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LONG-LASTING MEMORY ABNORMALITIES FOLLOWING EXPOSURE TO THE MOUSE DEFENSE TEST BATTERY

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Introduction

- Dysfunctional memory processes are thought to play a crucial role in the development and maintenance of post traumatic stress disorder (PTSD). Patients persistently re-experience the traumatic event particularly on exposure to trauma-related cues and display disturbances of concentration and memory which are evident when they process neutral, non traumatic information [1]. In addition, clinical studies report differences in the way individuals with and without PTSD process and learn in the presence of trauma-related distractors in comparison to a neutral environment resulting in enhanced learning of trauma related as compared to neutral information in PTSD patients [2, 3].
- In this context, the objective of the present study was to investigate the potential long-term effects of predatory stress exposure on behavior and on short-term episodic memory in mice by evaluating their cognitive performance following or not trauma context exposure.
- The mouse defense test battery (MDTB) was used as stressful situation. This test has been designed to investigate defensive responses of mice confronted with a predator, i.e. a rat. It has the advantage of providing a precise delineation of defensive behaviors, including flight, avoidance, risk assessment, sonic vocalization, defensive threat and attack, and escape attempts. [4].

Methods

ANIMALS

- Experiments were conducted in male Swiss mice purchased from Charles River (France). They were maintained under a 12:12 Light/Dark cycle (light on at 7:00 a.m.), housed individually and used for experiments during the light period, in accordance with the "Guide and Care and Use of Laboratory Animals" (National Institute of Health) and were approved by the in-house Animal Ethics Committee.
- Male Long-Evans rats, weighing 500-600g at time of testing, were used as threat stimulus in the MDTB. They were killed by CO_2 inhalation 10 min prior to the test session began in order to minimize their discomfort.

STRESS PROCEDURE

The test was performed in an oval runway. It involves a number of phases:

- **Three-minute pre/post test.** Exploration and escape attempts during the pre-rat period provided baseline activity data. The same measures, during the post-test period were compared to the pre-test period to provide an index of enhance contextual defense following rat exposure.
- **Rat avoidance test.** A dead rat is introduced at the opposite end of the apparatus and brought up to the subject at a speed of approximately 0.5 m/s. If the mouse flees, avoidance distance is recorded. This is repeated five times.
- **Chase/flight test.** The hand-held euthanized rat is brought up to the subject at a speed of approximately 2 m/s during 2 complete laps of the runway or 2 min. Stops, reversals and head orientations of the fleeing mouse were used as measures of risk assessment.
- **Straight alley test.** The runway is then converted to a straight alley of 80 cm long. The rat is placed at one end while the mouse is at the other. Measures are taken for 30 s and include freezing and the number of approaches/withdrawals (measures of risk assessment).
- **Forced contact test.** The rat is brought up to contact the subject fives times. Bites, vocalisations upright postures and jump attacks by the mouse are recorded and used as measures of defensive aggression.

CONTEXT RE-EXPOSURE

- Two weeks after runway exposure, stressed animals and naive control mice were placed in the MDTB apparatus for 3 minutes. Locomotion and escape attempts from the runway were measured. These data were compared to the pre- and post-test measures obtained 2 weeks earlier.

OBJECT AVOIDANCE TASK IN MICE

- The test was performed at day 14 and 15 following stress and began 2 hours after animals have been re-exposed to the MDTB. During the first session, mice were allowed to become familiar with the experimental environment for 7 minutes. Time spent in activity was measured. Twenty-four hours later, mice were again placed in the enclosure in the presence of two identical objects until they had explored them for 10 s. After a forgetting interval of 60 min, mice were placed again in the enclosure with a previously presented object and a new object for 5 min. Time spent exploring the familiar and the new objects were recorded. Under a short-term forgetting delay, during the recall session, normal mice spent more time exploring the new object compared to the familiar one, reflect a remembering of the familiar object. Animals displaying impaired recall performance spent the same amount of time exploring both objects, reflecting a forgetting of the familiar object (short-term visual memory deficit).

References

[1] Diagnostic and Statistical Manual of Mental Disorders, 2000, 4th edition, TR, American Psychiatric Association.

[2] Morey RA et al. 2009, Journal of traumatic stress 12:309-326.

[3] Chemtob CM et al. 1999, J Trauma Stress. Apr;12(2):309-26.

[4] Griebel G et al. 1995, Neuropharmacology. 12:1625-1623..

Results

Figure 1 Experimental timelines

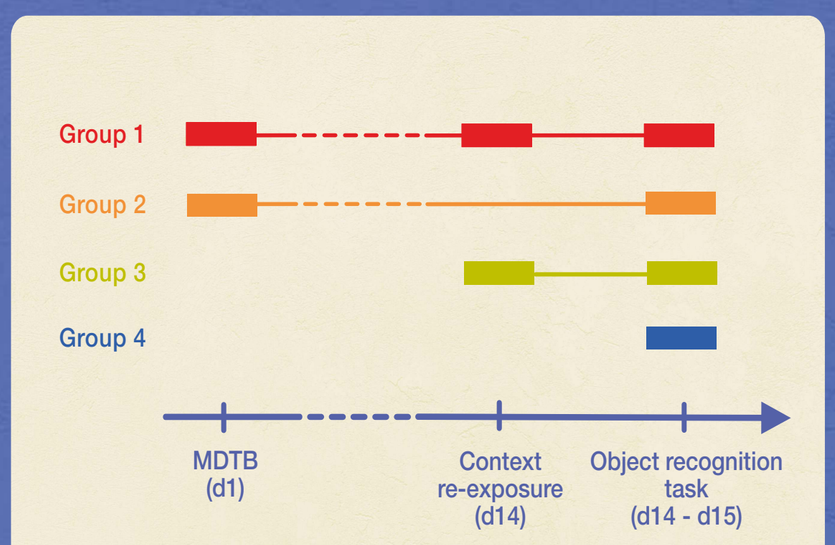
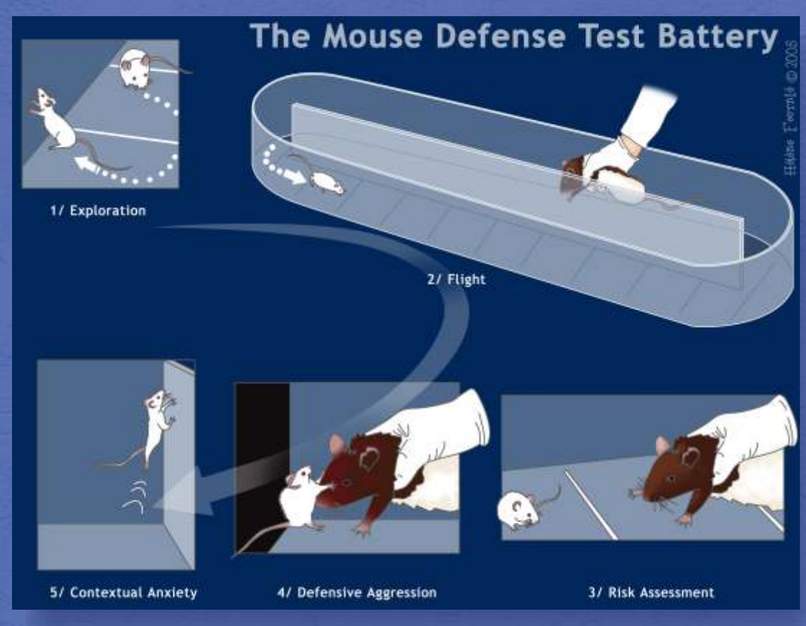
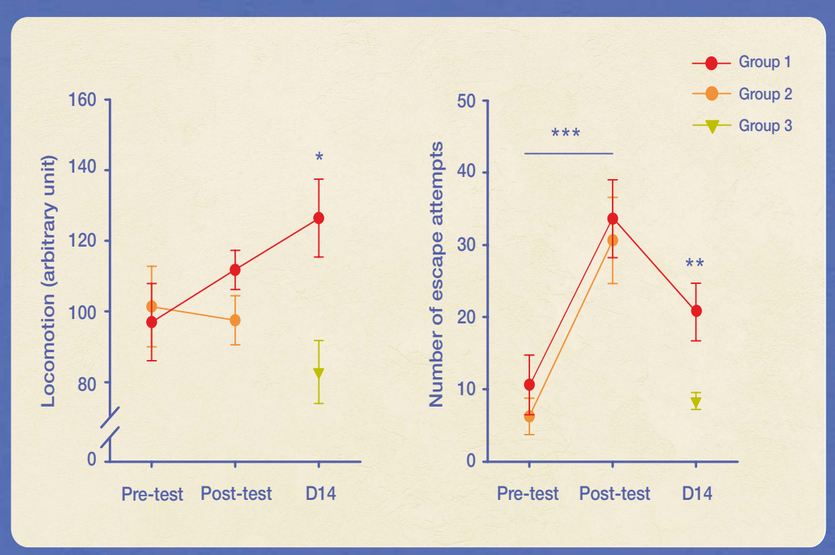


Figure 2 The Mouse Defense Test Battery procedure



BEHAVIORAL EFFECTS OF CONTEXT RE-EXPOSURE

Figure 3 Comparison of behaviors between the pre-test, the post-test and 14 days following predatory stress exposure.



- **Mice exposed to the MDTB displayed increased escape attempts from the apparatus following the removal of the rat.**
- **When mice were exposed again to the MDTB apparatus two weeks later, they displayed significantly more horizontal ambulation and escape attempts than naive animals.**

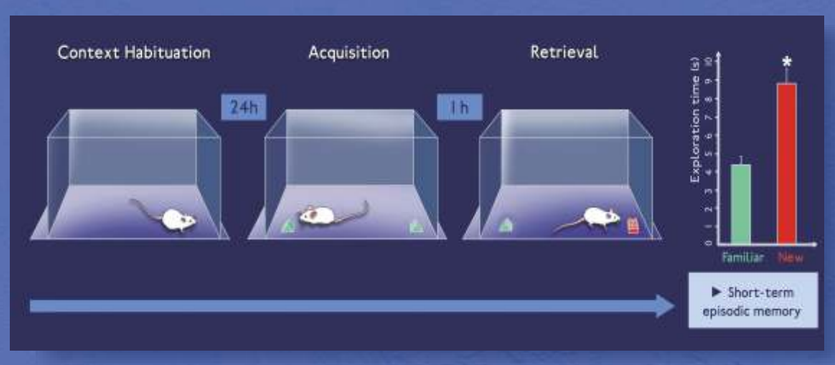
* $p \leq 0.05$ vs control group (one-way ANOVA)

** $p \leq 0.01$ vs control group (one-way ANOVA)

*** $p \leq 0.001$ between pre- and post-tests (two-way ANOVA with repeated measures on factor "mouse")

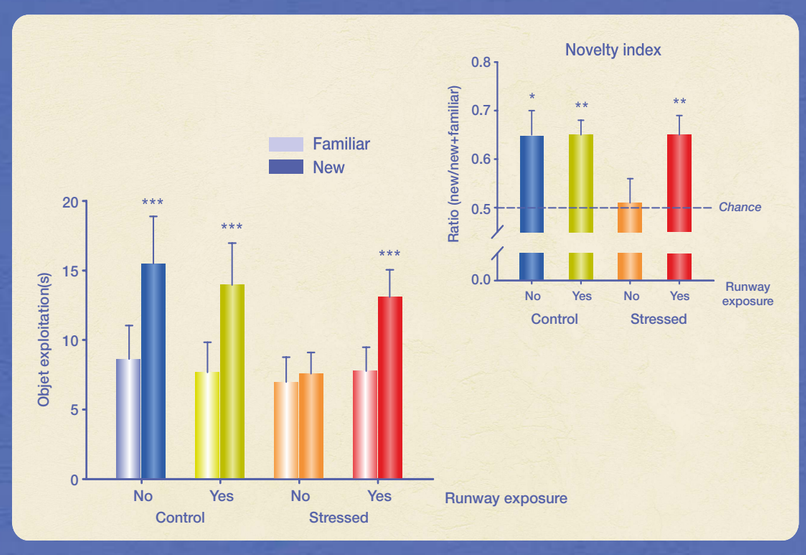
OBJECT RECOGNITION TASK

Figure 4 Experimental procedure



- **Short-term episodic memory was impaired following rat exposure when measured 15 days after stress.**
- **Exposure to the context alone before the object recognition task did not alter memory performance.**
- **Mice exposed to the context in which they had encountered the rat two weeks before, displayed normal memory performance.**

Figure 5 Effect of re-exposure to trauma context on long lasting short-term episodic memory impairment induced by rat exposure in mice.



Bars represent means (± s.e.m.) of time spent exploring new or familiar objects.

***p < 0.001, new versus familiar object. Two-way ANOVA with repeated measures on factor « object » (n = 7-8).

Insert graph: bars represent means (± s.e.m.) of Novelty Ratio. *p < 0.05, **p < 0.01 vs 0.5.

Conclusion

- These findings confirm that exposure to the MDTB induces behavioral alterations reminiscent of increased anxiety.
- Re-exposure to the stress context shows that the increase of escape attempts from the runway, i.e. an area in which a danger has been previously encountered, persists two weeks later.
- The deficit in cognitive performance after stress exposure can be alleviated by prior exposure to the traumatic environment in the absence of stressor. It can be hypothesized that re-exposure to the context resulted in an increase of arousal, vigilance and/or attention, which subsequently led to an improvement in cognitive performance.

- **Taken together, this procedure reproduces some of the symptoms observed in patients suffering from PTSD and may thus be of interest for further studies on the complex interaction between emotion and memory and the mechanisms underlying memory abnormalities in PTSD.**

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No potential conflict of interest