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

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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

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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

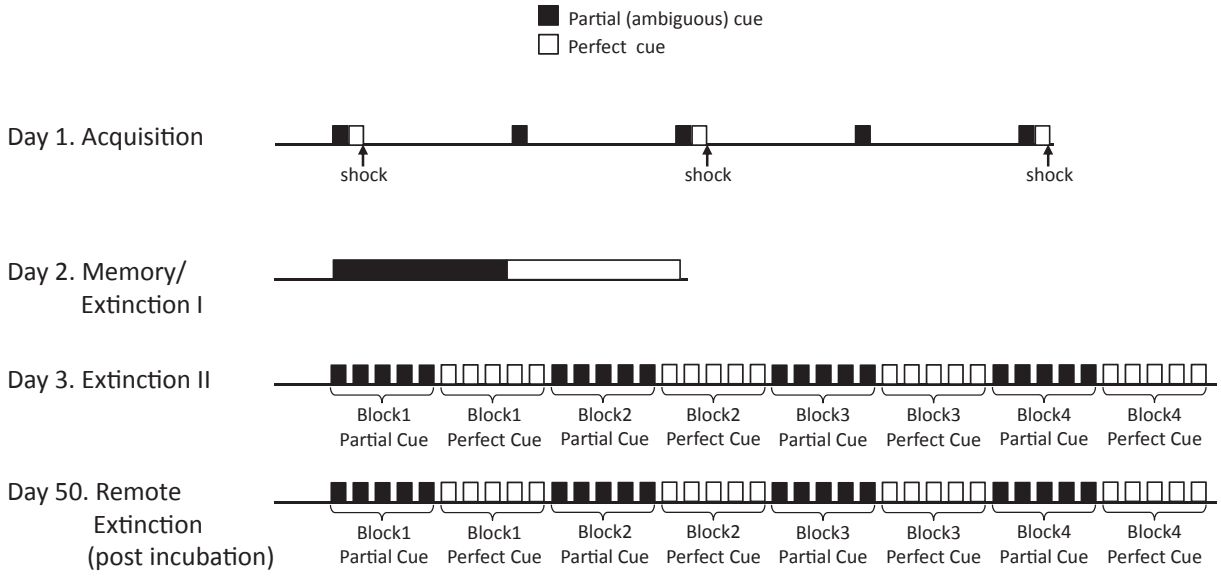

Fig. 1. Stimulus presentation scheme. Rats learn the association between cues (either tone or light, counterbalanced) and footshocks on Day 1, conditioning. A perfectly contingent cue (white) predicts shocks more reliably than a partially contingent, i.e. ambiguous cue (black). On days 2, 3, and 50, rats are re-exposed to both cues, without footshocks.

percentage of freezing was calculated separately for each cue [freezing (s) during cue/cue duration (s)].

For the behavioral tests above, freezing was manually scored blind the experimental conditions with the aid of a computer program (software developed internally by Velibor Ilić (Ilić, 2005)), during stimulus presentations, inter-trial intervals, and prior to the first cue to study generalization to a novel context).

2.3. Monoamine oxidase a assay

Thirty minutes after the end of the fear incubation test session, the subjects were sacrificed, brains were extracted and frozen in isopentane on dry ice for storage at −80 °C until further processing. In order to evaluate regional monoamine oxidase A activity, using a previously published protocol (Poirier et al., 2014), tissue from separate regions was obtained by selectively punching samples of prelimbic (PL), infralimbic (IL), septal (dorsal) hippocampal, temporal (ventral) hippocampal, central amygdala (CeA), and basolateral amygdala (BLA; Harris Uni-Core, Ted Pella, Redding, California, US) from 50 to 200 μm slices obtained on a cryostat (Leica CM3050S) covering the extent of each subregion according to atlas landmarks (Paxinos and Watson, 1997).

Mitochondrial, cytoplasmic and nuclei-enriched fractions were obtained using a differential centrifugation protocol. Sample lysates were obtained using a Teflon pestle in ice-cold IM homogenization buffer 20 mM Tris HCl, 10 mM KOAc, 1 mM EDTA, 0.25% NP40, containing freshly added 1 mM dithiothreitol (DTT) and a proteinase and phosphatase inhibitor cocktail (Complete EDTAfree, Roche Diagnostics GmbH, Rotkreuz, Switzerland). The samples were then centrifuged (20 min at 700 g, 4 °C). While the nuclei-enriched pellet was held back, the supernatant was collected and centrifuged again (15 min at 10 000 g, 4 °C). The supernatant and the pellet obtained were cytoplasmic- and mitochondrial-enriched fractions, respectively. The mitochondrial pellet was resuspended (75 mM mannitol, 25 mM sucrose, 5 mM KH_2PO_4, 20 mM Tris HCl, 0.5 mM EDTA, 100 mM KCl, 0.1% bovine serum albumen; MSK buffer) and used fresh.

Protein in the sample lysates was quantified using the detergent-compatible Bio-Rad DC protein assay (Bio-Rad Laboratories AG, Reinach, Switzerland). Protein samples were prepared in order to obtain equal concentrations by H_2O dilution. The average amount of protein in the mitochondrial fraction obtained for the prelimbic cortex (PL), infralimbic cortex (IL), anterior cingulate cortex (ACC), septal hippocampal, temporal hippocampal, central amygdala, and basolateral amygdala punches was respectively 40.0 (±0.8), 37.0 (±1.0), 64.1 (±5.0), 57.4 (±3.5), 65.2 (±9.6), 43.1 (±10.2) μg.

MAOA activity was evaluated in 5 μg of mitochondrial protein extracts (volume adjusted for concentration) using the luminescent MAOGlo Kit (Promega #V1401, Dübendorf, Switzerland), according to the manufacturer's instructions. Samples were processed in duplicates, and after a 30 min incubation with the luciferin detection reagent in a white opaque-wall small volume Greiner plate (HuberLab, Aesch, Switzerland), the luminescent signal was detected using the Infinite F500 detection platform managed with iControl 1.7.1.12 (Tecan, Männedorf, Switzerland), integrated over 200 ms. The sensitivity of the assay was confirmed using MAO A and B inhibitors, respectively clorgyline and deprenyl (Sigma-Aldrich, data not shown). A standard curve was produced, representing luminescence according to amount of MAOA enzyme (Promega #V1452). For each sample the luminescence value was converted to an equivalent MAOA quantity, obtained by interpolation in the linear range of the standard curve of activity.

2.4. Statistical analyses

Statistical analyses were performed with Statistics Package for the Social Sciences (SPSS 17.0; Zürich, Switzerland). Freezing to the ambiguous cue was analyzed using a mixed-design Analysis of Variance, with Stage as within-subject measure [acquisition, extinction I, extinction II (recall), and remote testing (*post* incubation)], and Remote freezing vulnerability (ambiguous cue freezing at remote test; low vs high median split). The assumption of sphericity was confirmed (Mauchly's test), a Sidak adjustment was applied for follow-up simple comparisons. Neural measures were examined with sub-group comparisons. For freezing levels, Tone and Light counterbalanced subgroups were analyzed indiscriminately as no main effect of Cue Modality (Tone or Light) was seen for freezing levels either at acquisition (Cue Modality and Cue Modality*Contingency, both $F < 1$) or remote extinction (Stimulus,

F < 1; and Stimulus*Contingency, both F1,14 = 3.9, p = 0.069).

Brain region MAO-A function was evaluated using a mixed-design analysis of variance on standardized values (Z-scores), with remote ambiguous freezing level as between-subjects factor and regions of interest as within-subjects factors. Remote perfect cue response was used as a covariate in order to help isolate effects distinct from the contribution of that specific cue, and thus potentially uncover distinct mechanisms. Separate analyses were conducted for prefrontal cortex regions (PL, IL, ACC), hippocampal segments (septal and temporal), and amygdala nuclei (basolateral and central). The results were considered statistically significant if $p < 0.05$.

3. Results

3.1. Freezing response for low and high responders to ambiguous cue at remote testing

Cue Contingency (perfect vs. Partial), Stage (Acquisition, Extinctions I, II, and Remote), and Group (high or low responders on the remote ambiguous cue) interacted significantly ($F_{3,42} = 5.5$, $p = 0.003$. The results of the remaining effects were as follows: main effect of Group, $F_{1,14} = 20.7$, $p < 0.001$; main effect of Stage, $F_{3,42} = 70.7$, $p < 0.001$; Stage x Cue, $F_{3,42} = 5.4$, $p = 0.003$; all other effects, $p > 0.07$). As shown in Fig. 2A (left), for the ambiguous cue, a difference in freezing emerged with extinction trials (Stage x Remote response Group, $F_{3,42} = 6.3$, $p = 0.001$; main effect of Stage, $F_{3,42} = 43.5$, $p < 0.001$; main effect of Remote response, $F_{1,14} = 24.7$, $p < 0.001$). A within-subjects contrasts analysis revealed that a quadratic progression across stages was different between the Groups ($F_{1,14} = 8.7$, $p = 0.011$). This observation was supported by follow-up simple effects analyses determining that for the high responders, freezing increased at the first extinction test (compared to acquisition, $p < 0.001$), whereas for the low responders, freezing levels between these two stages were not different; $p > 0.9$). Furthermore, while the Groups exhibited equivalent freezing at Acquisition ($p > 0.8$), high responders were elevated at all extinction stages (Extinction I, II, and Remote Extinction respectively $p = 0.001$, 0.011, and <0.001). In contrast, as seen in Fig. 2A (right) for the perfect cue there was a only main effect of Group ($F_{1,14} = 10.6$, $p = 0.006$. While there was also a main effect of Stage ($F_{3,42} = 48.7$, $p < 0.001$), no interaction was apparent ($F_{3,42} = 1.1$, $p > 0.38$).

In order to better understand the enhanced response pattern for the High responders, the longer protocols used in Extinction II and Remote Extinction were analysed by presentation block (Supplementary Fig. 1). At Extinction II, for the ambiguous cue in addition to the expected Group effect ($F_{1,14} = 8.6$, $p = 0.011$), there is an effect of presentation Block ($F_{3,42} = 9.4$, $p < 0.001$) and interaction achieved significance (Block*Group ($F_{3,42} = 3.1$, $p = 0.036$). Follow-up analyses revealed a significant augmentation of freezing for the High responders only in Block 1 ($p = 0.002$; other p all > 0.17). In contrast, for the perfect cue, although again the expected Group effect ($F_{1,14} = 4.7$, $p = 0.048$) and effect of presentation Block were present ($F_{3,42} = 14.8$, $p < 0.001$), but in this case the interaction did not achieve significance (Block*Group (F < 1).

At the remote extinction (data not shown), for the ambiguous cue in addition to the expected Group effect (F1,14 = 32.4, p < 0.001), there is an effect of presentation Block (F3,42 = 3.1, p = 0.037) but interaction did not achieve significance (Block*-Group (F3,42 = 2.4, p = 0.080). For the perfect cue, in addition to the expected Group effect (F1,14 = 17.1, p = 0.001), there is an effect of presentation Block (F3,42 = 3.6, p = 0.020) but interaction did not achieve significance (Block*Group (F < 1).

The enhanced threat response was also accompanied by a generalization of freezing to the different context at baseline, as seen in Fig. 2B (Remote response Group, $F_{1,14} = 11.4$, $p = 0.004$; Stage, $F_{3,42} = 33.6$, $p < 0.001$; Stage x Remote response Group,

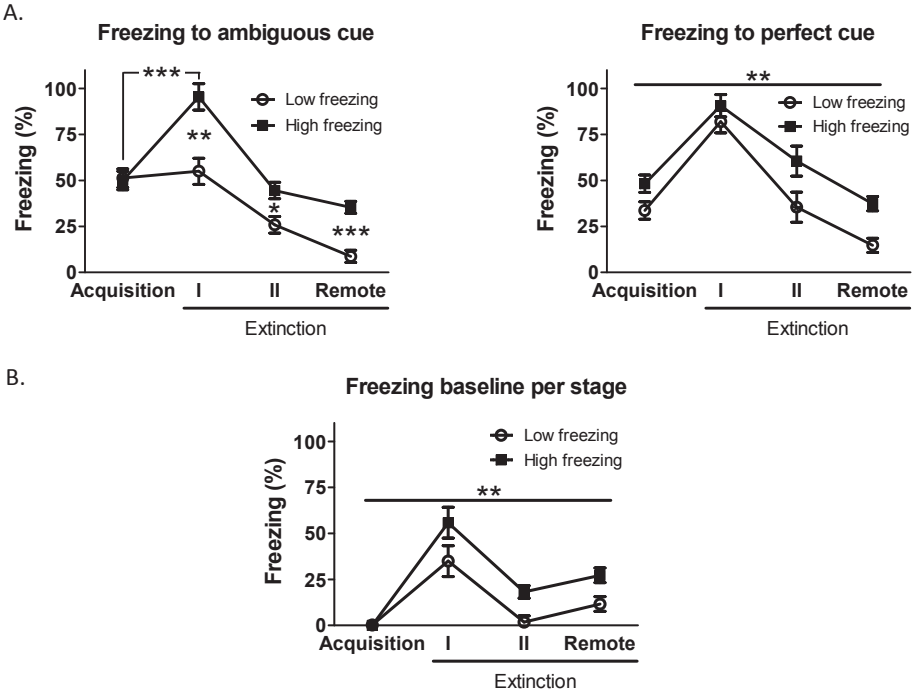

Fig. 2. Perfect and ambiguous cue freezing. The response to the cues is presented according to the Groups based on remote ambiguous cue reactivity. Vulnerable individuals present a response to the ambiguous cue that resembles that to the perfect cue. A. (Left) High responders only exhibit more freezing to the ambiguous cue in extinction training, and especially at Extinction I. (Right) High responders also exhibit more freezing to the perfect cue overall. B. Baseline freezing per stage according to remote response to ambiguous cue. Individuals expressing enhanced remote response to the ambiguous cue also freeze more prior to the cue presentations. *, $p < 0.05$. **, $p < 0.01$. ***, $p < 0.001$.

$F_{3,42} = 1.9$, $p = 0.15$). Likewise, as presented in Supplemental Fig. 2, enhanced freezing for high responders was observed during the ITIs for both the partial [$F_{1,14} = 20.8$, $p < 0.001$; effects of Time and any interaction with Group all $p > 0.26$) and the perfect cue [$F_{1,14} = 10.6$, $p = 0.006$; effects of Time and any interaction with Group all $p > 0.13$).

3.2. MAOA prefrontal cortex activity distinguishes low and high responders to ambiguous cue at remote testing

Analyses of MAOA activity (Fig. 3) revealed that when controlling for remote perfect cue response, the impact of ambiguous Group level differed according to prefrontal cortex region (Ambiguous Group x Region, $F_{2,26} = 5.4$, $p = 0.011$; Region, $F_{2,26} = 2.7$, $p = 0.085$; Region x Perfect Group, $F_{2,26} = 2.9$, $p = 0.076$; Ambiguous Group, $F_{1,13} = 0.2$, $p = 0.656$). Follow-up analyses revealed that higher prelimbic cortex MAOA activity was the only significant regional effect (PL $p = 0.030$; IL $p > 0.8$; ACC $p > 0.18$. No differences were apparent for the other regions of interest [main effects and interactions for HPC segments (septal and temporal), all $p \geq 0.296$ and for AMY nuclei (BLA and CeA), all $p \geq 0.325$]. Values for all regions prior to covariate ANOVA are shown in Suppl. Fig. 3. Considering instead a correlative approach with all subjects, no correlations were apparent for total freezing % for the ambiguous cue, but for the perfect cue, a correlation was observed with infralimbic cortex MAO-A ($\rho = -0.67$, $p = 0.005$).

4. Discussion

The present study examined at what stage differential vulnerability to persistent responding to a no longer threatening cue emerges, and whether it is associated with brain MAOA activity. Unlike freezing to the perfect cue, where differences between low and high responders are apparent already during acquisition, when freezing to the ambiguous cue is considered a vulnerability appeared not from acquisition of the conditioned response, but at subsequent stages—thus rather implicating processes of extinction, consolidation, and/or retrieval. In parallel, this pattern was associated with a generalization of the freezing response to the different context, prior to cue re-exposure, which could be attributable to an induced sensitivity not necessarily accompanied by fear (Siegmund and Wotjak, 2007; Pamplona et al., 2011), a "conditioned cognitive generalization" described to yet occur in the absence of hypothalamo-pituitary-adrenal axis engagement (Daviu et al., 2010, 2014). When controlling for the perfect cue response at that stage, greater freezing response to the ambiguous cue after an incubation period following repeated extinction sessions was associated with higher prelimbic cortex MAOA activity.

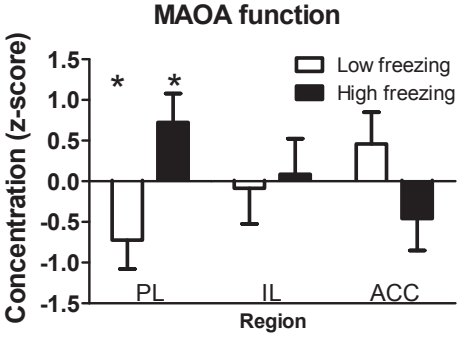

Fig. 3. MAOA function. The remote ambiguous response level interacted with brain region for MAOA activity, with prelimbic cortex the only significantly different region (controlling for the remote perfect cue response). *, $p < 0.05$. PL, prelimbic cortex; IL, infralimbic cortex; ACC, anterior cingulate cortex.

Previous studies with MAOA/B knockout mice using perfect predictors found heightened fear conditioning (Kim et al., 1997; Singh et al., 2013). In parallel, previous fear cue ambiguity research considering extinction of recent conditioning has shown that serotonin transporter (5HTT)-overexpressing mice presented less freezing to uncertain outcome (20% cue contingency, McHugh et al., 2015) and conversely Htr1a knockout mice exhibited selectively more freezing to ambiguous cues (Klemenhagen et al., 2006), and like here to partially contingent cues, but no difference to perfectly contingent fear cue conditioning (Tsetsenis et al., 2007). Extending these findings, our results supporting a qualitative difference between perfect and partial (ambiguous) threat cue contingencies, with further work disentangling the molecular mechanisms undoubtedly important for mechanistic understanding and intervention purposes.

We also found that individuals exhibiting higher levels of freezing to this ambiguous cue after the incubation of the aversive experience presented equivalent acquisition. It is possible that the stimulus presentation scheme may have affected the predictability of both cues, in terms of probabilistic and second-order uncertainty, respectively for the ambiguous and the unambiguous cues. This notion is supported by the observation that the freezing levels do not appear different between the cues during acquisition. Yet, at the Memory/Extinction I session, the High responders expressed almost twice the levels of freezing shown at initial acquisition, levels that while decreasing remained above those of more resilient adaptive individuals in subsequent extinction sessions. For the perfect cue, freezing levels at Memory/Extinction I also considerably increased—while at that stage group differences numerically exhibited the weakest difference, even though here no significant interaction was observed. Regarding the ambiguous cue response pattern, a parallel may be drawn with previous fear conditioning research identifying subgroups with a bimodal distribution of total freezing in extinction but not acquisition (Galatzer-Levy et al., 2013). Together these findings may be relevant to anxiety disorders such as PTSD, where individuals may be distinguished by poorer extinction over time, not necessarily greater acquisition (e.g. reviewed in Rothbaum and Davis, 2003). Where some individuals exposed to the same conditioning experiences yet present poorer extinction (Bush et al., 2007; Galatzer-Levy et al., 2013), the present results suggest that such an individual vulnerability may be partly underpinned by a poorer ability to update environmental priors with experience (Bach, 2015). Here, specifically, probabilistic uncertainty may be the form of ambiguity for which the individuals here exhibited poorer updating.

The brain regions engaged in fear conditioning are also different in subjects with PTSD, in comparison to both typical adults and relative to other anxiety disorders (social anxiety and phobias). In a meta-analysis, only PTSD patients were found to show hypoactivity of ventral ACC, dorsal ACC, and ventromedial prefrontal cortex (vmPFC, Etkin and Wager, 2007). As for extinction recall, in typical adults the vmPFC is recruited (Milad et al., 2007), but less so in subjects with PTSD, for whom dorsal anterior cingulate cortex is also hyper-responsive (Milad et al., 2009). The rat homologs of dorsal anterior cingulate cortex and subgenual vmPFC are prelimbic and infralimbic cortex, respectively (Nieuwenhuis and Takashima, 2011; Milad and Quirk, 2012), each in turn necessary for the expression or the extinction of fear (e.g. Milad and Quirk, 2002; Vidal-Gonzalez et al., 2006; Laurent and Westbrook, 2009; Sierra-Mercado et al., 2011). Here we found MAOA differences in prelimbic cortex, but none in either infralimbic or anterior cingulate cortex (nor the hippocampus or amygdala), for either the typical, unambiguous cue ("perfect"), or the ambiguous alternative at remote testing.

The ambiguity of the associations in the present paradigm potentially stems from various sources of uncertainty, probabilistic and second-order, including likely sensory, state, and rule uncertainty, all of which can implicate the anterior cingulate cortex (Bach and Dolan, 2012). There is evidence in humans ambiguity may recruit prefrontal-parieta-striatial networks (Lopez Paniagua and Seger, 2013), and that probabilistic uncertainty may be preferable over conflicting information, each respectively associated with prefrontal and striatal network engagement (Pushkarskaya et al., 2015).

In humans, dorsal anterior cingulate cortex may be implicated in revaluation, in effortful dampening of negative emotion, as proposed as a result of meta-analytic findings (Diekhof et al., 2011). Part of the dorsal anterior cingulate, the supragenual portion mediates the link between MAOA genetic variation and temperament, including harm avoidance (Buckholtz et al., 2007). Level of trauma and MAOA gene expression (according to functional polymorphisms) were previously shown to interact, with the latter moderating behavioral outcomes according to trauma level. Reactive aggression is greater in individuals with low activity MAOA-uVNTR, in proportion to level of provocation (e.g. Kuepper et al., 2013). It has been proposed that low activity allele of MAOA may predispose to enhanced reactivity to social threat, even if ambiguous (Buckholtz and Meyer-Lindenberg, 2008). The present findings suggest that a similar relationship may exist for the response to ambiguous threat cues in non-social situations. In relation to the aforementioned “conditioned cognitive generalization”, human neuroimaging studies have suggested that with uncertainty individuals may adopt a (meta)cognitive coping state, associated with prefrontal cortex activity (Paul et al., 2015). Additionally, a relationship between intolerance to uncertainty and poorer extinction of unambiguous fear cue associated with greater amygdala and vmPFC activation was previously reported in human work (Morriss et al., 2015). The association of this temperament with ambiguous fear cues remains to be explored, with present rodent findings predicting (dorsal) anterior cingulate engagement may be implicated in humans.

In summary, the partially contingent cues in fear conditioning represent an uncertain threat situation. Even after repeated extinction tests, in some vulnerable individuals a threat response may be heightened and persistent. This behavioral expression typical of individuals with an anxiety disorder may be linked with alterations in MAOA function in the human homolog of the rodent prelimbic cortex (i.e., dorsal anterior cingulate cortex). The present study may thus help bridge the findings of functional neuroimaging, genetic, and neurochemistry studies, providing useful routes of investigation for the development of interventions to mitigate the impact of traumatic experiences.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ynstr.2016.08.005.

References

Atwoli, L., Stein, D.J., Koenen, K.C., McLaughlin, K.A., 2015. Epidemiology of posttraumatic stress disorder: prevalence, correlates and consequences. Curr. Opin. psychiatry 28, 307–311.
Bach, D.R., 2015. Anxiety-like behavioural inhibition is normative under environmental threat-reward correlations. PLoS Comput. Biol. 11 e1004646.
Bach, D.R., Dolan, R.J., 2012. Knowing how much you don't know: a neural organization of uncertainty estimates. Nat. Rev. Neurosci. 13, 572–586.
Beckers, T., Krypotos, A.-M., Boddez, Y., Effting, M., Kindt, M., 2013. What's wrong with fear conditioning? Biol. Psychol. 92, 90–96.
Bonanno, G.A., Mancini, A.D., 2012. Beyond resilience and PTSD: mapping the heterogeneity of responses to potential trauma. Psychological Trauma: theory, Research. Pract. Policy 4, 74.
Breslau, N., Davis, G.C., Andreski, P., Peterson, E., 1991. Traumatic events and posttraumatic stress disorder in an urban population of young adults. Arch. Gen. Psychiatry 48, 216–222.
Breslau, N., Kessler, R.C., Chilcoat, H.D., Schultz, L.R., Davis, G.C., Andreski, P., 1998. Trauma and posttraumatic stress disorder in the community: the 1996 detroit area survey of trauma. Arch. Gen. Psychiatry 55, 626–632.
Buckholtz, J.W., Callicott, J.H., Kolachana, B., Hariri, A.R., Goldberg, T.E., Genderson, M., Egan, M.F., Mattay, V.S., Weinberger, D.R., Meyer-Lindenberg, A., 2007. Genetic variation in MAOA modulates ventromedial prefrontal circuitry mediating individual differences in human personality. Mol. Psychiatry 13, 313–324.
Buckholtz, J.W., Meyer-Lindenberg, A., 2008. MAOA and the neurogenetic architecture of human aggression. Trends Neurosci. 31, 120–129.
Bush, D.E., Sotres-Bayon, F., LeDoux, J.E., 2007. Individual differences in fear: isolating fear reactivity and fear recovery phenotypes. J. Trauma. stress 20, 413–422.
Choi, K.H., Le, T., McGuire, J., Coyner, J., Higgs, B.W., Diglisic, S., Johnson, L.R., Benedek, D.M., Ursano, R.J., 2012. Expression profiles of mitochondrial genes in the frontal cortex and the caudate nucleus of developing humans and mice selectively bred for high and low fear. PloS one 7 e49183.
Daviu, N., Andero, R., Armario, A., Nadal, R., 2014. Sex differences in the behavioural and hypothalamic–pituitary–adrenal response to contextual fear conditioning in rats. Hormones Behav. 66, 713–723.
Daviu, N., Fuentes, S., Nadal, R., Armario, A., 2010. A single footshock causes long-lasting hypoactivity in unknown environments that is dependent on the development of contextual fear conditioning. Neurobiol. Learn. Mem. 94, 183–190.
Dębiec, J., Bush, D.E.A., LeDoux, J.E., 2011. Noradrenergic enhancement of reconsolidation in the amygdala impairs extinction of conditioned fear in rats—a possible mechanism for the persistence of traumatic memories in PTSD. Depress. Anxiety 28, 186–193.
Desmedt, A., Marighetto, A., Piazza, P.-V., 2015. Abnormal fear memory as a model for posttraumatic stress disorder. Biol. psychiatry 78, 290–297.
Diekhof, E.K., Geier, K., Falkai, P., Gruber, O., 2011. Fear is only as deep as the mind allows: a coordinate-based meta-analysis of neuroimaging studies on the regulation of negative affect. NeuroImage 58, 275–285.
Etkin, A., Wager, T.D., 2007. Functional neuroimaging of anxiety: a meta-analysis of emotional processing in PTSD, social anxiety disorder, and specific phobia. Am. J. Psych. 164.
Fani, N., Jovanovic, T., Ely, T.D., Bradley, B., Gutman, D., Tone, E.B., Ressler, K.J., 2012. Neural correlates of attention bias to threat in post-traumatic stress disorder. Biol. Psychol. 90, 134–142.
Galatzer-Levy, I., Bonanno, G., Bush, D., LeDoux, J., 2013. Heterogeneity in threat extinction learning: substantive and methodological considerations for identifying individual difference in response to stress. Front. Behav. Neurosci. 7.
Galatzer-Levy, I.R., Burton, C.L., Bonanno, G.A., 2012. Coping flexibility, potentially traumatic life events, and resilience: a prospective study of college student adjustment. J. Soc. Clin. Psychol. 31, 542.
Garcia, R., Chang, C.H, Maren, S., 2006. Electrolytic lesions of the medial prefrontal cortex do not interfere with long-term memory of extinction of conditioned fear. Learn. Mem. 13, 14–17.
Garpenstrand, H., Annas, P., Ekblom, J., Oreland, L., Fredrikson, M., 2001. Human fear conditioning is related to dopaminergic and serotonergic biological markers. Behav. Neurosci. 115, 358.
Herry, C., Bach, D.R., Esposito, F., Di Salle, F., Perrig, W.J., Scheffler, K., Lüthi, A., Seifritz, E., 2007. Processing of temporal unpredictability in human and animal amygdala. J. Neurosci. 27, 5958–5966.
Hitora-Imamura, N., Miura, Y., Teshirogi, C., Ikegaya, Y., Matsuki, N., Nomura, H., 2015. Prefrontal dopamine regulates fear reinstatement through the down-regulation of extinction circuits. Elife 4 e08274.
Holmes, A., Singewald, N., 2013. Individual differences in recovery from traumatic fear. Trends Neurosci. 36, 23–31.
Huettel, S.A., Song, A.W., McCarthy, G., 2005. Decisions under uncertainty: probabilistic context influences activation of prefrontal and parietal cortices. J. Neurosci. 25, 3304–3311.
Ilić V. Clicker v1.13. [computer software]. Lausanne, 2005.
Kessler, R.C., Berglund, P., Demler, O., Jin, R., Merikangas, K.R., Walters, E.E., 2005. Lifetime prevalence and age-of-onset distributions of dsm-iv disorders in the national comorbidity survey replication. Arch. Gen. Psychiatry 62, 593–602.
Kim, J.J., Shih, J.C., Chen, K., Chen, L., Bao, S., Maren, S., Anagnostaras, S.G.,

Fanselow, M.S., De Maeyer, E., Seif, I., 1997. Selective enhancement of emotional, but not motor, learning in monoamine oxidase A-deficient mice. Proc. Natl. Acad. Sci. 94, 5929–5933.
Klemenhagen, K.C., Gordon, J.A., David, D.J., Hen, R., Gross, C.T., 2006. Increased fear response to contextual cues in mice lacking the 5-HT1A receptor. Neuropsychopharmacology 31, 101–111.
Kuepper, Y., Grant, P., Wielpuetz, C., Hennig, J., 2013. MAOA-uVNTR genotype predicts interindividual differences in experimental aggressiveness as a function of the degree of provocation. Behav. Brain Res. 247, 73–78.
Laurent, V., Westbrook, R.F., 2009. Inactivation of the infralimbic but not the prelimbic cortex impairs consolidation and retrieval of fear extinction. Learn. Mem. 16, 520–529.
Lissek, S., Pine, D.S., Grillon, C., 2006. The strong situation: a potential impediment to studying the psychobiology and pharmacology of anxiety disorders. Biol. Psychol. 72, 265–270.
Lopez Paniagua, D., Seger, C., 2013. Coding of level of ambiguity within neural systems mediating choice. Front. Neurosci. 7.
Mahan, A.L., Ressler, K.J., 2012. Fear conditioning, synaptic plasticity and the amygdala: implications for posttraumatic stress disorder. Trends Neurosci. 35, 24–35.
Maroun, M., 2013. Medial prefrontal cortex: multiple roles in fear and extinction. Neuroscientist 19, 370–383.
McHugh, S.B., Barkus, C., Lima, J., Glover, L.R., Sharp, T., Bannerman, D.M., 2015. SERT and uncertainty: serotonin transporter expression influences information processing biases for ambiguous aversive cues in mice. Genes Brain Behav. 14, 330–336.
McNally, G.P., Johansen, J.P., Blair, H.T., 2011. Placing prediction into the fear circuit. Trends Neurosci. 34, 283–292.
Milad, M.R., Pitman, R.K., Ellis, C.B., Gold, A.L., Shin, L.M., Lasko, N.B., Zeidan, M.A., Handwerger, K., Orr, S.P., Rauch, S.L., 2009. Neurobiological basis of failure to recall extinction memory in posttraumatic stress disorder. Biol. psychiatry 66, 1075–1082.
Milad, M.R., Quirk, G.J., 2002. Neurons in medial prefrontal cortex signal memory for fear extinction. Nature 420, 70–74.
Milad, M.R., Quirk, G.J., 2012. Fear extinction as a model for translational neuroscience: ten years of progress. Annu. Rev. Psychol. 63, 129–151.
Milad, M.R., Wright, C.I., Orr, S.P., Pitman, R.K., Quirk, G.J., Rauch, S.L., 2007. Recall of fear extinction in humans activates the ventromedial prefrontal cortex and hippocampus in concert. Biol. Psychiatry 62, 446–454.
Monfils, M.-H., Cowansage, K.K., Klann, E., LeDoux, J.E., 2009. Extinction-Reconsolidation boundaries: key to persistent attenuation of fear memories. Science 324, 951–955.
Morriss, J., Christakou, A., van Reekum, C.M., 2015. Intolerance of uncertainty predicts fear extinction in amygdala-ventromedial prefrontal cortical circuitry. Biol. Mood Anxiety Disord. 5, 1–13.
Nader, K., Balleine, B., 2007. Ambiguity and anxiety: when a glass half full is empty. Nat. Neurosci. 10, 807–808.
Nieuwenhuis, I.L., Takashima, A., 2011. The role of the ventromedial prefrontal cortex in memory consolidation. Behav. Brain Res. 218, 325–334.
Norris, F.H., 1992. Epidemiology of trauma: frequency and impact of different potentially traumatic events on different demographic groups. J. Consult. Clin. Psychol. 60, 409.
O'Donnell, M.L., Alkemade, N., Nickerson, A., Creamer, M., McFarlane, A.C., Silove, D., Bryant, R.A., Forbes, D., 2014. Impact of the diagnostic changes to posttraumatic stress disorder for DSM-5 and the proposed changes to ICD-11. Br. J. Psychiatry 205, 230–235.
Pamplona, F.A., Henes, K., Micale, V., Mauch, C.P., Takahashi, R.N., Wotjak, C.T., 2011. Prolonged fear incubation leads to generalized avoidance behavior in mice. J. psychiatric Res. 45, 354–360.
Parsons, R.G., Ressler, K.J., 2013. Implications of memory modulation for posttraumatic stress and fear disorders. Nat. Neurosci. 16, 146–153.
Paul, E.J., Smith, J.D., Valentin, V.V., Turner, B.O., Barbey, A.K., Ashby, F.G., 2015. Neural networks underlying the metacognitive uncertainty response. Cortex 71, 306–322.
Pawlak, C.R., Karrenbauer, B.D., Schneider, P., Ho, Y.-J., 2012. The elevated plus-maze test: differential psychopharmacology of anxiety-related behavior. Emot. Rev. 4, 98–115.
Paxinos, G., Watson, C., 1997. The Rat Brain in Stereotaxic Coordinates. Academic Press, San Diego, CA.
Poirier, G.L., Imamura, N., Zanoletti, O., Sandi, C., 2014. Social deficits induced by peripubertal stress in rats are reversed by resveratrol. J. psychiatric Res. 57, 157–164.
Pushkarskaya, H., Smithson, M., Joseph, J.E., Corbly, C., Levy, I., 2015. Neural correlates of decision-making under ambiguity and conflict. Front. Behav. Neurosci. 9, 325.
Resnick, H.S., Kilpatrick, D.G., Dansky, B.S., Saunders, B.E., Best, C.L., 1993. Prevalence of civilian trauma and posttraumatic stress disorder in a representative national sample of women. J. Consult Clin. Psychol. 61, 984–991.
Rothbaum, B.O., Davis, M., 2003. Applying learning principles to the treatment of Post-Trauma reactions. Ann. N. Y. Acad. Sci. 1008, 112–121.
Rushworth, M.F.S., Behrens, T.E.J., 2008. Choice, uncertainty and value in prefrontal and cingulate cortex. Nat. Neurosci. 11, 389–397.
Sarinopoulos, I., Grupe, D.W., Mackiewicz, K.L., Herrington, J.D., Lor, M., Steege, E.E., Nitschke, J.B., 2010. Uncertainty during anticipation modulates neural responses to aversion in human insula and amygdala. Cereb. Cortex 20, 929–940.
Shiraishi, H., Suzuki, A., Fukasawa, T., Aoshima, T., Ujiie, Y., Ishii, G., Otani, K., 2006. Monoamine oxidase A gene promoter polymorphism affects novelty seeking and reward dependence in healthy study participants. Psychiatr. Genet. 16, 55–58.
Shumake, J., Furgeson-Moreira, S., Monfils, M.H., 2014. Predictability and heritability of individual differences in fear learning. Anim. Cogn. 17, 1207–1221.
Siegmund, A., Wotjak, C.T., 2007. A mouse model of posttraumatic stress disorder that distinguishes between conditioned and sensitised fear. J. Psychiatr. Res. 41, 848–860.
Sierra-Mercado, D., Padilla-Coreano, N., Quirk, G.J., 2011. Dissociable roles of prelimbic and infralimbic cortices, ventral hippocampus, and basolateral amygdala in the expression and extinction of conditioned fear. Neuropsychopharmacology 36, 529–538.
Singh, C., Bortolato, M., Bali, N., Godar, S.C., Scott, A.L., Chen, K., Thompson, R.F., Shih, J.C., 2013. Cognitive abnormalities and hippocampal alterations in monoamine oxidase A and B knockout mice. Proc. Natl. Acad. Sci. 110, 12816–12821.
Soliman, A., Udemgba, C., Fan, I., Xu, X., Miler, L., Rusjan, P., Houle, S., Wilson, A.A., Pruessner, J., Ou, X.-M., 2012. Convergent effects of acute stress and glucocorticoid exposure upon MAO-A in humans. J. Neurosci. 32, 17120–17127.
Steimer, T., 2011. Animal models of anxiety disorders in rats and mice: some conceptual issues. Dialogues Clin. Neurosci. 13, 495–506.
Toth, I., Neumann, I.D., Slattery, D.A., 2012. Central administration of oxytocin receptor ligands affects cued fear extinction in rats and mice in a timepoint-dependent manner. Psychopharmacology 223, 149–158.
Tsetsenis, T., Ma, X.-H., Iacono, L.L., Beck, S.G., Gross, C., 2007. Suppression of conditioning to ambiguous cues by pharmacogenetic inhibition of the dentate gyrus. Nat. Neurosci. 10, 896–902.
Tsuchimine, S., Yasui-Furukori, N., Kaneda, A., Saito, M., Nakagami, T., Sato, K., Kaneko, S., 2008. Association between monoamine oxidase A (MAOA) and personality traits in Japanese individuals. Prog. Neuropsychopharmacol. Biol. Psychiatry 32, 1932–1935.
Vidal-Gonzalez, I., Vidal-Gonzalez, B., Rauch, S.L., Quirk, G.J., 2006. Microstimulation reveals opposing influences of prelimbic and infralimbic cortex on the expression of conditioned fear. Learn. Mem. 13, 728–733.
Werner, E.E., 1989. High-risk children in young adulthood: a longitudinal study from birth to 32 years. Am. J. Orthopsychiatry 59, 72.
Yu, Y.W., Yang, C.W., Wu, H.C., Tsai, S.J., Hong, C.J., Chen, M.C., Chen, T.J., 2005. Association study of a functional MAOA-uVNTR gene polymorphism and personality traits in Chinese young females. Neuropsychobiology 52, 118–121.