Neurobiology of Stress 5 (2016) 1–7

Contents lists available at ScienceDirect

Neurobiology of Stress

journal homepage: http://www.journals.elsevier.com/neurobiology-of-stress/

# Emergence in extinction of enhanced and persistent responding to ambiguous aversive cues is associated with high MAOA activity in the prelimbic cortex

Guillaume L. Poirier*, Natsuko Hitora-Imamura [1], Carmen Sandi

*Laboratory of Behavioral Genetics, Brain Mind Institute, École Polytechnique Fédérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland*

ARTICLE INFO

*Article history:*
Received 18 May 2016
Received in revised form
11 August 2016
Accepted 30 August 2016
Available online 31 August 2016

*Keywords:*
Adversity
Stress
Vulnerability
Resilience
Personality
Post-traumatic stress disorder

ABSTRACT

There is a great deal of individual variability in the emotional outcomes of potentially traumatic events, and the underlying mechanisms are only beginning to be understood. In order to further our understanding of individual trajectories to trauma, its vulnerability and resilience, we adapted a model of fear expression to ambiguous vs perfect cues in adult male rats, and examined long-term fear extinction, 2, 3, and 50 days from acquisition. After the final conditioned fear test, mitochondrial enzyme monoamine oxidase A (MAOA) function was examined. In order to identify associations between this function and behavioral expression, an *a posteri* median segregation approach was adopted, and animals were classified as high or low responding according to level of freezing to the ambiguous cue at remote testing, long after the initial extinction. Those individuals characterized by their higher response showed a freezing pattern that persisted from their previous extinction sessions, in spite of their acquisition levels being equivalent to the low-freezing group. Furthermore, unlike more adaptive individuals, freezing levels of high-freezing animals even increased at initial extinction, to almost double their acquisition session levels. Controlling for perfect cue response at remote extinction, greater ambiguous threat cue response was associated with enhanced prelimbic cortex MAOA functional activity. These findings underscore MAOA as a potential target for the development of interventions to mitigate the impact of traumatic experiences.



## 1. Introduction

Much progress has been made in our understanding of emotional trauma. Key neural substrates of fear, from acquisition to its recall, have been delineated (e.g. reviews by Mahan and Ressler, 2012; Milad and Quirk, 2012; Holmes and Singewald, 2013). In parallel, there is substantial evidence that not all those experiencing a potentially traumatic experience develop psychological trauma (e.g. Werner, 1989; Norris, 1992; Galatzer-Levy et al., 2012), and that when so its development is not uniform (Bonanno and Mancini, 2012). The lifetime incidence of post-traumatic stress syndrome varies between groups, and in the general population estimates approximate 6–12% in the U.S. (Breslau et al., 1991, 1998; Resnick et al., 1993; Breslau et al., 1998; Kessler et al., 2005; O'Donnell et al., 2014), ranging between 1 and 9% in other countries (Atwoli et al., 2015)—with debilitating consequences a public health issue with costly ramifications. Much work is still needed for broadly successful or even personalized interventions.

Potential routes to success in mitigating trauma vulnerability and enhancing recovery from trauma may be uncovered by the identification and characterization of differentially responding groups of individuals (e.g. Bush et al., 2007; Galatzer-Levy et al., 2013; Shumake et al., 2014), and the identification of associated physiological correlates. In order to identify potentially relevant associations, an *a posteri* segregation approach that stratifies individuals according to their sustained maladaptive fear responses is warranted (reviewed in Steimer, 2011; Pawlak et al., 2012; Desmedt et al., 2015). Importantly, traumatic memories frequently involve exaggerated responses not only to perfect signals or predictors (i.e., conditioned stimuli), but also to partially contingent cues (Lissek et al., 2006; Nader and Balleine, 2007; Beckers et al., 2013). Yet

* Corresponding author. Current address: Center for Comparative NeuroImaging, Department of Psychiatry, University of Massachusetts Medical School, Worcester, MA, USA.
*E-mail address:* guillaume.poirier@umassmed.edu (G.L. Poirier).
[1] Current address: Department of Pharmacology, Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo, Japan.

http://dx.doi.org/10.1016/j.ynstr.2016.08.005

another important consideration may be one of time, for those with PTSD are distinguished by poorer extinction over time, not necessarily greater acquisition, and early treatment is more effective than later attempts (e.g. reviewed in Rothbaum and Davis, 2003).

In the present study, in order to help further our understanding of post-traumatic stress disorder (PTSD) processes, particularly those leading to persistent responding, we studied the maintenance of fear conditioned responses. We sought to distinguish adaptive from maladaptive fear responses (Desmedt et al., 2015) by applying a rodent model of fear expression to fully and partially predicting cues (Tsetsenis et al., 2007). Specifically, we were particularly interested in the extinction of the ambiguous cue; i.e., the partial predictor cue that at training either was presented before the perfect one, whose presentation always co-terminated with a footshock, or alone and not followed by footshock. Thus, we considered individual differences in the remote expression of fear conditioning, a time frame relatively uncommonly studied in the animal literature (e.g. Siegmund and Wotjak, 2007; Monfils et al., 2009; Pamplona et al., 2011), yet critical given the DSM-5 diagnostic criterion of symptom persistence for over a month, combined with aforementioned greater challenge for delayed interventions.

Furthermore, we investigated the possible association between differences in long-term responses to conditioned ambiguous cues and expression levels of monoamine oxidase A (MAOA) in relevant brain regions. The rationale for this study was based on several observations. Mice selectively bred for high fear conditioning were shown to display abnormal developmental expression of mitochondrial genes, including MAO, in the prefrontal cortex (Choi et al., 2012). Conversely, genetic deletion studies revealed that MAO-A or -A/B deficient mice present amplified and less specific fear acquisition, while displaying normal spatial memory and motor abilities (Kim et al., 1997; Singh et al., 2013). In humans, studies of genetic variability of MAOA has revealed association with personality patterns (Shiraishi et al., 2006; Tsuchimine et al., 2008). Notably, MAOA-uVNTR polymorphisms have been related to high self-reported harm avoidance trait (Yu et al., 2005; Buckholtz et al., 2007). Furthermore, individuals with lower platelet MAO activity were found to exhibit stronger fear conditioning (Garpenstrand et al., 2001), while stress and glucocorticoids were reported to decrease MAOA activity and binding pervasively in the human brain (Soliman et al., 2012). In the present study, MAOA enzymatic activity was evaluated after a long-term conditioned fear test in the amygdala, hippocampus, infralimbic, prelimbic, and anterior cingulate cortex, as these are some of the major brain regions implicated in the expression and extinction of fear (McNally et al., 2011; Sierra-Mercado et al., 2011; Fani et al., 2012; Maroun, 2012; Parsons and Ressler, 2013; Hitora-Imamura et al., 2015), in addition to their recruitment in responding to ambiguity conferred by unpredictability and uncertainty (e.g. Huettel et al., 2005; Herry et al., 2007; Tsetsenis et al., 2007; Rushworth and Behrens, 2008; Sarinopoulos et al., 2010). The present study examined when persistent responding to a no longer threatening cue emerges, and whether it is associated with brain MAOA activity. On the basis of the MAO knockout mouse data, we hypothesized that poorer long-term fear extinction (i.e. greater persistence of freezing) would be associated with lower MAOA in these brain regions of interest.

## 2. Materials and methods

### 2.1. Subjects

The experimental subjects were the offspring (n = 16) of Wistar Han rats (Charles River Laboratories, L'Arbresle, France), bred in our animal house. At weaning, male rats from different litters were mixed and housed three per standard plastic cage on a 12 h light−dark cycle (lights on at 0700 h). Fear conditioning procedures were initiated in adult rats (postnatal day ≥115). Food and water were available *ad libitum*. All procedures were conducted in conformity with the Swiss National Institutional Guidelines on Animal Experimentation and approved by a license from the Swiss Cantonal Veterinary Office Committee for Animal Experimentation.

### 2.2. Behavioral testing

Associative learning of cue and aversive footshock was conducted according to the experimental design illustrated in Fig. 1, with an acquisition stage followed by three extinction tests. In order to examine individual response variability in freezing responses to shock conditioning, and the sensitivity to conditioned cue accuracy, i.e. the ability to discriminate between good and poor signals, a fear conditioning protocol comprising within-subjects both a perfect and a partially predictive shock cue (i.e. an ambiguous cue) (Tsetsenis et al., 2007) was adapted and extended to test individual variability in fear acquisition (Day 1, extinction, as well as incubation (respectively Days 2, 3, and 50). Training and testing took place in a Panlab (Spain) apparatus, comprising a (30 × 37 × 25 cm) chamber equipped to deliver a scrambled foot shock via the 20 rods (3-mm diameter) composing the floor. Each chamber was cleaned with 5% ethanol and dried thoroughly between each test. On testing days, rats were transported from the colony room to the adjacent behavioral laboratory in their cage on a transport rack, before being placed in conditioning chamber.

Fear Conditioning: The training/acquisition session lasted for 20 min, with 180s habituation, followed by the presentation of two cues. The first cue, perfectly contingent (i.e. unambiguous), was presented three times for 30 s at 210, 600 and 990s, co-terminating with a 0.6 mA, 1s foot shock. A partially contingent cue was presented five times for 30s, co-terminating thrice with the first cue onset and twice alone at 390 and 780 s. The latter cue provided ambiguity in the likelihood of shock co-occurrence, i.e. probabilistic uncertainty. The ambiguous and unambiguous cues were either a light presentation (28 V DC, 100 mA) or a tone (3 kHz, 85 dB), counterbalanced for among individuals within each group. Ventilation fans provided background noise of 68 dB, large shelving unit was apparent in one corner of room, lit by green ambient lighting, and acetic acid was wiped onto the apparatus. Fear extinction: Three extinction tests were carried out: on days 2, 3, and 50 from fear acquisition, respectively for memory/extinction (Extinction I), extinction recall (Extinction II), and long-term (remote) extinction after a fear incubation period (adapted from Garcia et al., 2006; Monfils et al., 2009; Dębiec et al., 2011; Toth et al., 2012). In order to minimize contextual conditioning responses during fear extinction, extinction recall, and remote recall, these test phases were carried out with different visual, olfactory and tactile cues (i.e., in chamber an insert with smooth gray plastic floor and, perforated metal walls, along with lemon rather than acetic acid odor; distal cues consisting of shelving unit moved across room to opposite corner; white lighting). The rats were placed in the same chambers, but in this novel context. A 3 min baseline preceded stimulus presentation. For extinction I, cues were presented each for 6 min, in a counterbalanced order. Each of the subsequent test sessions (Extinction II and Remote extinction) lasted 27 min. The cues were presented as four blocks each comprising five 30 s presentations of one cue separated by 5 s intervals, followed by five 30 s presentations of the second cue separated by 5 s intervals (for each cue a block thus lasting 2 min 55 s), in the absence of any foot shock throughout the entire session. The order of presentation of each cue was counterbalanced within each group. Sessions were video recorded and time spent freezing was quantified and