Neurobiology of Learning and Memory 94 (2010) 199–205

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

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

Substantia nigra, nucleus basalis magnocellularis and basolateral amygdala roles in extinction of contextual fear conditioning in the rat

Elisabetta Baldi, Corrado Bucherelli *

Department of Physiological Sciences, University of Florence, Viale G.B. Morgagni 63, I-50134 Florence, Italy

ARTICLE INFO

Article history:
Received 15 February 2010
Revised 12 May 2010
Accepted 15 May 2010
Available online 20 May 2010

Keywords:
Contextual fear conditioning
Extinction
Substantia nigra
Nucleus basalis magnocellularis
Basolateral amygdala

ABSTRACT

Fear conditioning is accepted as a useful experimental paradigm to investigate anxious disorders following stress. In this field it is important to understand the mechanisms underlying the extinction of conditioned fear. In the rat it has been shown that the amygdalar basolateral nucleus plays a crucial role in all memorization phases of this type of memory (acquisition, consolidation, retrieval, and also reconsolidation and extinction). Recent results show that both the substantia nigra and nucleus basalis magnocellularis, two sites strongly connected with the basolateral amygdala are also involved in the consolidation of contextual fear conditioning.

The aim of the present work is to investigate if latter two sites, besides the basolateral amygdala, are also involved in the extinction of the conditioned fear response. The results show that tetrodotoxin-induced inactivation of post-extinction training of either site does not impair the extinction process, which instead is impaired by inactivation of the basolateral amygdala. Thus, the present results confirm previous ones which show that diverse memorization phases (post-acquisition consolidation, extinction, reconsolidation) may be sustained by different neural sites and circuits.

1. Introduction

Pavlovian fear conditioning is a very useful tool for investigating neural and behavioral fear mechanisms. In this simple experimental paradigm the animal (quite often a rodent) is given a neutral conditioned stimulus (CS, a sound or a distinctive environment) coupled with an aversive non-conditioned stimulus (US, quite often an electrical footshock). This association of stimuli is learned when fear conditioned responses are recorded after the presentation of the CS only. Long-term memory results from the consolidation or stabilization process of the memory trace or engram during which it is strengthened and stabilized for a long time. It has already been shown that in the rat, besides the basolateral amygdala (BLA), the substantia nigra (SN) and the nucleus basalis magnocellularis (NBM) also play important roles in contextual fear conditioning consolidation. In fact, bilateral post-acquisition inactivation of these sites, even if not performed immediately after initial training, is followed by memory deficits at retention testing (Baldi, Mariottini, & Bucherelli, 2007a, 2007b; Sacchetti, Ambrogi Lorenzini, Baldi, Tassoni, & Bucherelli, 1999b). NBM and SN are strongly connected to BLA: the NBM is the main source for the cholinergic innervation of the BLA (Schauz & Koch, 1999; Woolf, 1998; Woolf & Butcher, 1982) and the SN provides an important dopaminergic projection to the whole BLA group (Fallon & Loughlin, 1985).

Interest has recently been renewed in the topic of conditioned responses extinction. This phenomenon has been investigated both in general and with particular reference to fear conditioning. Extinction is present when the subject's conditioned response to CS diminishes because the contingent CS–US relationship becomes weaker. To obtain extinction of context fear responses it may be sufficient to expose the animal to the context without administering the US. Thus, fear response extinction is an interesting paradigm for the investigation of inhibitory learning. Moreover, it is accepted that fear conditioning extinction is a useful experimental model for the study of human anxiety disorders (Cain, Godsil, Jami, & Barad, 2005; Lin, Yeh, Lu, & Gean, 2003; Myers & Davis, 2007).

An early explanation of the extinction phenomenon was given by Rescorla and Wagner (1972). Extinction would be the result of the weakening and final erasing of the learned CS–US relationship. This approach may be thought of as being simplistic and incomplete. In fact, it has been shown that the coupled CS–US retraining is not necessary to revive the fear responses to an "extinct" CS (Bouton, Westbrook, Corcoran, & Maren, 2006). Modalities such as renewal, reinstatement and spontaneous recovery show that the original CS–US coupling is not totally erased, even when the CS can no longer evoke the fear response. It has been suggested that extinction implies "new" learning. This new learning would coexist with the older one and inhibit it (Barad, Gean, & Lutz, 2006; Myers & Davis, 2002, 2007; Quirk & Mueller, 2008).

* Corresponding author. Address: Dipartimento di Scienze Fisiologiche, Viale G.B. Morgagni 63, Firenze I-50134, Italy. Fax: +39 55 4379506.
E-mail address: corrado.bucherelli@unifi.it (C. Bucherelli).

1074-7427/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.nlm.2010.05.007

Another point of interest is whether the molecular mechanisms and neural sites involved in engram post-acquisition consolidation are the same as those involved in the extinction of the identical engram (Bahar, Samuel, Hazvi, & Dudai, 2003; Berman & Dudai, 2001; Cain, Blouin, & Barad, 2002; Lattal & Abel, 2001; Lin et al., 2003; Myers & Davis, 2002, 2007; Ponnusamy, Nissim, & Barad, 2005). It has been shown that the two processes may have some features in common, e.g. both necessitate the activation of NMDA receptors (Cammarota et al., 2008; Davis, 2002; Ledgerwood, Richardson, & Cranney, 2003; Lin et al., 2003; Santini, Muller, & Quirk, 2001; Szapiro, Vianna, McGaugh, Medina, & Izquierdo, 2003), of PI-3 kinase (Chen et al., 2005; Fischer et al., 2007; Lin et al., 2001; Lin et al., 2003), of MAPK (Atkins, Selcher, Petraitis, Trzaskos, & Sweatt, 1998; Lin et al., 2003; Lu, Walker, & Davis, 2001; Szapiro et al., 2003) and of protein synthesis (Lin et al., 2003; Schafe & LeDoux, 2000; Vianna, Szapiro, McGaugh, Medina, & Izquierdo, 2001; but see Lattal & Abel, 2001). On the other hand, the two processes also exhibit quite different features, e.g. a different sensitivity to actinomycin D blockade (Lin et al., 2003), and to CREB phosphorylation affected by calcineurin (Myers & Davis, 2007). Similar questions have arisen when confronting post-acquisition consolidation and reconsolidation of contextual fear conditioning traces in rodents (Alberini, 2005, 2007; Bucherelli, Baldi, Mariottini, Passani, & Blandina, 2006; Nader, 2007).

BLA has been shown to be involved both in post-acquisition consolidation (LaLumiere, Buen, & McGaugh, 2003; McGaugh, 2002; Sacchetti et al., 1999b) and extinction of contextual fear conditioning (Barad et al., 2006; Berlau & McGaugh, 2006; Falls, Miserendino, & Davis, 1992; Laurent & Westbrook, 2008; Lee & Kim, 1998; Myers & Davis, 2002, 2007; Quirk & Mueller, 2008). SN and NBM are respectively the main sources of BLA dopaminergic and cholinergic innervation, but it is not evident whether these neural sites, besides being actively involved in contextual fear conditioning post-acquisition consolidation, also play a significant role during extinction of the same mnemonic trace. The systemic administration of the muscarinic antagonist scopolamine impairs extinction in a passive avoidance paradigm (Prado-Alcalà, Haiek, Rivas, Roldan-Roldan, & Quirarte, 1994; Roldan, Cobos-Zapiain, Quirarte, & Prado-Alcalà, 2001) and intra-BLA infusions of the muscarinic agonist oxotremorine increase contextual fear extinction, demonstrating the importance of the BLA cholinergic system in fear extinction (Boccia, Blake, Baratti, & McGaugh, 2009). Otherwise NBM lesion studies appear to indicate that this site is not crucial in the extinction process of several learned responses (passive avoidance, active avoidance, operant discriminations) (Butt & Hodge, 1995; Flicker, Dean, Watkins, Fisher, & Bartus, 1983; Miyamoto, Kato, Narumi, & Nagaoka, 1987). Concerning the dopaminergic system, the systemic administration of its enhancers impairs fear extinction (Borowski & Kokkinidis, 1998; Nader & LeDoux, 1999; Willick & Kokkinidis, 1995) while the systemic administration of sulpiride (D2 antagonist) improves extinction (Ponnusamy et al., 2005). Otherwise intra-BLA administration of the D1 antagonist SCH 23390 negatively affects fear conditioning extinction (Hikind & Maroun, 2008).

The aim of the present work was to clarify whether the rat NBM and SN (like the BLA) are involved not only in the memorization but also in the extinction of the contextual fear mnemonic trace. BLA, NBM and SN were bilaterally inactivated by the stereotaxic administration of tetrodotoxin (TTX) performed immediately after post-extinction training in rats having undergone context fear conditioning.

2. Materials and methods

2.1. Animals

Seventy-day old male albino Wistar rats (average body weight 290 g) (Morini, San Polo d'Enza, Reggio Emilia, 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, 1999a; Sacchetti et al., 1999b). Box dimensions were $29 \times 31 \times 26$ 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 ($3.5 \times 1.8 \times 2.1$ (h) m), kept at a constant temperature of 20 ± 1 °C. Illumination inside the room was 60 lux.

2.2.2. Conditioning

On day 1 the rat was gently taken manually from the home cage, placed in a bucket and carried from the housing room to the soundproof room. Once there, it was placed inside the conditioning apparatus and left undisturbed for 3 min. After this time, seven electrical foot-shocks (0.8 mA, 1 s) at 30-s intervals were delivered. The rat was left undisturbed for 2 min after the end of the stimulation sequence. Freezing duration was measured. Rats were brought back to the home cage immediately thereafter.

2.2.3. Extinction

To induce extinction of contextual fear conditioning, 94 h after conditioning, the rats were placed in the conditioning apparatus and left inside for 120 min without receiving any foot-shocks. They were then returned to the home cage.

2.2.4. Contextual fear conditioning retention trial

To measure contextual freezing 7 days (96 h + 72 h) after conditioning, the animals were again placed inside the conditioning apparatus and left there for 6 min. While they were there, no foot-shocks were administered. After that time they were returned to the home cage.

The rat's behavior was recorded by means of a closed circuit TV system. Freezing (immobility) was defined as the complete absence of somatic motility except for respiratory movements (LeDoux, Sakaguchi, & Reis, 1983). Measurements were performed by means of a stop-watch by personnel who did not know to which experimental group each animal belonged. Total accumulated freezing time (i.e. total seconds spent freezing during each period) was measured.

2.2.5. Surgery and drug administration

SN functional inactivation was induced by the injection of 8 ng TTX (Sigma, Italy) dissolved in 0.8 μl saline, into points with the following stereotaxic coordinates: antero-posterior (AP) = −5.5, lateral (L) = ±2.2, and ventral (V) = 8.1 as in previous studies (Ambrogi Lorenzini, Baldi, Bucherelli, & Tassoni, 1994; Baldi et al., 2007a) according to Paxinos and Watson (1986). NBM functional inactivation was induced by the injection of 4 ng TTX (Sigma, Italy) dissolved