Behavioural Brain Research 304 (2016) 20–23

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Short communication

Distinct state anxiety after predictable and unpredictable fear training in mice

Thomas Seidenbecher*,[1], Jasmin Remmes[1], Thiemo Daldrup, Jörg Lesting, Hans-Christian Pape

Institute of Physiology I, Westfälische Wilhelms-University Münster, Germany

HIGHLIGHTS

- Predictable and unpredictable CS-US timing induced phasic and sustained fear in mice.
- State anxiety was assessed during phasic and sustained fear using the elevated plus-maze test.
- Expression of phasic fear was associated with anxiolytic behavior,sustained component of fear was associated with enduringstate anxiety.

ARTICLE INFO

Article history:
Received 11 November 2015
Received in revised form 3 February 2016
Accepted 7 February 2016
Available online 10 February 2016

Keywords:
Conditioning
Behavior
Learning
Predictability
Unpredictability

ABSTRACT

Sustained fear paradigms in rodents have been developed to monitor states of anxious apprehension and to model situations in patients suffering from long-lasting anxiety disorders. A recent report describes a fear conditioning paradigm, allowing distinction between phasic and sustained states of conditioned fear in non-restrained mice. However, so far no prospective studies have yet been conducted to elucidate whether induction of phasic or sustained fear can affect states of anxiety. Here, we used CS (conditioned stimulus) and US (unconditioned stimulus) pairing with predictable and unpredictable timing to induce phasic and sustained fear in mice. State anxiety during various fear response components was assessed using the elevated plus-maze test. Training with unpredictable CS-US timing resulted in CS-evoked sustained components of fear (freezing), while predictable CS-US timing resulted in rapid decline. Data suggested the influence of training procedure on state anxiety which is dependent on progression of conditioned fear during fear memory retrieval. Animals trained with unpredictable CS-US timing showed an unchanged high anxiety state throughout behavioral observation. In contrast, mice trained with predictable CS-US timing showed anxiolytic-like behavior 3 min after CS onset, which was accompanied by a fast decline of the fear conditioned response (freezing). Further systematic studies are needed to validate the phasic/sustained fear model in rodents as translational model for anxiety disorders in humans.

Over the last decades, Pavlovian fear conditioning has become one of the standard behavioral paradigms to study fear- and anxiety-related processes in rodents and humans [1,2]. In rodents, conditioned fear is observed upon pairing of a neutral (conditioned, CS) stimulus (e.g., tone) with an aversive (unconditioned, US) stimulus (e.g., footshock). After conditioning, during fear memory retrieval, presentation of the CS alone elicits a variety of defensive behaviors (e.g., freezing), indicating states of conditioned fear. Although symptoms of fear and anxiety are very similar, they differ distinctly in behavioral expressions and underlying neuronal mechanisms (for excellent reviews see Refs. [3–7]). Fear is defined as an adaptive state of apprehension that develops rapidly and declines quickly once the threatening stimulus is absent; a physiological state called phasic fear. Phasic fear can be measured using a short, discrete cue, that is predictably paired with an aversive event (e.g., footshock) [4,8], whereas more diffuse cues or less predictable threats can induce more sustained states of fear [9,10]. To distinguish between phasic and sustained fear and their underlying molecular and neuronal mechanisms, appropriate animal models of anxiety disorders are essential. In this regard, Davis and Walker initially developed the sustained fear model in rats, using fear-potentiated startle responses as measure of fear, to have a more valid and useful model of a long-lasting clinical situation in patients suffering from anxiety disorders, than it would be reflected by

* Corresponding author at: Institut für Physiologie I, Westfälische Wilhelms-Universität Münster, Robert-Koch-Str. 27a, D-48149 Münster, Germany.
E-mail address: seidenbe@uni-muenster.de (T. Seidenbecher).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bbr.2016.02.009
0166-4328/

only short-lasting phasic fear responses (for review see Ref. [4]). Recently, we have described a modified training protocol to assess phasic and sustained fear in freely behaving mice, using freezing (in addition to startle responses) as measure of fear [8]. Although, recent data have shown that phasic and sustained fear in rats are dissociable pharmacologically [11], the evidence, that conditioned phasic and sustained states of fear can affect anxiety-like behavior, has not yet been assessed in detail. Thus, evaluation of an altered state of anxiety by the phasic/sustained fear paradigm in mice would further promote this approach as an appropriate translational model for human anxiety disorders. Therefore, in the present study, we used the recently established phasic/sustained fear paradigm in freely behaving (non-restrained) mice for the assessment of state anxiety in the elevated plus-maze (EPM) during various components of phasic and sustained fear. In this study, we tested the hypothesis, that phasic and sustained components of fear, after predictable or unpredictable fear training, can affect anxiety-like behavior in the EPM.

Experiments were performed in accordance to the European Communities Council directive (86/609/EEC), with the regulations of German law and as approved by the local animal care committee LANUV NRW (AZ 84-02.042012.A206). Animals were kept in a 12 h light/dark cycle provided with water and food (Altromin 1324, Altromin GmbH, Lage, Germany) ad libitum. Experiments were conducted with 8–10 weeks old male C57BL/6J mice (n = 54, Charles River, Germany). One week before start of experiments (adaptation, fear conditioning, retrieval, EPM), mice were separated from group housing and single housed in standard Macrolon cages type III (38 × 22 × 15 cm) with sawdust as bedding material (Allspan, Höveler GmbH & Co.KG, Langenfeld, Germany). Mice were trained as described previously [8]. Briefly, on day 1, mice were adapted to the test cage for one minute (context A) followed by 36 startle-eliciting white noise bursts (85 dB, 50 ms duration, inter-burst interval (ISI) 30 s). On day 2, fear conditioning was performed in a standardized fear conditioning chamber (context B, TSE, Germany). For the induction of sustained fear, mice were allowed a two-minute adaptation to the fear conditioning apparatus followed by the presentation of four 10 kHz tones as conditioning stimulus (CS, 75 dB, pseudo-randomized stimulus presentation with variable (unpredictable) duration of 29, 9, 19 and 14 s (ISI 30 s). An unconditioned stimulus (US, footshock, 0.4 mA, duration 1 s) coincided with the termination of each CS. A second session was repeated 6 h later with CS duration in altered order (19, 14, 29 and 9 s) (unpredictable CS-US timing). For the induction of phasic fear, a second group of animals (predictable CS-US timing) was confronted to four 10 kHz tone presentations at 75 dB with constant (predictable) duration of 10 s. Each tone was followed by a footshock (US, 0.4 mA, 1 s). ISIs were pseudo-randomized (15 s, 20 s and 19 s) and presented in a pseudorandomized order between the first and a second training session 6 h later. Twenty-four hours after fear conditioning (day 3), single animals were transferred to the retrieval environment (context A), habituated over a period of 1 min before being exposed to the phasic/sustained fear retrieval protocol. A 75 dB, 10 kHz sine wave stimulus was used as CS. For more details see recent publication by Ref. [8]. Freezing behavior (% of time averaged in 30 s bins) was analyzed over the entire retrieval session. Freezing (immobility except for respiratory movements), as indicator of behavioral fear, was used and evaluated offline (% per 30 s time bins) using the multidimensional software tool MOVE [12]. In brief, this approach is a semi-automated system, supported by multi-angle video recording for motion detection which works with pixel differences on a frame-by-frame basis. In addition, a key logger option can be used to manually score different types of behaviors (e.g., freezing, grooming or sniffing), by pressing keyboard buttons to monitor behavioral expressions. To assess the influence of phasic and sustained fear on state anxiety, we evaluated the effect of training to

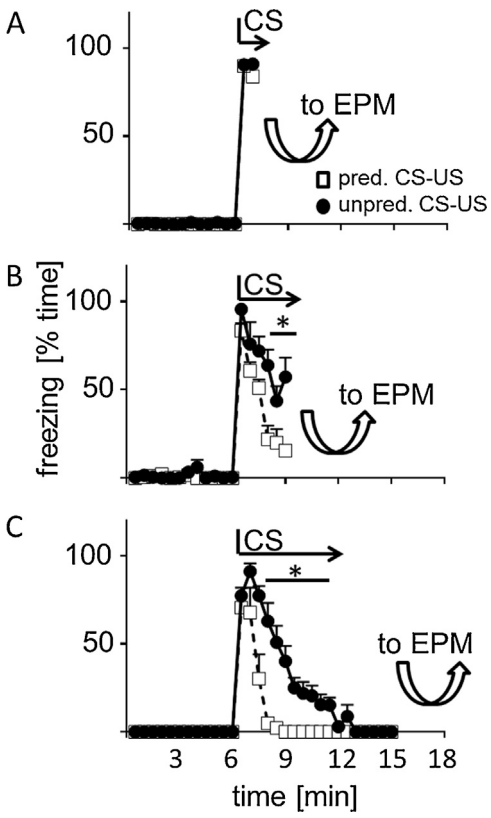

Fig. 1. Freezing during fear memory retrieval 24 h after predictable/unpredictable traing (cf. [8]). Fraction of time spent freezing (% of 30 s time bins) before, during and after CS presentation for animals trained to predictable (open squares) or unpredictable threat (black dots). Time point of interruption within the retrieval session and transfer to the EPM is marked (A: 1 min after CS onset, B: 3 min after CS onset, C: 3 min after CS offset, each group n = 6). Values are mean + SEM (Bonferroni post-hoc test: $^{*}p < 0.05$).

predictable and unpredictable CS-US timing and the progression of fear response during retrieval on anxiety in the EPM at different time points during fear memory retrieval. The following time points within the fear memory retrieval protocol were selected (see Fig. 2): (B) 3 min before CS onset, (C) 1 min after CS onset, (D) 3 min after CS onset, and (E) 3 min after CS termination. At the given time points, single animals were transferred in their home cage from retrieval context A to a neutral context (context C) in a neighboring laboratory. There, the EPM test was started within 30 s, without continuation of CS presentation. Basal state fear was tested in naïve animals (n = 6) (Fig. 2A). The EPM, elevated 75 cm above the floor and light intensity on the open arms 120 lux, consisted of two open (30 × 5 cm) and two wall-enclosed arms (30 × 5 × 25 cm) connected by a central platform (5 × 5 cm). Behavioral testing was started by placing the mouse in the central area, facing a closed arm. Numbers of entries into open/closed arms, time spent on open/closed arms as well as total locomotor activity were monitored (Video-Mot II, TSE, Bad Homburg, Germany) over a period of 5 min. Data were analyzed by Statistica (Stat Soft Inc. Tulsa, USA) using ANOVA followed by Bonferroni post-hoc test and paired t-test for open/closed arm ratio. In all experiments, differences were considered statistically significant at $p < 0.05$.

Confirming the results from a recent publication [8], fear conditioned animals expressed a high percentage of freezing in response to the onset of the conditioned stimulus 24 h after fear conditioning, irrespective of predictable or unpredictable fear training (Fig. 1A). However, dependent on the progression of CS presentation, animals trained with unpredictable CS-US timing displayed prolonged and sustained freezing throughout the entire CS presen-