023062, São Paulo, SP, Brazil.

E-mail addresses: [thayscr@gmail.com](mailto:thayscr@gmail.com) (T.B. Santos), [julianacarlota@gmail.com](mailto:julianacarlota@gmail.com) (J.C. Kramer-Soares), [vm.favaro@gmail.com](mailto:vm.favaro@gmail.com) (V.M. Favaro), [gabi.oliveira@gmail.com](mailto:gabi.oliveira@gmail.com) (M.G.M. Oliveira).

These stable and multimodal stimuli change as the animal navigates around the environment, forming different configurations over time (Hartley, Lever, Burgess, & O'Keefe, 2014; Hyman, Ma, Balaguer-Ballester, Durstewitz, & Seamans, 2012). The contextual representation prevalent at the time of the US occurrence will be associated with it (Fanselow, 2010; O'Reilly and Rudy, 2001; Rudy, 2009; Rudy & O'Reilly, 1999, 2001), constituting what is designated as contextual fear conditioning. The question raised in the present study is whether a configuration assembled some time before the US presentation would be associated with this US. In other words, a transient memory could also store a multimodal, complex, assembled stimulus and, if so, are the mechanisms used similar to those in unimodal stimuli? This question aims to better understand the power of predictability of different type of stimuli, and how associations are made and govern our behavior in situations in which different contexts are successively experienced.

To address this question, we took advantage of the context pre-exposure facilitation effect (CPFE). In this paradigm, a pre-exposure to the context is separated by an interval of 24 h from the US-context association session. It is thought that the animal forms a representation of the context during the pre-exposure session. This contextual representation must be recalled from long-term memory to be associated with the US. Usually, a re-exposure time in context before the US or the exposure to a transport cage previously associated with the context is used to reactivate the contextual memory (Fanselow, 1990; Rudy & Wright-Hardesty, 2005).

The retention of the contextual memory follows a U-shaped function varying the time interval in this paradigm. When the interval between pre-exposure and context-US association is in the range of seconds or hours, a robust conditioned response is observed. However, if this interval is of some minutes, no conditioning is obtained (Rudy & Wright-Hardesty, 2005). According to the authors, the descendent and ascendant arms of the U-shaped curve could reflect a discontinuity between two memory processes. The first arm could be related to synaptic processes that are modified quickly, but have a fast decay function. If this is the case, we hypothesize that neural substrates involved in tasks that depend on maintaining information over time would also be critical to the maintenance of the contextual representation for some seconds before its association with the US, but not for long periods.

Prelimbic cortex (PL) activity has been related to the acquisition of tasks that depend on maintaining information over time, such as trace fear conditioning (Gilmartin & Helmstetter, 2010; Gilmartin, Kwapis, & Helmstetter, 2012; Gilmartin & McEchron, 2005; Gilmartin, Miyawaki, Helmstetter, & Diba, 2013; Reis, Jarome, & Helmstetter, 2013; Runyan & Dash, 2004; Runyan, Moore, & Dash, 2004). The PL is a subdivision of the so-called medial pre-frontal cortex (mPFC), which is anatomically and functionally subdivided, from dorsal to ventral, into the medial agranular cortex (AGm), the anterior cingulate cortex (AC), the PL and the infralimbic cortex (IL) in rodents (Heidbreder & Groenewegen, 2003).

The main purpose of the present study was to determine the parameters in which contextual fear conditioning occurs between stimuli with short and long time intervals of discontinuity, and the possible involvement of the PL in this form of conditioning. To do so, we chose CPFE as a starting approach. We first verified in CPFE if a contextual CS can be associated to a US without the need for context reactivation by transport cues or re-exposure time to context, but instead by its maintenance during the time interval. We compared the effects of CPFE on contextual fear conditioning acquisition with a 5 s or 24 h interval. Because the PL is necessary for the maintenance of a discrete CS during the time interval until its association to the US, we evaluated if reversible bilateral inactivation of the PL with the GABA<sub>A</sub> agonist muscimol before training would impair the CPFE in fear conditioning acquisition with a 5 s interval. For comparison, control groups of animals were submitted

to regular delay contextual fear conditioning or CPFE paradigms. We also verified if the CS needed to be presented again simultaneously to the US presentation for conditioning to occur.

## 2. Material and methods

### 2.1. Subjects

Male Wistar rats weighing 250–330 g (CEDEME, Centro de Desenvolvimento de Modelos Experimentais para Biologia e Medicina, Universidade Federal de São Paulo, Brazil) were housed in groups of 5 in Plexiglas-walled cages with corn-cob bedding on the floor. After surgery, the animals were housed individually. The room temperature was controlled ( $22\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ ), and a light-dark cycle was maintained on a 12 h on-off cycle (07:00–19:00 h lights on). Food and water were available *ad libitum* throughout the study. The Ethical Committee for Animal Research of Universidade Federal de São Paulo approved the study under the protocol number 964017122013. The experiments were performed in compliance with the recommendations of the “Brazilian Guidelines for Care and Use of Animals for Scientific and Educational Purposes” (CONCEA, Conselho Nacional de Controle de Experimentação Animal, Brazil).

### 2.2. Apparatus

The conditioning chamber, referred to as Context A, was a  $22 \times 27 \times 45$  cm acrylic box with black walls and a transparent cover. The floor was composed of parallel stainless steel bars of 0.4 cm in diameter connected to an electric generator (AVS Projetos, São Paulo, Brazil). For the context named Context B, a  $35 \times 60$  cm cylindrical chamber with white walls and floor and a transparent cover was used to provide a different contextual and spatial configuration from Context A. Context A was cleaned with a 20% ethanol solution, and Context B was cleaned with a 10% sodium hypochlorite aqueous solution after each experimental session. Both chambers were located in the same experimental room in all experiments. A video camera attached to the cover was used for behavioral records in both contexts. Transport cage 1 was used in habituation and training sessions, and it was similar to the Plexiglas-walled home cage. This transport cage was also used as the transition box, in which the animal was placed between the pre-exposure and re-exposure to context during the interval of discontinuity in training sessions. Transport cage 2 was a white wooden box with paper towel bedding and was used in the test sessions.

### 2.3. Drug

Muscimol conjugated to tetramethyl-rhodamine (BODIPY<sup>®</sup> TMR-X conjugate, synthesized by Molecular Probes, Oregon, United States) was dissolved in 1 ml of ACSF (artificial cerebrospinal fluid), resulting in a 1 mg/ml concentration, and was stored at  $-20\text{ }^{\circ}\text{C}$  until use. We chose muscimol labeled with a fluorescent molecule for its slower and more limited diffusion area, probably due to its higher molecular weight and dissolution in myelinated fibers and membrane lipids (Allen et al., 2008). This allowed selective study of the PL and drug pattern diffusion.

### 2.4. Stereotactic surgery

Rats were anesthetized with ketamine hydrochloride (90 mg/kg) and xylazine (10 mg/kg) (Vertbrands) administered intraperitoneally (IP), and then fixed in the stereotaxic frame (Insight, São Paulo, Brazil) by the upper incisors. The cranium was set in a hor-

Download English Version:

<https://daneshyari.com/en/article/5043105>

Download Persian Version:

<https://daneshyari.com/article/5043105>

[Daneshyari.com](https://daneshyari.com)