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ScienceDirect
Neurobiology of Learning and Memory 87 (2007) 133–139
Neurobiology of Learning and Memory
www.elsevier.com/locate/ynlme

Substantia nigra role in fear conditioning consolidation

Elisabetta Baldi, Chiara Mariottini, Corrado Bucherelli *

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

Received 9 May 2006; revised 5 July 2006; accepted 24 July 2006
Available online 15 September 2006

Abstract

The substantia nigra (SN) is known to be involved in the memorization of several conditioned responses. To investigate the role of the SN in fear conditioning consolidation this neural site was subjected to fully reversible tetrodotoxin (TTX) inactivation during consolidation in adult male Wistar rats which had undergone fear training to acoustic CS and context. TTX was stereotaxically administered to different groups of rats at increasing intervals after the acquisition session. Memory was assessed as conditioned freezing duration measured during retention testing, always performed 72 and 96 h after TTX administration. In this way there was no interference with normal SN function during either acquisition or retrieval phases, so that any amnesic effect could be due only to consolidation disruption. The results show that SN functional integrity is necessary for contextual fear response consolidation up to the 24-h after-acquisition delay. On the contrary SN functional integrity was shown not to be necessary for the consolidation of acoustic CS fear responses. The present findings help to elucidate the role of the SN in memory consolidation and better define the neural circuits involved in fear memories.

Keywords: Context; Functional inactivation; Aversive responses; Nigrostriatal dopaminergic system

1. Introduction

The substantia nigra (SN) is a reticulated structure located in the brain stem. It is a section of a larger mesotelencephalic system with perikarya in ventral midbrain and projections to striatum, pallidum, basal forebrain, limbic structures and cerebral cortex (Moore & Bloom, 1978). It is well known that the nigrostriatal dopaminergic system exerts a critical influence on the responses of tonically active striatal neurons related to the control of fear, emotions, learning and memory. In particular, the SN is a key structure involved in diverse kinds of learning as spatial memory (Levin, Briggs, Christopher, & Auman, 1994; Miyoshi et al., 2002), rewarded operant conditioning (Correa, Mingote, Betz, Wisniecki, & Salamone, 2003; Dowd & Dunnett, 2005; Gulley, Kosobud, & Rebec, 2002; Trevitt, Carlson, Nowend, & Salamone, 2001) and aversive conditioning (Barth & Klingberg, 1988; Huston & Staubli, 1978; Kim & Routtenberg, 1976; LePiane & Phillips, 1978; Mitcham & Thomas, 1972; Pelleymounter, Schleisinger, Wehmer, Hall, & Stewart, 1988; Pezze & Feldon, 2004; Routtenberg & Holzman, 1973; Thompson, 1978).

Most of the results showing the involvement of SN in learning and memory were obtained by means of irreversible lesion techniques causing memory impairment (Dowd & Dunnett, 2005; Meloni & Davis, 2000; Mitcham & Thomas, 1972; Miyoshi et al., 2002). One limitation of the irreversible lesion technique is that it does not allow the assessment of the role played by the chosen site in the several distinct postulated stages of mnemonic processing (acquisition, consolidation, and retrieval). Instead, this type of analysis is made possible by the reversible functional inactivation technique (Bures & Buresova, 1990). Using this technique it has been shown that the SN is involved in the consolidation of aversive conditioning. In fact, the immediate post-training administration of picrotoxin (Cobos-Zapian et al., 1996; Kim & Routtenberg, 1976), bicuculline (Cobos-Zapian et al., 1996) or substance

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

1074-7427/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.nlm.2006.07.006

P (Huston & Staubli, 1978) in the SN induces amnesia of passive avoidance conditioning, as was confirmed by means of TTX administration (Ambrogi Lorenzini, Baldi, Bucherelli, & Tassoni, 1994).

Passive avoidance is an aversive conditioned response that evokes a state of fear (Kim & Jung, 2006). More recently fear conditioning, another aversive response, has been extensively employed. This paradigm is advantageous because it allows independent investigation of both the fear response to a specific CS and to a context (Corodimas & LeDoux, 1995; Kim & Fanselow, 1992; LeDoux, 2000; Maren & Fanselow, 1996; Sacchetti, Ambrogi Lorenzini, Baldi, Tassoni, & Bucherelli, 1999a, Sacchetti, Ambrogi Lorenzini, Baldi, Tassoni, & Bucherelli, 1999b). To assess these responses, freezing duration is measured. Freezing is an innate reaction to fear or danger, which may become a conditioned response after the experimental subject has undergone appropriate training (Fanselow, 1990; LeDoux, 2000; Maren & Fanselow, 1996; Sacchetti et al., 1999a). A further advantage is that in fear conditioning, as in passive avoidance conditioning, although only one training session is sufficient for the experimental subject to learn these associations, there are very well retained for a long time (Fanselow, 1990; Sacchetti et al., 1999a, 1999b, 2001). Since only one learning session is sufficient, there is the further advantage of knowing when mnemonic processing starts (Ambrogi Lorenzini, Baldi, Bucherelli, Sacchetti, & Tassoni, 1998, 1999; Bures & Buresova, 1990; Sacchetti et al., 1999b, 2001).

The reversible functional inactivation technique has been successfully employed to assess the time-course of mnemonic processing in several subcortical and cortical neural sites (Ambrogi Lorenzini, Baldi, Bucherelli, Sacchetti, & Tassoni, 1999; Bures & Buresova, 1990; Clark, Zhang, & Lavond, 1992; Kim & Thompson, 1997; Mamounas, Thompson, & Madden, 1987). If the inactivating agents are administered after the acquisition session, and retrieval testing is performed when all pharmacological effects have disappeared, the mnemonic processing in a given neural site may be selectively investigated during consolidation. It is accepted that during this phase early labile, short-term engrams are elaborated into much more stable, long-lasting ones (Ambrogi Lorenzini et al., 1998, 1999; McGaugh, 1966; Sacchetti et al., 1999b). Functional inactivation results have shown that SN plays a role during passive avoidance consolidation. From this finding stems the hypothesis that SN may play a role also in fear conditioning consolidation. In the present study, to investigate this hypothesis, SN was reversibly inactivated by the stereotaxic administration of TTX at several post-acquisition delays after the single training session. Retrieval testing (during which conditioned freezing durations to acoustic CS and to context were independently measured) was always performed when TTX effects had totally disappeared (72 and 96 h after TTX administration) (Ambrogi Lorenzini et al., 1999; Zhuravin & Bures, 1991). It should be kept in mind that consolidation is the phase of memory processing during which the possible motor effects of the inactivation of a neural structure (e.g. the SN) cannot be a source of error, since during this phase the animal is not called to perform any motor activity, as would occur both during acquisition and retrieval (Correa et al., 2003; Gulley et al., 2002; Trevitt et al., 2001).

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 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.

Context 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 subjects 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 one 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, Sakaguchi, & Reis, 1983; Sacchetti et al., 1999a, 1999b) lasting 6 s was administered seven times, at 30 s intervals. The last 1 s of each CS was paired with the US as an electric footshock. US intensity was 1.0 mA, as in previous experiments (Sacchetti et al., 2001). The rat was left undisturbed for 2 min after the end of the stimulation pattern. Freezing duration was measured during this period. Rats were brought back to the home cage immediately thereafter.