Neurobiology of Learning and Memory 90 (2008) 217–222

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

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

Microinfusion of the D1 receptor antagonist, SCH23390 into the IL but not the BLA impairs consolidation of extinction of auditory fear conditioning

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ARTICLE INFO

Article history:
Received 16 December 2007
Revised 17 March 2008
Accepted 17 March 2008
Available online 28 April 2008

Keywords:
IL
BLA
Extinction
Consolidation
Dopamine
D1 receptors
Rat

ABSTRACT

In auditory fear conditioning, repeated presentation of the tone in the absence of the shock leads to extinction of the acquired fear response. Both the medial prefrontal cortex (mPFC) and the basolateral amygdala (BLA) are involved in extinction. Here we examined this involvement by antagonizing D1 receptors in both regions, in the rat. We microinfused the D1 receptor antagonist, SCH23390, into the infra-limbic part of the mPFC (IL) or BLA at different time points. SCH23390 mircoinfused into the IL either before extinction acquisition or following short extinction training resulted in impairment of extinction consolidation. Microinfusion of SCH23390 into the BLA, prior to acquisition of extinction caused impairment in acquisition of extinction without affecting extinction consolidation. This is supported by the results showing that microinfusion of SCH23390 into the BLA following a short-training session did not affect consolidation. These results further strengthen the role of mPFC in consolidation of extinction while highlighting the role of the D1 receptors in this process.

1. Introduction

Dopamine (DA) is one of the transmitters that most potently modulate the mechanisms underlying states of fear and anxiety (Millan, 2003; Oei & King, 1980). Early reported findings that the D1 receptor antagonist SCH23390 inhibits fear-potentiated startle demonstrate a role for DA receptors in conditional fear in animals (Davis, Falls, Campeau, & Kim, 1993). Similarly, both D1 receptor stimulation and systemic injections of amphetamine were reported to attenuate fear extinction and to renew conditioned stimulus-potentiated startle in fear-extinguished rats (Borowski & Kokkinidis, 1998; Dieu, Seillier, Majchrzak, Marchand, & Di Scala, 2005; Willick and Kokkinidis, 1995).

Conversely, when testing D1-deficient mice, El-Ghundi, O'Dowd, and George (2001) found prolonged retention of fear responses and delayed extinction, suggesting that D1 receptors, when blocked or altogether absent, do not impair fear memories, but rather play an important role in extinguishing them.

Thus, so far the data regarding the role of DA transmission and the specific involvement of D1 receptors in the extinction of fear have yielded controversial findings.

Anatomical studies have shown D1 receptors to be highly expressed in the amygdala (Boyson, McGonigle, & Molinoff, 1986; Meador-Woodruff et al., 1991) and in the mPFC (Gaspar, Bloch, & LeMoine, 1995; Joyce, Goldsmith, & Murray, 1993; Lidow, Goldman Rakic, Gallager, & Rakic, 1991).

Furthermore, extinction of fear memory requires plasticity in both the infralimbic medial prefrontal cortex (IL) and the basolateral amygdala (BLA; Akirav, Raizel, & Maroun, 2006; Myers & Davis, 2007; Quirk & Mueller, 2008). However, as most studies have focused on the role of D1 receptors in fear acquisition, either in the BLA (Ciccocioppo, Sanna, & Weiss, 2001; Greba & Kokkinidis, 2000; Guarraci, Frohardt, & Kapp, 1999a, 1999b; Hurd, McGregor, & Pontén, 1997; Nader & LeDoux, 1999) or in the mPFC (Pezze, Bast, & Feldon, 2003), no evidence is available of the role they may play in extinction of fear.

Here, in order to shed light on the role of D1 receptors in the IL and the BLA in extinction of fear we antagonized D1 receptors in either IL or BLA by local microinfusion of the SCH23390 while using the same paradigm and temporal parameters of drug infusion.

Importantly, since DA may affect locomotion, some of the experiments in this study were designed to test the animals 24 h after the microinfusions of the drug so as to reduce the effects of the drug on locomotion during retrieval and to specifically target the consolidation time window.

Our results show that D1 receptors in the IL are important for consolidation of cued fear extinction while in the BLA these receptors may affect acquisition of fear extinction.

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doi:10.1016/j.nlm.2008.03.003

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (~60 d old, 200–250 g) were caged individually at 22 ± 2 °C under 12 h light/dark cycles. Water and food are available *ad libitum*.

All animal experiments were conducted according to the University of Haifa Ethics and Animal Care Committee, which are in complete accordance with the NIH guidelines for care and use of laboratory animals.

2.2. Drugs

The D1 receptor antagonist SCH23390 (0.25 μg/0.5 μl) from Sigma was used (St. Louis, MO). The drug was dissolved in sterile saline, which was used as control.

This dose is lower than those typically used in studies in which SCH23390 was injected intracranially to examine its effects on fear (see for example; Guarraci et al., 1999; Waddington Lamont & Kokkinidis, 1998). We have used this dose because we aimed to target the BLA without affecting other amygdaloid nucleus since SCH23390 rapidly diffuses away from its injection site (Caine, Heinrichs, Coffin, & Koob, 1995),

2.3. Surgery and drugs administration

Rats were anesthetized with 4.8 ml/kg Equithesin (2.12% w/v $MgSO_4$, 10% v/v ethanol, 39.1% v/v propylene glycol, 0.98% w/v sodium pentobarbital, and 4.2% w/v chloral hydrate), restrained in a stereotactic apparatus (Stoelting, USA), and implanted bilaterally with stainless steel guide cannula (23 gauge, thin wall) aimed to the IL (IL; anterior, 3 mm relative to bregma; lateral, ±0.5 mm; ventral, −5 mm) or to the BLA (BLA: anterior, −3 mm relative to bregma; lateral, ±5.3 mm; ventral, −7.6 mm). The cannulae were positioned in place with acrylic dental cement and secured by two skull screws. A stylus was placed in the guide cannula to prevent clogging. Animal were allowed one week to recover before being subjected to experimental manipulations.

For microinfusion the stylus was removed from the guide cannula, and a 28 gauge injection cannula, extending 1.0 mm from the tip of the guide cannula, was inserted. The injection cannula was connected via PE20 tubing to a Hamilton micro syringe driven by a micro infusion pump (CMA/100; Carnegie Medicin, Stockholm, Sweden). Microinfusion was performed bilaterally in a 0.5 μl volume per hemisphere delivered over 1 min. The infusion cannula was left in its position for one additional minute to minimize dragging of the infused liquid along the infusion tract.

2.4. Auditory fear conditioning

Animals were randomly assigned to two groups: control (Vehicle) and experimental (SCH23390). The conditioned stimulus (CS) was a 30-s tone (4 kHz; 80 dB sound pressure level) and the unconditioned stimulus (US) was 0.5 mA, 0.5 s foot-shock delivered to the floor bars, with the CS and US co-terminating. The inter-trial interval was 2 min throughout. Tones and shocks were applied in a conditioning chamber with floor grids and transparent plexiglas walls. In order to condition the rats specifically to the tone and minimize the effect of context, a white plastic "safe" box was inserted into the conditioning chamber whenever the tone was delivered without shock. Before each session rats were placed in the white box for 8 min habituation (no tone or shock). Between each session the shock grids and the white box were cleaned with odors clean wipes.

On Day 1, rats were placed in the white box twice a day for 5 min (habituation; no tone or shock). On Day 2 (*Conditioning*), rats were placed in the grid box and received 3 tones paired with foot-shock. Following conditioning animals were taken to their home. On Day 3 (*Ex1*), rats were placed in the white box and 15 tones or 5 tones [*Ex1-short*] without foot-shock were delivered (Tone: 4 kHz; 80 dB sound pressure level, ITI of 2 min). On Days 4 and 5 [*Ex2* and *Ex3*, respectively], rats received additional 15 tones without foot-shock in the white box.

Results are presented as the percent time spent freezing during the 30 s tone. The results for the 15 tones in the extinction training are presented as 5 blocks, each consisting of the average of 3 trials.

The results for the 5 tones in the extinction training are presented as 5 trials.

2.5. Automated measurement of activity and freezing

The automated digitized freezing system was kindly contributed by Prof. Joram Feldon [Laboratory of Behavioral Neurobiology, Swiss Federal Institute of Technology Zurich (ETH), Switzerland].

Briefly, the video images were transferred to a computer equipped with an analysis program (Image,) and a macroprogram (P. Schmid, Behavioral Neurobiology Laboratory, Swiss Federal Institute of Technology Zurich). The percentage of changed pixels between two adjacent 1 s images from one of the test boxes was used as a measure of activity. Freezing is commonly identified as cessation of any movement except for respiratory movements. If the percentage of changed pixels between two adjacent 1 s images was <0.05%, this corresponded well to such immobility and the behavior of the rat was scored as 'freezing' for the respective second.

2.6. Histology

At the completion of the behavioral experiments animals were anesthetized and microinfused into either the BLA or the IL with 0.5 μl of India ink. Cannula location was examined under a light microscope following Nissl staining. Fig. 1 shows schematic drawing of BLA (coronal view at position −3.14 and −3.30 mm posterior to bregma) and IL (coronal view at position +3.20 and +2.70 mm anterior to bregma) cannulae placements. Solid black circles indicate the locations.

2.7. Statistics

Differences among the groups were determined using ANOVA and *t* test.

3. Results

3.1. D-1 antagonist microinfused into the IL prior extinction training impairs consolidation of extinction of fear

Rats were trained for auditory fear conditioning and then divided into two groups [Vehicle, $n = 7$; DA antagonist SCH23390,

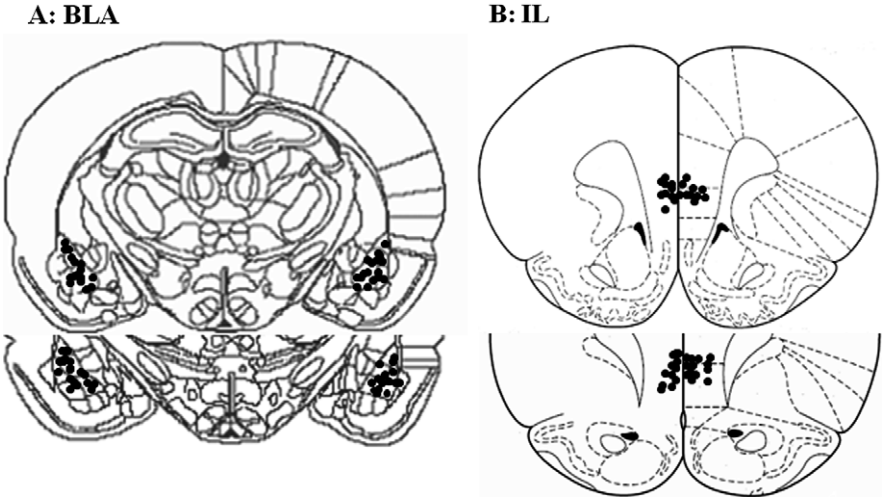

Fig. 1. (A) Solid black circles indicate BLA cannulae locations (coronal view at position 3.14 and 3.30 mm posterior to bregma; Paxinos & Watson, 1998). (B) Solid black circles indicate IL cannulae locations (coronal view at position 3.20 and 2.70 mm anterior to bregma; Paxinos & Watson, 1998).