Behavioural Brain Research 272 (2014) 40–45

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

Relief learning is distinguished from safety learning by the requirement of the nucleus accumbens

Milad Mohammadi[a,b], Jorge R. Bergado-Acosta[a], Markus Fendt[a,c,*]

[a] Institute for Pharmacology and Toxicology, Otto-von-Guericke University Magdeburg, Magdeburg, Germany
[b] Integrative Neuroscience Program, Otto-von-Guericke University Magdeburg, Magdeburg, Germany
[c] Center of Behavioral Brain Sciences, Otto-von-Guericke University Magdeburg, Magdeburg, Germany

HIGHLIGHTS

- Cues can be associated with relief or safety from an aversive event.
- The neural basis of relief and safety learning is poorly understood.
- Inactivation of the nucleus accumbens block relief but not safety learning.
- This demonstrates that these two forms of learning are neuronally distinct.

ARTICLE INFO

Article history:
Received 5 June 2014
Received in revised form 23 June 2014
Accepted 24 June 2014
Available online 1 July 2014

Keywords:
Anxiety
Fear
Rat
Reward
Startle

ABSTRACT

Aversive events induce aversive memories (fear learning) and can also establish appetitive memories. This is the case for cues associated with the cessation of an aversive event (relief learning) or occurring in an explicitly unpaired fashion (safety learning). However, the neural basis of relief and safety learning is poorly understood. In particular, it is not clear whether relief learning and safety learning are neuronally distinct. In the present study, we ask whether the nucleus accumbens is required for the acquisition of relief- and/or safety memory. Temporary inactivation of the nucleus accumbens by local injections of the GABA-A receptor agonist muscimol during the learning session abolished relief learning whereas safety learning was not affected. Thus, the requirement for a functional nucleus accumbens distinguishes relief from safety learning, showing that these two forms of learning are neuronally distinct.

1. Introduction

An aversive event leads to aversive memories. That is, stimuli preceding the aversive event typically become learned as signals for threat. This learning process is called fear learning and is well investigated in animals and humans [1–5]. However, it is less widely acknowledged that stimuli experienced upon the cessation of an aversive event, at the moment of relief, can lead to appetitive memories (summarized in [6]). This learning phenomenon is called relief learning and describes the ability to associate the cessation of an aversive event with a coincident stimulus [7–11]. Later, that stimulus is able to induce conditioned relief, which can be behaviorally measured as appetitive-like behavioral changes. Behavioral changes like startle attenuation in the stimuli's presence or as approach behavior toward it. Relief learning has been demonstrated in flies, rodents and humans, and it plays an important role of relief learning in normal and pathological emotions [6]. Yet, the neural mechanisms underlying it remain scarcely investigated. What is known is that the nucleus accumbens (NAC) is activated upon and is necessary for the retrieval of relief memory [8]. The NAC's involvement in reward processing [12–14] led to the hypothesis that "the 'signature' of relief memory thus corresponds to reward-memory" [6,8]. Whether this is also true regarding the establishment (acquisition) of relief memory (whether the NAC is also required during training) remains an open question.

Furthermore, there is uncertainty as to the psychological nature of the 'appetitive' memory observed after relief learning. That is, conceivably it is a special form of safety learning (see discussion in [6]). Safety learning is often induced by explicitly unpaired presentations of the to-be-learned stimulus and the aversive event [15,16]. Thus, the stimulus comes to predict a period of absence of an aversive event. It remains an open question whether the 'appetitive'

* Corresponding author at: Institute for Pharmacology and Toxicology, Otto-von-Guericke University Magdeburg, Leipziger Straße 44, D-39120 Magdeburg, Germany. Tel.: +49 391 67 21982; fax: +49 391 67 15869.
E-mail address: markus.fendt@med.ovgu.de (M. Fendt).

http://dx.doi.org/10.1016/j.bbr.2014.06.053
0166-4328/

behavior toward the stimulus observed after relief learning is based on such absence-prediction, or on its association with the relieving offset of the aversive event.

To answer both these open questions, we tested whether a functional NAC during training is required for the acquisition of relief and/or safety memory. Toward this end, we inactivated the NAC in two groups of rats by local injections of the GABA-A receptor agonist muscimol. Then, the animals were submitted to either relief or safety learning. One day later, retrieval of relief or safety memory, respectively, was tested in the two groups.

2. Materials and methods

2.1. Animals

Seventy adult male Sprague Dawley rats at an age between 2 and 3 months (250–350 g) at the time of the surgery were used in this experiment. They were kept in groups of 4 to 6 animals per cage under a light:dark cycle (h) of 12:12 (lights on 6:00 am) and had free access to water and food. All experiments and surgeries were done during the light phase. The experiments were performed in accordance with international ethical guidelines for the use of animals in experiments and were approved by the local ethical committee (Landesverwaltungsamt Sachsen-Anhalt, Az. 42502-2-1172 UniMD).

2.2. Stereotaxic surgery

The animals were anesthetized with isoflurane (Baxter Germany GmbH) mixed with pure Oxygen (5% isoflurane for induction, then 2.0–2.5%). Then, the animals were fixed into a rodent stereotaxic apparatus. The skull was exposed and stainless steel guide cannulas (custom-made; diameter: 0.7 mm, length: 8.0 mm) were bilaterally implanted aiming at NAC: 1.2 mm rostral, ±1.5 mm lateral, and 7.4 mm ventral to bregma [17]. Cannulas were fixed with dental cement to the skull and three anchoring screws. After the surgery, the animal was single caged and supervised for 4 h and then returned to colony. After the surgery, there was a recovery period of 5–7 days.

2.3. Apparatus

We used a startle system with eight chambers (35 cm × 35 cm × 35 cm; SR-LAB, San Diego Instruments, USA). Each chamber consisted of a stable platform holding a horizontal cylinder transparent animal enclosure with inner diameters of 9 cm and inner length of 16 cm. Underneath the platform, a piezoelectric motion sensor was mounted for measuring animal movements. The output signal of this sensor was digitalized with a sampling rate of 1 kHz and send to the computer. Beginning at startle stimulus or electric stimulus onset, respectively, consecutive 1-ms readings were recorded to obtain the magnitude of the animal's response to the startle stimulus or electric stimulus (arbitrary units). As startle magnitude, the average readout in the 'startle response peak window', 10–30 ms after startle stimulus onset, were taken. To measure reactivity to electric stimuli, the readouts for the period of the stimulus were added up.

For conditioning, aversive electric stimuli (US) and light stimuli (CS) were used. The light stimulus was produced by a 10 W bulb, had an intensity of ~1000 lx and a duration of 5 s. The electric stimuli were administered via a floor grid (6 bar with 5 mm diameter, distance: 10 mm), had an intensity of 0.4 mA and a duration of 0.5 s. For the application of acoustic stimuli, a loudspeaker mounted at the ceiling of the box was used. During all test, a background noise with an intensity of 50 dB SPL were presented to mask environmental noises. The acoustic startle stimulus was a noise burst with an intensity of 96 dB SPL and duration of 40 ms.

2.4. Behavioral protocol

The behavioral experiment was carried out in 3 sessions (see also Fig. 1). On the first day (baseline session), animals were put in the chambers and after 5 min of acclimation 10 startle stimuli were delivered with an intertrial interval of 30 s. Then, the animals were put back into their home cages. Based on the mean startle amplitude of this session, the animals were distributed into five groups with similar mean baseline startle amplitude (relief conditioning + vehicle; relief conditioning + 1.3 nmol muscimol; relief conditioning + 2.6 nmol muscimol; safety conditioning + vehicle; safety conditioning + 2.6 nmol muscimol).

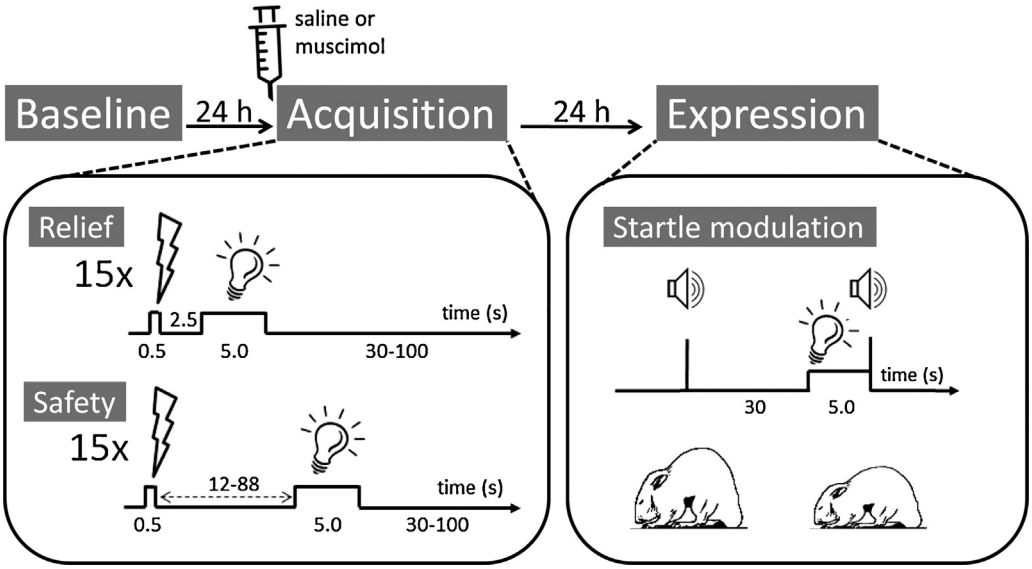

Fig. 1. Behavioral protocol. After intra-NAC injections (saline, muscimol), rats were either relief- or safety-conditioned. One day later, startle modulation by the relief- or safety-CS, respectively, were measured (for details, see main text).