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Entorhinal cortex contribution to contextual fear conditioning extinction and reconsolidation in rats

Elisabetta Baldi, Corrado Bucherelli *

Dipartimento di Medicina, Sperimentale e Clinica, Sezione di Fisiologia, Università degli Studi di Firenze, Viale G.B. Morgagni 63, I-50134 Firenze, Italy

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ABSTRACT

During contextual fear conditioning a rat learns a temporal contiguity association between the exposition to a previously neutral context (CS) and an aversive unconditioned stimulus (US) as a footshock. This condition determines in the rat the freezing reaction during the subsequent re-exposition to the context. Potentially the re-exposition without US presentation initiates two opposing and competing processes: reconsolidation and extinction. Reconsolidation process re-stabilizes and strengthens the original memory and it is initiated by a brief re-exposure to context. Instead the extinction process leads to the decrease of the expression of the original memory and it is triggered by prolonged re-exposure to the context. Here we analyzed the entorhinal cortex (ENT) participation in contextual fear conditioning reconsolidation and extinction. The rats were trained in contextual fear conditioning and 24 h later they were subjected either to a brief (2 min) reactivation session or to a prolonged (120 min) re-exposition to context to induce extinction of the contextual fear memory. Immediately after the reactivation or the extinction session, the animals were submitted to bilateral ENT TTX inactivation. Memory retention was assessed as conditioned freezing duration measured 72 h after TTX administration. The results showed that ENT inactivation both after reactivation and extinction session was followed by contextual freezing retention impairment. Thus, the present findings point out that ENT is involved in contextual fear memory reconsolidation and extinction. This neural structure might be part of parallel circuits underlying two phases of contextual fear memory processing.

1. Introduction

In rodents contextual fear conditioning is a paradigm useful to study emotional learning and memory (Anagnostaras, Gale, & Fanselow, 2001; LeDoux, 2000; Maren, 2001). This form of learning involves the association of an otherwise neutral context with an aversive stimulus, e.g. an electrical footshock (the unconditioned stimulus, US). After training, the context alone elicits a conditioned fear response such as freezing behavior, i.e. the suppression of all somatic movements, with the exception of respiration, behaving as a conditioned stimulus (CS) (Fanselow, 1980; LeDoux, Sakaguchi, & Reis, 1983; Sacchetti, Ambrogi Lorenzini, Baldi, Tassoni, & Bucherelli, 1999a). This form of memory can be easily maintained for a long time (LeDoux, 2000; Maren, 2001).

Long-term memory is generated through a process known as consolidation. According to the classical theory of memory consolidation, through this process the newly formed mnemonic trace, initially sensitive to disruption by several treatments (e.g. electroconvulsive shock, intracerebral or systemic pharmacological treatments), becomes stable over time (Dudai, 1996; McGaugh, 2000). Thus, once stabilized the engram remains insensitive to disruption. However, results have shown that after reaching a stable state, memory becomes transiently sensitive to disruption if it is reactivated (for instance by retrieval trial) (Bucherelli & Tassoni, 1992a; Judge & Quartermain, 1982; Misanin, Miller, & Lewis, 1968; Nader, Schafe, & LeDoux, 2000). In many instances the same treatments that disrupt consolidation are effective in disrupting a reactivated memory (Alberini, 2005; Dudai, 2004; Nader, 2003; Sara, 2000). The process by which a reactivated memory becomes again stable and insensitive to disruption has been termed reconsolidation (Alberini, 2005; Dudai, 2006; Nader, 2003; Tronson & Taylor, 2007). To induce contextual fear conditioning memory trace reactivation it is sufficient to expose the experimental subject to the training context (CS) in the absence of aversive US (footshock) (Nader, 2003; Tronson & Taylor, 2007). This type of trial can also be considered as an extinction trial. The mnemonic trace extinction results in the decrease of the conditioned fear response evoked by the context when the context no longer predicts footshock for the animal (Baldi & Bucherelli, 2010;

* Corresponding author. Fax: +39 55 4379506.
E-mail address: corrado.bucherelli@unifi.it (C. Bucherelli).

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Myers & Davis, 2007; Quirk & Mueller, 2008). This suggested that memory retrieval is a dynamic process that may potentially initiate two competing processes: reconsolidation and extinction (De la Fuente, Frendenthal, & Romano, 2011; Mamiya et al., 2009; Rossato, Bevilaqua, Izquierdo, Medina, & Cammarota, 2010; Suzuki et al., 2004). In fact, reconsolidation stabilizes and extinction weakens the expression of the original memory. An important determinant of subsequent engram processing following the retrieval is the temporal duration of re-exposure to the CS (context) without the exposition to the US: brief reactivation sessions lead to memory reconsolidation, whereas longer reexposition sessions lead to memory extinction (Barak & Hamida, 2012; de la Fuente et al., 2011; Debiec, LeDoux, & Nader, 2002; Eisenberg, Kobilo, Berman, & Dudai, 2003; Lee, Milton, & Everitt, 2006; Pedreira & Maldonado, 2003; Suzuki et al., 2004).

Current understanding of the neural basis of fear reconsolidation and extinction is much poorer compared with the acquisition/consolidation of conditioned fear. The current knowledge indicates that these different mnemonic phases are characterized by both distinctive and coincident features regarding anatomical and molecular requirements (Alberini, 2005; Berman & Dudai, 2001; Bucherelli, Baldi, Mariottini, Passani, & Blandina, 2006; Chen et al., 2005; Izquierdo et al., 2006; Lee, Everitt, & Thomas, 2004; Lin, Yeh, Lu, & Gean, 2003; Szapiro, Vianna, McGaugh, Medina, & Izquierdo, 2003; Vianna, Szapiro, McGaugh, Medina, & Izquierdo, 2001). Understanding the mechanisms of fear memory reconsolidation and extinction may have clinical relevance in treatment of human anxiety disorders such as post-traumatic stress disorder. Indeed, reconsolidation and extinction procedures may be used to reduce the expression of fear memory (Alberini, 2005; Auber, Tedesco, Jones, Monfils, & Chiamulera, 2013; Davis, Myers, Chhatwal, & Ressler, 2006; Hartley & Phelps, 2010; Monfils, Cowansage, Klann, & LeDoux, 2009; Nader, 2003; Parsons & Ressler, 2013; Quirk et al., 2010; Rao-Ruiz et al., 2011; Rossato et al., 2010; Schiller et al., 2010). Thus, the identification of both neural circuits underlying the reconsolidation and extinction processes and pharmacological agents that impair reconsolidation or potentiate extinction appears to be crucial.

Experimental results have shown that the basolateral amygdala (BLA) and hippocampus are involved in contextual fear conditioning consolidation (Anagnostaras et al., 2001; Kim & Fanselow, 1992; McGaugh, 2000; Sacchetti et al., 1999a), reactivation/reconsolidation (Baldi, Mariottini, & Bucherelli, 2008; Bucherelli et al., 2006; Debiec et al., 2002; Lee et al., 2004; Mamiya et al., 2009) and extinction (Baldi & Bucherelli, 2010; Fisher, Sananbenesi, Schrick, Spiess, & Radulovic, 2004; Fisher et al., 2007; Myers & Davis, 2007; Quirk & Mueller, 2008; Sananbenesi et al., 2007; Vianna et al., 2001). These neural structures have extensive reciprocal connections with the entorhinal cortex (ENT) (Amaral & Witter, 1989; McDonald & Mascagni, 1997; Pitkanen, Pikkarainen, Nurminen, & Ylinem, 2000; Swanson & Cowan, 1977; Witter, Wouterlood, Naber, & Van Haeften, 2000). It has been suggested an interplay between the BLA and ENT in the regulation of memory consolidation (Majak & Pitkanen, 2003; Roesler, Roozendaal, & McGaugh, 2002). Moreover, the ENT has a pivotal role in processing information that is critical to hippocampal functioning (Eichenbaum, Otto, & Cohen, 1994; Maren & Fanselow, 1997). Hence, it appears interesting to analyse whether this neural site is involved in the same phases of contextual fear memory in which BLA and hippocampus play a role. Our previous results have shown that ENT is involved in contextual fear consolidation in the rat. Post-acquisition bilateral ENT tetrodotoxin (TTX) inactivation up to 1.5 h after training results in retention deficit of contextual freezing (Baldi, Liuzzo, & Bucherelli, 2013). Regarding reconsolidation, there are few and contrasting data that do not provide direct evidence for a role of this neural site in contextual fear conditioning reconsolidation. In fact, in rats trained in an inhibitory avoidance task, the infusion of anisomycin (a protein synthesis inhibitor) into ENT performed 15 min before or 3 h after memory reactivation, did not affect subsequent memory retention (Cammarota, Bevilaqua, Medina, & Izquierdo, 2004). On the other hand, it has been shown that reconsolidation of long-term recognition memory is associated with BDNF and Egr-1 mRNA expression and hyperphosphorylation of ERK in the ENT (Kelly, Laroche, & Davis, 2003; Romero-Granados, Fontan-Lozano, Delgado-Garcia, & Carrion, 2010), whereas protein synthesis in the ENT does not seem necessary for reconsolidation of this type of memory (Lima et al., 2009). Finally up to now an active role of the ENT in memory extinction was only observed in tasks other than classical contextual fear conditioning. Lesions in the lateral entorhinal cortex increased resistance to extinction of an operant-conditioning task in mice (Gauthier, Destrade, & Soumiren-Mourat, 1983), and immediately post-extinction intra-ENT infusions of a NMDA antagonist, protein synthesis or CaMKII inhibitors impaired inhibitory avoidance extinction (Bevilaqua et al., 2006), that was associated with significant c-Fos expression in this neural site (Huang, Shyu, Hsiao, Chen, & He, 2013).

Because of the lack of direct evidence for ENT role in contextual fear memory reconsolidation and extinction, the aim of the present work was to inactivate the rat ENT by the stereotaxic administration of the depressor of neuron excitability tetrodotoxin (TTX) for studying ENT involvement in these memorization processes. The inactivation was bilaterally performed either immediately after trace reactivation or immediately after extinction training of contextual fear conditioning. In this way it has been possible to clarify the involvement of this brain site in these two memorization phases.

2. Materials and methods

2.1. Animals

Seventy-day old male albino Wistar rats (average body weight 290 g) (Harlan, Italy) were used. The animals were individually housed in stainless steel cages in a room with a natural light–dark cycle and constant temperature of 20 ± 1 °C. The rats had free access to food and water throughout the experiment. All animal care and experimental procedures were conducted in accordance with Italian legislation and the official regulations of the European Communities Council on use of laboratory animals (Directive of 24 November 1986; 86/609/EEC).

2.2. Behavioral procedures

2.2.1. Apparatus

As in previous experiments a basic Skinner box module (Modular Operant Cage, Coulbourn Instruments Inc.) was used to induce fear conditioning (Sacchetti, Ambrogi Lorenzini, Baldi, Tassoni, & Bucherelli, 1999b; Sacchetti et al., 1999a). Box dimensions were 29 × 31 × 26 cm. The top and two opposite sides were made of aluminum panels, the other two sides of transparent plastic and the floor of stainless steel rods connected to a shock delivery apparatus (Grid Floor Shocker, Coulbourn Instruments Inc., Model E13-08). The apparatus was connected to a stimulus programming device (Scatola di comando Arco 2340 – Ugo Basile) in order to predetermine number, duration and rate of US delivery. The apparatus was placed in an acoustically insulated room (3.5 × 1.8 × 2.1 (h) m), kept at a constant temperature of 20 ± 1 °C. Illumination inside the room was 60 lux.