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Differential roles of the basolateral amygdala and nucleus basalis magnocellularis during post-reactivation contextual fear conditioning reconsolidation in rats

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ABSTRACT

The roles of the basolateral amygdala and nucleus basalis magnocellularis in fear conditioning reconsolidation were investigated by means of tetrodotoxin bilateral inactivation performed 96 h after conditioning, immediately after reactivation training. Footshocks of 1.2 mA intensity were employed to induce the generalization phenomenon.

Basolateral amygdala inactivation disrupts the contextual fear response and its generalization but not acoustic CS trace retention, when measured 72 and 96 h after tetrodotoxin administration. Nucleus basalis magnocellularis functional inactivation does not affect memory post-reactivation phase of any of the three conditioned fear responses. The present findings show a differential role of the two structures in fear memory reconsolidation and can be a starting point for future investigation of the neural circuits subserving generalization.

1. Introduction

Initially, newly acquired mnemonic information is present in a transient labile condition in which the memory trace can still be disrupted by several factors, but later becomes resistant to disruption and stable enough for long-term storage. This process is called consolidation (McGaugh, 2000). Consolidated memories are not permanently immune to change but may be brought back to a labile or plastic state. For instance, retrieval can return them to a vulnerable state (Bucherelli & Tassoni, 1992; Misanin, Miller, & Lewis, 1968; Nader, Schafe, & LeDoux, 2000). Thus the hypothesis has been advanced that a process may begin by which the original memory trace once more becomes resistant (reconsolidation) (Sara, 2000). Nevertheless, it remains controversial whether established memories, once recalled, require "reconsolidation". The major point against this hypothesis is based on the reversibility of the reported effects due to this labile state (temporary amnesia). There are reports of memory recovery, over time, following post retrieval treatments that impair retention performance (Alberini, 2005; Biedenkapp & Rudy, 2004; Cammarota, Bevilaqua, Medina, & Izquierdo, 2004; Dudai & Eisenberg, 2004; Lattal & Abel, 2004; McGaugh, 2004; Millin, Moody, & Riccio, 2001; Nader, Hardt, & Wang, 2005; Power, Berlau, McGaugh, & Steward, 2006; Rudy, Biedenkapp, Moineau, & Bolding, 2006; Vianna, Szapiro, McGaugh, Medina, & Izquierdo, 2001).

Freezing is the temporary suppression of all somatic motility, except for respiratory movements (Fanselow, 1980; LeDoux, Sakaguchi, & Reis, 1983). Fear-conditioned experimental subjects (rats, mice) will exhibit a freezing response both to the specific CS previously associated with the administered aversive US (e.g., footshocks), and to exposure to the surroundings where they were subjected to the conditioning paradigm, i.e., to the "context" (CXT). The two freezing responses can be separately measured when appropriate experimental designs are employed (Anagnostaras, Maren, & Fanselow, 1995; Fanselow, Kim, Yipp, & De Oca, 1994; Phillips & LeDoux, 1992; Sacchetti, Ambrogi Lorenzini, Baldi, Tassoni, & Bucherelli, 1999a, 1999b; Sigmundi & Bolles, 1983; Sparks & LeDoux, 1995). In fact, when the experimental animal is placed again in the conditioning CXT, without acoustic CS, context freezing will appear. If the animal is placed in a CTX completely different from that of the conditioning trials, it will not exhibit freezing. In this experimental condition the animal will freeze only when the original acoustic CS is administered or if generalization to CXT is present. At least two functional characteristics coexist in generalization, i.e. the tendency to respond to stimuli other than the training stimulus that was associated with reinforcement and the tendency for the strength of response to decline as test stimuli become increasingly different from the training stimulus to rise to orderly sloped generalization gradients (Pavlov, 1927). Generalization appears in fear condition-

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ing when footshock intensity is beyond certain limits (Baldi, Ambrogi Lorenzini, & Bucherelli, 2004). If generalization occurs after contextual fear conditioning, animals show a freezing response to the conditioning CXT as well as to contexts differing from those used for conditioning. The freezing response was found to be larger in the training CXT than in a diverse CXT (contextual generalization) (Bolles & Collier, 1976; Fanselow, 1980). Generally speaking, researchers try to employ conditioning parameters low enough to avoid generalization. This caution is due to the fact that generalization hinders the correct evaluation of the memorization of the engram (in this case that of the original conditioning CXT). Consequently, generalization has seldom been investigated and very little is known of central neural structures involved in this phenomenon.

In the memorization of aversive responses a crucial role is played by the axis nucleus basalis magnocellularis (NBM)-basolateral amygdala (BLA) (Baldi, Mariottini, & Bucherelli, 2007b; Dumery & Blozovski, 1987; Power & McGaugh, 2002). The role of BLA in mediating consolidation and reconsolidation of fear conditioning has been repeatedly investigated. More recently it has been shown that NBM is involved in the consolidation of both CS and CXT engrams in fear conditioning. The NBM appears to play a role similar to that of BLA, but for a shorter time (Baldi et al. 2007b; Sacchetti et al. 1999b). Concerning the fear conditioning post-reactivation phase, BLA appears to be involved in both CS (Duvarci & Nader, 2004; Sacchetti, Sacco, & Strata, 2007) and CXT (Bucherelli, Baldi, Mariottini, Passani, & Blandina, 2006) mnemonic processing. On the other hand, if a strong punishment is employed during conditioning, the critical function of BLA during the post-reactivation phase disappears (Sacchetti et al., 2007). The role of the NBM during this one more labile phase and the roles of NBM and BLA in generalization have never been investigated.

Thus, the aims of the present work were to ascertain whether the NBM is involved in fear conditioning post retrieval trace reactivation, as it is in consolidation, and to better define the already known role of BLA in this process, especially in relation to the administration of footshocks of high intensity and the consequent generalization phenomenon. Tetrodotoxin (TTX) reversible inactivation of NBM and BLA were performed immediately after trace reactivation.

2. Method

2.1. Animals

Seventy-day old male albino Wistar rats (average body weight 290 g) (Morini, San Polo d'Enza, Reggio Emilia, Italy) were employed. 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 employed to induce fear conditioning (Sacchetti et al. 1999a, 1999b). Box dimensions were 29 × 31 × 26 cm. The top and two opposite sides were made of aluminum panels. The other two sides were made of transparent plastic. The floor was made of stainless steel rods connected to a shock delivery apparatus (Grid Floor Shocker, Coulbourn Instruments Inc., Model E13-08). There was a loudspeaker to emit acoustic stimuli of known intensity, frequency and duration. 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 CS-US couplings. 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.

CXT freezing response was measured in the same apparatus that was used for conditioning. As in previous experiments the freezing response to acoustic CS was measured in a totally different apparatus from that employed for conditioning (Sacchetti et al. 1999a, 1999b). The apparatus was a modified shuttle box apparatus (Ugo Basile) (20 × 47 × 20 cm). The walls were made of gray opaque plastic with black vertical stripes (width 1 cm, spaced 3 cm apart). The lid was made of transparent plastic and the floor of black opaque plastic. There was a loudspeaker to administer acoustic stimuli to the experimental animals in the apparatus. The apparatus was connected to a stimulus programming unit (Automatic Reflex Conditioner 7501, Ugo Basile) in order to predetermine CS (number of stimuli, duration of stimuli, rate of stimulation). The unit could also predetermine intensity and frequency of the acoustic stimulus. The apparatus was placed in an acoustically insulated room (3.5 × 3.6 × 2.1 (h) m) kept at a constant temperature of 20 ± 1 °C. Illumination inside the room was 10 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 appropriate soundproofed room. Once there, it was placed inside the conditioning apparatus. The rat was left undisturbed for 3 min. After this time, CS as an 800 Hz tone from a frequency generator, amplified to 75 dB (LeDoux et al., 1983; Sacchetti et al., 1999a, 1999b) lasting 6 s was administered 7 times, at 30 s intervals. The last 1 s of each CS was paired with the US as an electric footshock. US intensity was 1.2 mA. The rat was left undisturbed for 2 min after the end of the stimulation sequence. Freezing duration was measured during this period. Rats were brought back to the home cage immediately thereafter.

2.2.3. Reactivation

To induce reactivation, rats were again placed in the conditioning apparatus 96 h after the training trial. Each rat was left for 2 min within the apparatus. During this time three acoustic CSs were presented which were identical to those previously employed to condition the animals. The acoustic CS were presented at 30 s intervals, and were not coupled to electric footshocks. Rats were brought back to the home cage immediately thereafter.

2.2.4. Conditioned freezing measurement

Freezing duration was measured 72 and 96 h after TTX or saline administration. To measure contextual freezing the animals were again placed inside the conditioning apparatus and left there for 3 min. While they were there, neither electrical nor acoustic stimuli were administered. After that time they were brought back to the home cage. The rat's behavior was recorded by means of a closed circuit TV system. To measure acoustic CS freezing the animals were placed in the other apparatus to avoid the facilitation of acoustic CS retention due to contextual cues (Balaz, Capra, Kasprow, & Miller, 1982; Corodimas & LeDoux, 1995). Once inside the apparatus the animal was left undisturbed for 3 min. After this time, during a subsequent second 3-min period a series of seven acoustic stimuli was administered, identical to that used during the conditioning session (frequency, intensity, duration, intervals between stimuli). The rat's behavior was recorded for the entire 6-min period by means of a closed circuit TV system, after which