Neuroscience Research 95 (2015) 66–73

Contents lists available at ScienceDirect

Neuroscience Research

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

Brief fear preexposure facilitates subsequent fear conditioning

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ARTICLE INFO

Article history:
Received 22 October 2014
Received in revised form 30 January 2015
Accepted 4 February 2015
Available online 12 February 2015

Keywords:
Fear conditioning
Post traumatic stress disorder
N-methyl-D-aspartate receptor
Trauma
Mouse

ABSTRACT

Post-traumatic stress disorder (PTSD) is an anxiety disorder that occurs following an unexpected exposure to a severe psychological event. A history of a brief trauma is reported to affect a risk for future PTSD development; however, little is known about the mechanisms by which a previous trauma exposure drives the sensitivity to a late-coming trauma. Using a mouse PTSD model, we found that a prior foot shock enhances contextual fear conditioning. This shock-induced facilitation of fear conditioning (*i.e.*, priming effect) persisted for 7 days and was prevented by MK801, an *N*-methyl-D-aspartate receptor antagonist. Other types of trauma, such as forced swimming or tail pinch, did not induce a priming effect on fear conditioning. Thus, a trauma is unlikely generalized to modify the sensitivity to other traumatic experiences. The behavioral procedure employed in this study may be a useful tool to elucidate the etiology of PTSD.

1. Introduction

Fear is a normal defensive psychological reaction in which animals and humans may protect themselves from uncertain dangers. However, inappropriate regulation of fear causes anxiety disorders. One of the anxiety disorders is post-traumatic stress disorder (PTSD), which is triggered by a sudden experience of a severe traumatic event. PTSD is characterized by mental re-experiences of the traumatic event, avoidance of stimuli that may be related to the trauma, and symptoms of increased arousal, such as heightened startle and palpitation (DSM-V, 2013). The sensitivity to PTSD varies among individuals; even the same stressor may induce PTSD in some people but may not induce it in others. A number of risk factors have been implicated to contribute to the vulnerability to PTSD development. A recent study has suggested that one of the risk factors is a past trauma experience (Ozer et al., 2003). However, the mechanisms by which a memory of a previous stressor is stored in the neuronal circuitry and thereafter facilitates the formation of PTSD are not fully understood. This is, in part, because of a lack of animal models for history-dependent modulations of the PTSD development.

Fear conditioning is a behavioral test that is widely used to measure the strength of aversive memory. In a typical test of contextual fear conditioning, mice or rats that received aversive electric foot shocks in a chamber show "freezing" behaviors when they are placed in the same chamber that does not deliver foot shocks any longer. Thus, the fear conditioning paradigm captures some aspect of PTSD. In this study, we used the fear conditioning test and sought to establish an experimental animal model for trauma-induced sensitization of PTSD. Specifically, we examined the effect of a brief fear preexposure on subsequent fear conditioning. We found that mice that had received a prior single foot shock showed higher freezing responses, compared to intact mice. Because this phenomenon resembles a prior trauma-induced increase in the sensitivity to PTSD in humans, we further scrutinized this experimental model.

2. Materials and methods

2.1. Animal ethics

Experiments were performed with the approval of the animal experiment ethics committee at the University of Tokyo (approval number: P24-10) and according to the University of Tokyo guidelines for the care and use of laboratory animals. All efforts were made to minimize the animals' suffering and the number of animals used.

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http://dx.doi.org/10.1016/j.neures.2015.02.001
0168-0102/

2.2. Animals and drugs

Male C57BL/6J mice (SLC, Shizuoka, Japan) of 4–6 weeks old were housed under conditions of controlled temperature and humidity (23 ± 1 °C, 55 ± 5%), maintained on a 12:12-h light/dark cycle, and had access to food and water *ad libitum*. All behavioral tests were performed between 8 AM and 2 PM. Mice were acclimated by daily handling for 5 days before the behavioral experiments. MK801 (TOCRIS Bioscience, Bristol, UK), an antagonist of NMDA receptor, was diluted in saline and intraperitoneally injected at a dose of 1.0 mg/kg 30 min prior to the priming shock. We chose a relatively high dose (1.0 mg/kg) of MK801, because MK801 at a dose of 0.1 mg/kg is reported to be insufficient to inhibit the acquisition of contextual fear memory, whereas 1.0 mg/kg MK801 inhibited it (Gould et al., 2002).

2.3. Behavioral procedures

Fear conditioning was performed in a shock chamber consisting of a plastic box (18 cm in width, 11 cm in depth, and 11 cm in height) with transparent walls and a metal grid floor connected to a shock scrambler (SGA-2010, O'HARA, Tokyo, Japan). On day 1, mice were placed in a shock chamber, and a single electrical shock (1 mA, 2 s; priming shock) was immediately delivered to floor metal grids. Then, the mice were quickly removed from the chamber. All these procedures were completed within 10 s. Mice in the Conditioning-only group were placed in the chamber, but they did not receive the priming shock. The animals were soon returned to their home cages. This immediate foot shock is known to induce no contextual fear conditioning, because the animals cannot relate the aversive experience to the environmental context (Landeira-Fernandez et al., 2006). On day 2 (conditioning session), the mice in the Conditioning-only group and the Priming-shock + Conditioning group were allowed to freely explore the chamber for 5 min. During the following 4 min, they received 4 foot shocks (1 mA, 1 s; conditioning shock) at an interval of 1 min. Mice in the Priming-shock-only group were placed in the shock chamber but did not receive the conditioning shock. On day 3 (test session), the animals were re-placed in the same chamber for 5 min without any foot shocks. During both the fear conditioning and the test session, the animals were monitored at 2 Hz using a top-view digital camera. Freezing was automatically identified, using custom-made MATLAB routine (Sakaguchi et al., 2012; Ishikawa et al., 2014). After denoise, the mouse body was binarized at each video frame, and the body motion was detected by calculating the number of pixels in which the binary values flipped from 0 to 1 or from 1 to 0 between two successive video frames. Freezing time was defined based on the total number of frames in which the number of the pixel changes was below the threshold. The threshold was determined so that the calculated freezing time was comparable to that obtained manually by three well-trained observers.

2.4. Forced swimming and tail pinch

Mice were subjected to two aversive stressors, *i.e.*, the forced swimming and the tail pinch. The forced swimming is widely used as a behavioral despair model that produces acute stress (Porsolt et al., 1977). Individual mice were forced to swim inside a vertical Plexiglas cylinder (innerϕ = 12 cm). The water temperature was 22 ± 1 °C, the depth was 15 cm, and the above-water wall height was 8 cm. The mice were kept in the water for 15 min until they spent 60% of their time in immobility. The immobility time was evaluated using a video-based automatic detection system (Ishikawa et al., 2014). The mice were then returned to their home cage. The water was changed for each mouse. For the tail pinch, an artery clip (4.5 cm in length) was placed on the base of the tail for 5 s. Mice that did not try to remove the tail pinch within 1 s were excluded from the following experiments.

2.5. Elevated-plus maze

The mice were placed in the center of a maze with four arms arranged in the shape of a plus sign. The maze consisted of a central quadrangle (8 cm in width and 8 cm in length), two opposing open arms (25 cm in length and 8 cm in width), and two opposing closed arms. These four arms were identical in size, but the closed arms were equipped with 25-cm-high walls at both the sides and the far end. The floorboard was made of white plastic, the walls were made of opaque gray plastic, and the floorboard was elevated 25 cm above the ground. At the beginning of each trial, the mice were placed on the central quadrangle facing an open arm. The movements of the mice during a period of 5 min were recorded by a camera positioned above the center of the maze. The number of entries into the open arms and the closed arms and the time spent in each arm were manually determined. An entry into an arm was defined as placement of the four paws on that arm (Kumakura et al., 2010). The time spent in the open arms was expressed as a percentage of the total time spent in the open and closed arms, *i.e.*, open-arm time/(open-arm time + closed-arm time) × 100. The number of open arm entries were expressed as a percentage of the total arm entries, *i.e.*, open-arm entries/(open-arm entries + closed-arm entries) × 100. Mice whose total number of entries into arms was less than 10 were excluded.

2.6. Acetic acid-induced writhing test

Pain sensitivity was evaluated by referring to acetic acid-induced writhing responses. Acetic acid (0.9%) was intraperitoneally injected at a volume of 10 ml/kg into mice, and they were placed in a vertical Plexiglas cylinder (inner ϕ = 12 cm) 5 min before the test. Mice were habituated to these cylinders for 30 min before injection. Their movements were video-recorded for 10 min. A stretching behavior of the hind limbs accompanied by a contraction of the abdominal muscles was defined as a writhing response. From each video, a total of 30 time periods (5 s in length) were extracted at an interval of 20 s. A blinded observer judged whether the writhing behaviors were present or absent in these 5-s video clips. The writhing frequency was expressed as the percentage of the video clips with writhings to the total 30 videos.

2.7. Plasma corticosterone concentration

The blood was collected from the abdominal inferior vena cava of anesthetized mice with diethyl ether, incubated at 37 °C for 30 min, and centrifuged at 3000 × *g* for 10 min. The supernatant was collected as blood serum. The blood was obtained within 15 min after acute stresses, during which corticosterone is reported to exhibit the peak response (Anisman et al., 1998; Shanks et al., 1990). The concentration of corticosterone was measured with Corticosterone ELISA kit (Assaypro).

2.8. Statistical analyses

Statistical analyses were performed using R software. All data are demonstrated as means ± SEMs. Student's *t*-test was used for comparisons between two independent groups. One-way analysis of variance (ANOVA) and Tukey's *post hoc* test were used for comparisons among more than two independent groups. Statistical significance was set at $P < 0.05$.