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PREVENTING LONG-LASTING FEAR RECOVERY USING BILATERAL ALTERNATING SENSORY STIMULATION: A TRANSLATIONAL STUDY

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Abstract—Posttraumatic stress disorder (PTSD) is a highly debilitating and prevalent psychological disorder. It is characterized by highly distressing intrusive trauma memories that are partly explained by fear conditioning. Despite efficient therapeutic approaches, a subset of PTSD patients displays spontaneous recurrence of traumatic memories after successful treatment. The development of animal behavioral models mimicking the individual variability in treatment outcome for PTSD patients represent therefore an important challenge as it allows for the identification of predicting factors of resilience or susceptibility to relapse. However, to date, only few animal behavioral models of long-lasting fear recovery have been developed and their predictive validity has not been tested directly. The objectives of this study were twofold. First we aimed to develop a simple animal behavioral model of long-lasting fear recovery based on auditory cued fear conditioning and extinction learning, which recapitulates the heterogeneity of fear responses observed in PTSD patients after successful treatment. Second we aimed at testing the predictive validity of our behavioral model and used to this purpose a translational approach based (i) on the demonstration of the efficiency of Eye Movement Desensitization and Reprocessing (EMDR) therapy to reduce conditioned fear responses in PTSD patients and (ii) on the implementation in our behavioral model of an electrical bilateral alternating stimulation of the eyelid which mimics the core feature of EMDR. Our data indicate that electrical bilateral alternating stimulation of the eyelid during extinction learning alleviates long-lasting fear recovery of conditioned fear responses and dramatically reduces inter-individual variability. These results demonstrate the face and predictive validity of our animal behavioral model and provide an interesting tool to understand the neurobiological underpinnings of long-lasting fear recovery.

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Key words: fear recovery, fear conditioning, PTSD, bilateral alternating stimulation, Eye Movement Desensitization and Reprocessing.

INTRODUCTION

Anxiety disorders are among the most frequent psychiatric conditions with a lifetime prevalence of around 6–8% in the population worldwide (Breslau et al., 1998; Kessler, 2000). In particular, posttraumatic stress disorder (PTSD) represents one of the most frequent anxiety disorders, which can develop following the experience of a traumatic event. PTSD patients exhibit a number of symptoms including re-experiencing of the traumatic event (flashback, nightmare), avoidance of places or objects associated with the initial trauma, fear generalization and hyperarousal (APA, 2000). Although, current therapeutic approaches for anxiety disorders are often associated with short-term improvement of these anxiety-related symptoms, a fraction of PTSD patients display long-lasting relapse of traumatic memories after successful treatment (Rachman, 1979; Foa et al., 1991; Rodriguez et al., 1999; Resick et al., 2002, 2012; Boschen et al., 2009; Vervliet et al., 2013). Thus it is of strong clinical interest to develop animal models reproducing human fear relapse to further understand and identify the underlying neurobiological mechanisms. Over the past years, several animal models of PTSD have been developed using various stressors, which

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Abbreviations: BAS, bilateral alternating stimulations; CS, conditioned stimulus; EMDR, Eye Movement Desensitization and Reprocessing; IL, infralimbic; ITI, intertrial interval; MPSS, Modified PTSD Symptoms Scale; PL, prelimbic; Post-FC, Post-Fear Conditioning; PTSD, posttraumatic stress disorder; SC, skin conductance; SCL, SC level; SCR, SC response; US, unconditioned stimulus.

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reproduced specific PTSD symptoms such as generalization of fear responses to non traumatic places or stimuli, resistance to extinction (an analog of exposure therapies in humans), hyperarousal, and avoidance of trauma-related stimuli (Siegmund and Wotjak, 2006; Goswami et al., 2013; Goode and Maren, 2014). To date however, only few attempts have been made to develop animal models mimicking PTSD long-lasting relapse of traumatic fear memories following successful treatment (Deschaux et al., 2011; Goode and Maren, 2014).

In the laboratory, traumatic aversive experiences are usually induced using the classical auditory fear-conditioning paradigm which consists in the repetitive association between a neutral stimulus (the conditioned stimulus (CS), usually a tone or a light) with a mild electrical footshock (the unconditioned stimulus (US)). Following conditioning, re-exposure to the CS induces a broad range of conditioned fear responses including an immobilization reaction labeled freezing, which represents a reliable behavioral measure of the learned association. Inhibition of conditioned fear behavior can be observed following repetitive exposure to the CS without the US in a context different from the original conditioning context, a process labeled fear extinction. Interestingly, following extinction learning, re-exposure to the extinction context, the original conditioning context, or unsignaled footshocks can lead to relapse of fear behavior (Myers and Davis, 2007; Herry et al., 2010; Goode and Maren, 2014), although it is not clear if this occurs in all the individuals tested. Individual variability is nowadays considered as a critical component of PTSD animal models because it allows identifying predicting factors of resilience or susceptibility to traumatization (Cohen et al., 2012; Goswami et al., 2013; Daskalakis and Yehuda, 2014). Unfortunately, most of the animal models of PTSD currently available do not evaluate individual susceptibility to relapse after successful fear extinction (but see Goswami et al., 2010).

In the present manuscript, we pursue to main objectives. First we developed a simple behavioral model of long-lasting fear recovery using auditory cued fear conditioning and extinction learning based on individual variability. Second, we validated our model using a translational approach based on (i) the identification of a valid therapeutic approach to reduce conditioned fear responses in PTSD patients and (II) on the implementation in our behavioral model of this therapeutic approach developed in humans. Our behavioral results in mice indicate that following successful extinction, mice display either maintenance of fear extinction or long-lasting fear recovery, which replicates the heterogeneity of fear responses observed in PTSD patients after successful treatment. We next tested the validity of our behavioral model and used a translational approach to this purpose. We first evaluated in PTSD patients undergoing classical fear conditioning and extinction, the efficiency of Eye Movement Desensitization and Reprocessing (EMDR) therapy to reduce PTSD symptoms and conditioned fear reactions. Among the therapeutic approaches to treat PTSD patients, EMDR is one of the most efficient and recommended therapies (Foa et al., 2009; WHO, 2013). In summary, it consists first in the assessment of cognitive, emotional and physical aspects of actual distress to traumatic scenes, and second in imaginal exposure to the traumatic event in association with bilateral alternating stimulations (BAS) (i.e. either auditory, visual, or somatosensory stimuli alternating between the two sides of the body) (Servan-Schreiber et al., 2006). The major therapeutic action of EMDR is thought to be the association of the patient traumatic memory with BAS (Shapiro, 1996; Herkt et al., 2014). In a second step, we implemented in behaving animals an electrical BAS of the eyelid applied during fear extinction, which mimics the core feature of the EMDR procedure, to evaluate if this stimulation alleviates long-lasting fear recovery, and therefore inter-individual variability to relapse, in our behavioral model.

EXPERIMENTAL PROCEDURE

Animals

Male C57BL6/J mice (3 months old, Janvier) were individually housed for 7 days prior to all experiments, under a 12-h light/dark cycle, and provided with food and water *ad libitum*. All studies took place during the light portion of the cycle. Mice were gently handled for 2–3 min/day during 5 days, to minimize nonspecific stress. All animal procedures were performed in accordance with standard ethical guidelines (European Communities Directive 86/60-EEC) and were approved by the committee on Animal Health and Care of Institut National de la Santé et de la Recherche Médicale and French Ministry of Agriculture and Forestry (authorization A3312001).

Surgery

Mice were anesthetized with isoflurane (induction 3%, maintenance 1.5%) in O_2. Body temperature was maintained with a temperature controller system (FHC, Bowdoin, ME, USA). Mice were secured in a stereotaxic frame and bilaterally implanted in muscle above the eyelid with stimulating electrodes. The electrodes consisted of silver wires (127-μm inner diameter, Phymep, Paris, France) and were attached to a four pins connector (Omnetics, Minneapolis, MN, USA). All implants were secured using Super-Bond cement (Sun Medical, Moriyama, Shiga, Japan). After surgery mice were allowed 7 days to recover and habituated to handling. Analgesia was applied before, and 1 day after surgery (Metacam, Boehringer, Ingelheim am Rhein, Germany).

Animal behavioral apparatus

Fear conditioning and extinction took place in two different contexts (A and B). The conditioning and extinction boxes were cleaned with 70% ethanol or 1% acetic acid before and after each session, respectively. To score freezing behavior an automated infrared beam detection system located on the bottom of the experimental chambers was used (Coulbourn Instruments, Whitehall, PA, USA). The animals were considered to be freezing if no movement was detected for 2 s.

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