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# Persistence of amygdala gamma oscillations during extinction learning predicts spontaneous fear recovery

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ABSTRACT

Extinction of auditory fear conditioning induces a temporary inhibition of conditioned fear responses that can spontaneously reappear with the passage of time. Several lines of evidence indicate that extinction learning relies on the recruitment of specific neuronal populations within the basolateral amygdala. In contrast, post-extinction spontaneous fear recovery is thought to result from deficits in the consolidation of extinction memory within prefrontal neuronal circuits. Interestingly, recent data indicates that the strength of gamma oscillations in the basolateral amygdala during auditory fear conditioning correlates with retrieval of conditioned fear responses. In the present manuscript we evaluated the hypothesis that post-extinction spontaneous fear recovery might depend on the maintenance of gamma oscillations within the basolateral amygdala by using single unit and local field potential recordings in behaving mice. Our results indicate that gamma oscillations in the basolateral amygdala were enhanced following fear conditioning, whereas during extinction learning gamma profiles were more heterogeneous despite similar extinction learning rates. Remarkably, variations in the strength of gamma power within the basolateral amygdala between early and late stages of extinction linearly predicted the level of post-extinction spontaneous fear recovery. These data suggest that maintenance of gamma oscillations in the basolateral amygdala during extinction learning is a strong predictive factor of long term spontaneous fear recovery.



## 1. Introduction

In mammals, the mechanisms underlying emotional learning are often evaluated using auditory fear conditioning, a form of Pavlovian learning that consists of associating a conditioned stimulus (the CS, usually a tone) with a mild aversive unconditioned stimulus (the US, usually a footshock). Following fear conditioning, repeated presentation of the CS alone progressively inhibits conditioned fear responses, a phenomenon labeled fear extinction (Myers & Davis, 2007). Fear extinction is a form of new learning known to be encoded in specific neuronal networks including the basolateral amygdala (BLA) and the medial prefrontal cortex (mPFC) (Burgos-Robles, Vidal-Gonzalez, Santini, & Quirk, 2007; Courtin, Bienvenu, Einarsson, & Herry, 2013; Herry et al., 2010; Holmes et al., 2012; Klavir, Genud-Gabai, & Paz, 2012; Maroun, Kavushansky, Holmes, Wellman, & Motanis, 2012; Milad & Quirk, 2002; Orsini & Maren, 2012).

Notably, several studies have consistently demonstrated that the BLA is a key structure involved in the initial stages of extinction memory formation. Several lines of evidence suggest that cellular plasticity in the BLA underlies the acquisition of extinction memory. For instance, pharmacological studies indicate that local interference with glutamatergic synaptic plasticity in the BLA, such as infusion of NMDA receptors antagonists or blockers of ERK/MAPK signaling prevents or attenuates extinction learning (Falls, Miserendino, & Davis, 1992; Herry, Trifilieff, Micheau, Luthi, & Mons, 2006; Zimmerman & Maren, 2010). Moreover, fear extinction learning is correlated with an increase in CS-evoked activity within a subpopulation of neurons in the basal nuclei of the amygdala (Herry et al., 2008). Persistence of extinction memories is usually evaluated during a post-extinction retrieval test, where the extinguished CS is presented one to several days after extinction learning. Several studies have indicated the existence of large individual differences in the response to non-reinforced CS presentations during retrieval despite similar extinction learning rates (Burgos-Robles et al., 2007; Herry & Mons, 2004; Milad & Quirk, 2002). Indeed, during post-extinction retrieval of extinction memories, some animals display spontaneous recovery of conditioned fear responses (high fear recovery), whereas others present low fear responses upon presentation of the extinguished CS (low fear recovery). The heterogeneous distribution of conditioned fear responses during post-extinction retrieval could be mediated by

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specific functional changes displayed at the time of the retrieval session or acquired prior to the retrieval session.

In direct support of the first possibility, recent data indicate that enhanced correlations between BLA and mPFC theta oscillations during retrieval are correlated with the expression of previously acquired fear memories (Lesting et al., 2011). In contrast, the loss of such correlations between these structures is associated with retrieval of extinction memories (Lesting et al., 2011). Moreover synaptic plasticity in the mPFC-BLA pathway has been shown to be enhanced during post-extinction fear recovery whereas synaptic plasticity in the BLA-mPFC pathway displayed the opposite pattern (Vouimba & Maroun, 2011). Only few studies have evaluated the predictive factors of long term fear recovery following extinction learning. For instance, studies have revealed that long-term retention of extinction memory was correlated with the thickness of the ventromedial prefrontal cortex in healthy humans (Milad et al., 2005) and that amygdala activation during extinction in patients suffering from post-traumatic stress disorder is associated with high fear recovery (Bremner et al., 2005; Milad et al., 2009).

Although the neuronal mechanisms of post-extinction fear recovery are still poorly understood, recent data suggested that amygdala gamma oscillations might play a key role in this phenomenon. Indeed, several lines of evidence indicate that (i) amygdala activation during acquisition of emotional memories strongly correlates with long-term memory retrieval (Cahill et al., 1996; Hamann, Ely, Grafton, & Kilts, 1999), (ii) processing of biologically significant stimuli is associated with amygdala gamma oscillations (Sato et al., 2011a, 2011b; 2012) and (iii) the strength of gamma oscillations during memory acquisition predicts subsequent memory retrieval (Headley & Weinberger, 2011; Osipova et al., 2006; Sederberg, Kahana, Howard, Donner, & Madsen, 2003; Sederberg et al., 2007b). Together these data suggest the possibility that the maintenance of gamma oscillations during extinction might predict spontaneous post-extinction fear recovery. The present study evaluated this hypothesis using single unit and local field potential recordings in the BLA of mice following auditory fear conditioning and extinction learning.

## 2. Material and methods

### 2.1. Subjects

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).

### 2.2. Behavior

Habituation and fear conditioning took place in context A consisting of a square transparent Plexiglas box (25 cm side, 80 cm high) with a shock grid floor made of stainless steel rods placed inside a sound attenuating and temperature regulated cubicle. The walls of the cubicle were made of sound attenuating black foam. Inside the cubicle the floor and walls of the Plexiglas cylinder were cleaned with a 70% ethanol solution before and after each session. Extinction learning and post-extinction fear retrieval were performed in context B consisting of a square transparent Plexiglas cylinder (25 cm diameter, 40 cm high) with a grey plastic floor placed inside a sound attenuating and temperature regulated cubicle. The walls of the cubicle were made of sound attenuating white foam and the light intensity was reduced. The floor and walls of the Plexiglas square were cleaned with a 1% acetic acid solution before and after each session.

To score freezing behavior an automated infrared beam detection system located on the bottom of the experimental chambers was used (Coulbourn Instruments). The animals were considered to be freezing if no movement was detected for 2 s. On day 1, 11 C57BL6/J mice were submitted to a habituation session in context A, in which they received 4 presentations of the $CS^+$ and of the $CS^-$ (total CS duration: 30 s, consisting of 50 ms pips repeated at 0.9 Hz, 2 ms rise and fall, pip frequency: 7.5 kHz or white-noise, 80 dB sound pressure level, CS were counterbalanced across animals). Discriminative fear conditioning was performed on the same day by pairing the $CS^+$ with a US (1 s foot-shock, 0.6 mA, 5 $CS^+$/US pairings; inter-trial interval: 20–180 s). The onset of the US coincided with the offset of the $CS^+$. The $CS^-$ was presented after each $CS^+$/US association but was never reinforced (5 $CS^-$ presentations, inter-trial interval: 20–180 s). The frequencies used for $CS^+$ and $CS^-$ were counterbalanced across animals. On days 2 and 3, conditioned mice were submitted to extinction training (Early and Late Extinction sessions) in context B during which they received 4 and 12 presentations of the $CS^-$ and $CS^+$, respectively. Retrieval of extinction was tested 7 days later in context B, with 4 presentations of the $CS^-$ and the $CS^+$.

### 2.3. Surgery and recordings

Mice were anesthetized with isoflurane (induction 3%, maintenance 1.5%) in $O_2$. Body temperature was maintained with a temperature controller system (FHC). Mice were secured in a stereotaxic frame and unilaterally implanted in the BLA with a multi-wire electrode aimed at the following coordinates (Franklin & Paxinos, 1997): 1.7 mm posterior to bregma; 3.2 mm lateral to midline and 4.2 mm below the cortical surface. The electrodes consisted of 16 individually insulated nichrome wires (13 μm inner diameter, impedance 30–100 KΩ; Kanthal) contained in a 26 gauge stainless steel guide cannula. The wires were attached to an 18 pin connector (Omnetics). All implants were secured using Super-Bond cement (Sun Medical). After surgery mice were allowed 7 days to recover and habituated to handling. Analgesia was applied before, and 1 day after surgery (Metacam, Boehringer). Electrodes were connected to a headstage (Plexon) containing 16 unity-gain operational amplifiers. The headstage was connected to a 16-channel preamplifier (gain 100x, bandpass filter from 150 Hz to 9 kHz for single unit activity and from 0.7 Hz to 170 Hz for field potentials, Plexon). Spiking activity was digitized at 40 kHz and bandpass filtered from 250 Hz to 8 kHz, and isolated by time–amplitude window discrimination and template matching using a Multichannel Acquisition Processor system (Plexon).

### 2.4. Single unit and local field potential analysis

Single-unit spike sorting was performed using Off-Line Spike Sorter (OFSS, Plexon). Principal component scores (PC) were calculated for unsorted waveforms and plotted in a 3D PC space; clusters containing similar valid waveforms were manually defined. A group of waveforms were considered to be generated from a single neuron if the waveforms formed a discrete, isolated, cluster in the PC space and did not contain a refractory period less than 1 ms, as assessed by using auto-correlogram analyses. To avoid analysis of the same neuron recorded on different channels, we computed cross-correlation histograms. If a target neuron presented a peak of activity at a time that the reference neuron fired, only one of