



Sex differences in learned fear expression and extinction involve altered gamma oscillations in medial prefrontal cortex



Georgina E. Fenton^{a,1}, David M. Halliday^b, Rob Mason^c, Timothy W. Bredy^{d,e}, Carl W. Stevenson^{a,*}

^a School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, UK

^b Department of Electronics, University of York, Heslington, York YO10 5DD, UK

^c School of Life Sciences, University of Nottingham, Queen's Medical Centre, Nottingham NG7 2UH, UK

^d Queensland Brain Institute, University of Queensland, Brisbane, Queensland 4072, Australia

^e Department of Neurobiology and Behavior, University of California, Irvine, Irvine, CA 92697, United States

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ABSTRACT

Sex differences in learned fear expression and extinction involve the medial prefrontal cortex (mPFC). We recently demonstrated that enhanced learned fear expression during auditory fear extinction and its recall is linked to persistent theta activation in the prelimbic (PL) but not infralimbic (IL) cortex of female rats. Emerging evidence indicates that gamma oscillations in mPFC are also implicated in the expression and extinction of learned fear. Therefore we re-examined our *in vivo* electrophysiology data and found that females showed persistent PL gamma activation during extinction and a failure of IL gamma activation during extinction recall. Altered prefrontal gamma oscillations thus accompany sex differences in learned fear expression and its extinction. These findings are relevant for understanding the neural basis of post-traumatic stress disorder, which is more prevalent in women and involves impaired extinction and mPFC dysfunction.

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1. Introduction

Women show a greater prevalence of post-traumatic stress disorder (PTSD) than men (Glover, Jovanovic, & Norrholm, 2015; Maeng & Milad, 2015), yet the neural mechanisms underlying this sex difference remain unclear. PTSD is characterized by impaired fear extinction (Jovanovic & Norrholm, 2011; Milad et al., 2009). This is the reduction in learned fear that results from repeated non-reinforced presentations of the conditioned stimulus (CS) (Herry et al., 2010). PTSD is also associated with dysfunction of the medial prefrontal cortex (mPFC), a heterogeneous brain area important for mediating the expression and extinction of learned fear. Whereas the anterior cingulate cortex (ACC) is involved in learned fear expression, its extinction requires the ventromedial prefrontal cortex (vmPFC) (Linnman et al., 2012; Mueller, Panitz, Hermann, & Pizzagalli, 2014). Importantly, ACC and vmPFC dysfunction are observed in PTSD (Milad et al., 2009; Shin et al.,

2009). Accumulating evidence indicates sex differences in fear extinction that involve mPFC (Baker-Andresen, Flavell, Li, & Bredy, 2013; Baran, Armstrong, Niren, & Conrad, 2010; Merz et al., 2012; Rey, Lipps, & Shansky, 2014; Zeidan et al., 2011). However, the functional roles of different mPFC subregions in mediating sex differences in learned fear expression and extinction remain unclear.

Using *in vivo* electrophysiology, we have recently shown that sex differences in learned fear expression are linked to altered mPFC theta oscillations (4–12 Hz) in rats (Fenton et al., 2014). Compared to males, females exhibited more fear during auditory fear extinction and its recall which was accompanied by persistent theta activation in prelimbic (PL) cortex, the rodent homolog of ACC. In contrast, we found no sex differences in theta activity in infralimbic (IL) cortex, the homologous area to vmPFC (Sierra-Mercado, Padilla-Coreano, & Quirk, 2011; Vidal-Gonzalez, Vidal-Gonzalez, Rauch, & Quirk, 2006). Compared to males, females also showed more contextual fear before extinction and extinction recall associated with persistent PL theta activation, whereas there was no accompanying sex difference in IL theta activation. Given the lack of sex differences in IL theta activity observed before and during extinction and its recall, we speculated that the enhanced fear shown by females was due to impaired contextual

* Corresponding author.

E-mail addresses: gef8@leicester.ac.uk (G.E. Fenton), carl.stevenson@nottingham.ac.uk (C.W. Stevenson).

¹ Current address: Department of Neuroscience, Psychology and Behaviour, University of Leicester, University Road, Leicester LE1 9HN, UK.

regulation of extinction rather than an extinction deficit *per se*. However, another possibility is that other types of oscillatory activity in mPFC are linked to sex differences in fear extinction.

Gamma oscillations (30–120 Hz) play a key role in prefrontal-dependent cognitive functions such as attentional processing (Benchenane, Tiesinga, & Battaglia, 2011). Recent studies also indicate that both theta and gamma synchrony between mPFC and other inter-connected areas are involved in various memory processes (Harris & Gordon, 2015), including learned fear inhibition (Courtin, Karalis, Gonzalez-Campo, Wurtz, & Herry, 2014; Lesting et al., 2011; Stujenske, Likhtik, Topiwala, & Gordon, 2014; Wang, Yuan, Keinath, Ramos Álvarez, & Muzzio, 2015). Interestingly, emerging evidence indicates that oscillatory activity at lower gamma frequencies (~30–50 Hz) in mPFC plays a role in the extinction of learned fear. In mice, impaired fear extinction is associated with enhanced gamma activation in PL (Fitzgerald et al., 2014). In humans, gamma activation in vmPFC accompanies the recall of fear extinction, while unsuccessful fear extinction recall is associated with a failure of vmPFC gamma activation (Mueller et al., 2014). Therefore gamma oscillations in PL and IL might also be implicated in sex differences in learned fear expression and its extinction. Here we re-examined the local field potential (LFP) activity data from our recent study and analyzed PL and IL gamma oscillations before and during auditory fear extinction and its recall in males and females.

2. Material and methods

2.1. Animals and surgery

Young adult male and age-matched naturally cycling female Lister hooded rats (Harlan, UK) were used in these experiments, which were conducted with internal ethical approval and in accordance with the Animals (Scientific Procedures) Act 1986, UK. Electrodes were implanted into PL and IL under anesthesia and with analgesia as previously described (Fenton et al., 2014). Rats were singly housed during recovery and behavioural testing, which started 10–14 days after surgery.

2.2. Behavioural testing

Auditory fear conditioning, extinction and extinction recall testing were conducted using two chambers described in detail elsewhere (Stevenson, Spicer, Mason, & Marsden, 2009). On Day 0 rats were habituated to contexts A and B (15 min each). On Day 1 rats underwent tone habituation (five tones alone; 30 s, 80 dB, 4 kHz, 2 min inter-trial interval (ITI)) followed by auditory fear conditioning (five tones co-terminating with footshock; 1 s, 0.5 mA, 2 min ITI) in context A. On Days 2 and 15 rats underwent extinction training and recall testing, respectively (30 tones alone; 30 s ITI), in context B (Fig. 1A). Freezing during tone alone presentations and tone-shock pairings on Day 1, and before (i.e. 2 min prior to the first tone onset) and during tone presentations on Days 2 and 15, was scored manually and served as an index of learned fear.

2.3. In vivo electrophysiology

During behavioural testing LFP activity was recorded by connecting the electrodes to a preamplifier linked to a Plexon Recorder system (Plexon Inc, TX), via a headstage and a commutator. LFPs were band-pass filtered at 0.7–170 Hz and digitized at 1 kHz. All electrode placements in PL and IL were verified histologically as described previously (Fenton et al., 2014). Only data from rats with confirmed electrode placements in PL and IL (Fig. 2A) was included in the analysis.

2.4. Behavioural data analysis

The mean of freezing during two consecutive tones on Days 2 and 15 was used in the statistical analysis. Spontaneous fear recovery was calculated by dividing mean freezing during the first two tones on Day 15 by mean freezing during the last two tone-shock pairings on Day 1 and expressed as a percentage. Contextual fear before extinction and extinction recall was determined by assessing freezing before the first tone onset on Days 2 and 15. Freezing and spontaneous fear recovery were expressed as the mean + SEM. Sex differences in tone-induced freezing during conditioning, extinction and extinction recall were analyzed separately using two-way analysis of variance (ANOVA). Sex differences in spontaneous fear recovery were analyzed using an unpaired *t*-test. Differences in tone-induced freezing between extinction and extinction recall were analyzed separately in males and females using two-way ANOVA. Sex differences in contextual fear before extinction and extinction recall were analyzed using two-way ANOVA. Post-hoc comparisons were conducted using Bonferroni's testing. The significance level for all comparisons was set at $P < 0.05$.

2.5. In vivo electrophysiology data analysis

Using multi-taper spectral analysis (Fenton, Spicer, Halliday, Mason, & Stevenson, 2013), spectral estimates of LFP activity in PL and IL (Fig. 2B) were generated during early and late extinction and extinction recall by taking the mean of the first two and last two tones on Days 2 and 15 and pooling across males or females. Similarly, spectral estimates of LFP activity in the 2 min periods before tone onset on Days 2 and 15 were generated and pooled across males or females. Differences in LFP power during early vs late extinction, early vs late extinction recall, and before extinction vs before extinction recall were each determined separately in males or females using the log ratio difference of spectra test (Diggle, 1990) and quantified statistically using 95% confidence intervals (Fenton et al., 2013, 2014; Stevenson, Halliday, Marsden, & Mason, 2007, 2008). The LFP analysis was restricted to the 30–45 Hz band, which coincides with the lower gamma frequencies recently implicated in fear extinction (Fitzgerald et al., 2014; Mueller et al., 2014).

3. Results

3.1. Females exhibit enhanced learned fear expression during extinction and extinction recall

Before presenting our mPFC gamma activity findings we first summarize the behavioural results from our recent study (Fenton et al., 2014). We found no differences in freezing between males ($n = 9$) and females ($n = 10$) during the presentation of tones alone (data not shown) or tone-shock pairings during fear conditioning (Fig. 1B). Females did show significantly greater tone-induced freezing during extinction (main effect of sex: $F_{(1,17)} = 13.63$, $P < 0.01$; Fig. 1C) and extinction recall (main effect of sex: $F_{(1,17)} = 27.41$, $P < 0.001$; Fig. 1D), indicating that females showed enhanced learned fear expression during extinction and its later recall. Females also showed enhanced spontaneous recovery of fear over time after extinction, as indicated by a significantly increased percentage of fear recovered during early extinction recall relative to late fear conditioning ($t_{17} = 2.39$, $P < 0.05$; Fig. 1E). Despite this sex difference in spontaneous fear recovery both males (Fig. 1F) and females (Fig. 1G) showed savings of extinction, as indicated by a significant decrease in tone-induced freezing during extinction recall

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