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Research report

Effects of multiple brief exposures to trauma-associated cues on traumatized resilient and vulnerable rats

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ABSTRACT

Intrusive re-experiencing of a trauma is a core symptom in post-traumatic stress disorder (PTSD), and is often triggered by contextual cues associated with the event. It is not yet established if intrusive re-experiencing is the consequence of PTSD, or if it could contribute to the development of PTSD following a traumatic event. The present study (1) examined the impact of repeated brief re-exposures to trauma reminders on the strength of PTSD-like symptoms, as well as on their time-development and (2) investigated the reactivity over time to these cues in trauma resilient and vulnerable rats, defined on the basis of the PTSD-like symptoms they demonstrated.

Rats were exposed to a Single Prolonged Stress, combining three different stresses (2-h restraint, 20-min forced swim and CO_2 unconsciousness) delivered together with tone and odor cues and preceded by an inhibitory avoidance conditioning or a control procedure. During the following two weeks, reminded rats were briefly re-exposed to trauma-associated cues either 4 or 8 times.

The results indicated that 4 re-exposures to the same cue strengthened PTSD-like symptoms (anxiety, arousal, fear to trauma-cue). However 8 re-exposures to similar or different trauma-cues did not alter PTSD-like symptoms and led to a rapid extinction of the fear reactivity to these cues. The present results further indicated that shortly after trauma, both resilient and vulnerable rats strongly reacted to trauma-associated cues, while only vulnerable rats reacted long after the trauma, suggesting a slower loss of fear responses to trauma cues in these rats.

We concluded that re-experiencing may participate in, but cannot be solely responsible for, the development of long-term PTSD effects.

1. Introduction

Post-traumatic stress disorder (PTSD) is a debilitating disorder that develops in some people who have experienced a traumatic event. PTSD causes intrusive symptoms such as re-experiencing the traumatic event through vivid nightmares, flashbacks, or thoughts that occur in response to trauma cues. Re-experiencing which constitutes the core of the pathology, can be so realistic that the subjects often think they are living the trauma all over again, feeling the same emotions and the same physical sensations of what happened. The vividness of the re-experiencing leads patients to be exposed to repeated and persistent high levels of stress, and to developing anxiety, which might be associated with the progression of trauma-related pathologies and the development of related disorders (Pitman and Delahanty, 2005; Pitman, 1988).

In animals, intrusive re-experiencing can be addressed by the use of brief exposures to situational reminders of the traumatic episode. Some studies indicate that re-exposures to reminders relative to an aversive conditioning can durably increase the strength of the fear responses (Inda et al., 2011). Concerning PTSD models, Pynoos et al. (1996) developed a procedure in mice combining a strong aversive stimulus with repeated (1–6) exposures to the experimental context in which this stimulus was delivered. They showed that this combination, thought to mimic trauma and re-experiencing, was associated with PTSD-like symptoms that were not obtained without any re-exposure to the context. This animal model has then been repeatedly used to investigate the biological basis of post-traumatic stress disorder (Cleren et al., 2013; Corral-Frias et al., 2013; Hawley et al., 2013; Olson et al., 2011). However, although these studies strongly suggest that repeated brief exposures to situational reminders can increase the strength of an aversive experience and transform it in a traumatic experience, they did not directly address the long term consequences that re-experiencing may have on PTSD development. This issue is the main goal of the present study. We recently showed that rats exposed to an animal

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model of PTSD, the Single Prolonged Stress (SPS; Liberzon et al., 1997), combining 2-h restraint stress, followed by a 20-min forced-swim and a loss of consciousness produced by CO_2, delivered together with tone and odor cues, developed PTSD-like symptoms (Toledano and Gisquet-Verrier, 2014). We further showed that a brief re-exposure to each of the different trauma-associated cues induced transient fear reactions, suggesting that these re-exposures were able to reactivate the trauma memory and thus to mimic re-experiencing in human (Le Dorze and Gisquet-Verrier, 2016).

In the present study, we explored the possibility that repeated brief exposures to trauma-associated cues, delivered shortly after the trauma, could durably increase PTSD-like symptoms. In addition, we explored whether spontaneous loss as well as extinction of the fear responses elicited by these different reminder cues in rats resulted in their being resilient or vulnerable to the trauma on the basis of their symptoms (Le Dorze and Gisquet-Verrier, 2016). For that purpose, rats were exposed to a SPS, preceded by weak inhibitory avoidance conditioning in order to induce additional cues, or a control procedure, involving the same conditions with no electrical shock. During the first two weeks, rats received 4 or 8, brief re-exposures to either identical or different trauma-associated cues. Re-exposed and non re-exposed rats were then evaluated for their reactivity to trauma-associated cues and to PTSD-like symptoms in order to determine the role that re-experiencing may have had on the strength of the pathology.

2. Results

2.1. Effect of re-exposures to the same reminder

2.1.1. Experiment 1: Effects of 4 re-exposures on PTSD-like symptoms

In the first Experiment (Fig. 1), rats underwent a Single Prolonged Stress (SPS) or a control procedure (CTRL), and half of them were re-exposed 4 times (4R) to the predictive box (on day 3, 6, 9, and 12), while the other half remained in their cages (NR, not re-exposed). Rats were thus divided into 4 groups (CTRL-NR, CTRL-4R, SPS-NR, and SPS-4R; ns=8), according a 2 (trauma conditions) by 2 (reminder conditions) experimental design. Rats were then tested with the Elevated Plus Maze (EPM) and Acoustic Startle Response (ASR) tests..

2.1.1.1. Anxiety: Elevated Plus Maze (EPM-D15, Fig. 1B). A two-way ANOVA performed on percent of the time spent in open arms of the EPM revealed a SPS effect ($F(1\text{-}28)=5.6$, $p=0.024$), with no Reminder effect ($F < 1$), and no significant interaction between these two factors ($F < 1$). Planned comparisons further indicated that the difference between SPS and CTRL groups reached a significant level only for the reminded rats ($F(1\text{-}14)=7.46$, $p=0.015$).

2.1.1.2. Arousal: Acoustic Startle Response (ASR-D17, Fig. 1C). A two-way ANOVA performed on the 3 last blocks of the startle response test revealed a Reminder effect ($F(1\text{-}28)=3.68$, $p=0.05$), with no SPS effect ($F < 1$), but a significant interaction between these two factors ($F(1\text{-}28)=8.63$, $p=0.006$). Complementary analyses indicated that SPS tended to reduce the startle response in the non re-exposed (NR) rats ($F(1\text{-}14)=3.43$, $p=0.08$), confirming our previous results, but significantly increased the ASR in the re-exposed (4R) rats ($F(1\text{-}14)=5.26$, $p=0.03$). This opposite effect led to a significant difference between the SPS NR and SPS 4R groups ($F(1\text{-}14)=13.68$, $p=0.002$).

Experiment 1. *indicates that four re-exposures to the predictive box tended to increase anxiety and significantly altered arousal in SPS traumatized rats.*

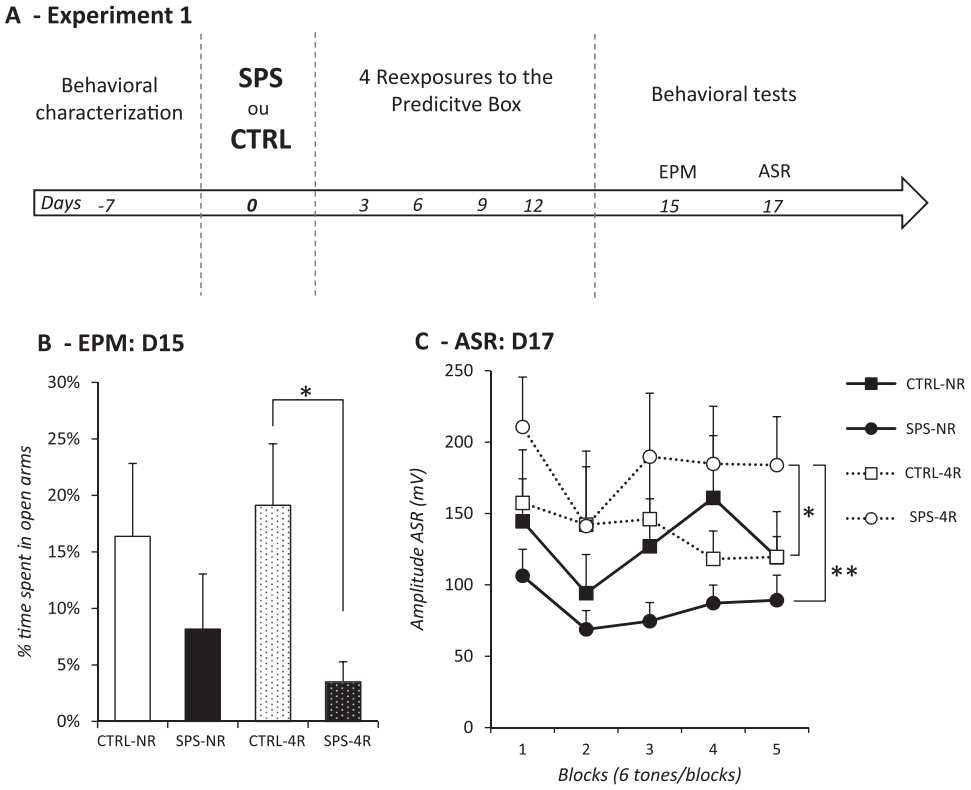

Fig. 1. Experiment 1**:** Behavioral performance of CTRL and SPS groups re-exposed (4R) four times to the predictive box (PB), or not re-exposed (NR). **A) Timeline of general protocol:** after a behavioral characterization (D-7), rats received a Single Prolonged Stress (SPS) at Day 0, or a control procedure (CTRL). From D3 to 12, rats were re-exposed or not to the predictive box. PTSD-like symptoms tests were investigated with an Elevated Plus Maze (EPM), and an Acoustic Startle Response (ASR) tests. **B) EPM, performed at Day 15:** percentage of time spent in open arms over a 5-min period. **C) ASR performed at Day 17:** mean startle amplitude (mV) obtained during the five blocks of six 115 dB tones. Data are expressed as mean ± standard error of the mean (SEM). *.05 > p > 0.01; **0.01 > p > .001; ***p < .001.