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RESEARCH ARTICLE

The effects of muscimol and AMN082 injections into the medial prefrontal cortex on the expression and extinction of conditioned fear in mice

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SUMMARY

The medial prefrontal cortex (mPFC), in particular its infralimbic part, is a key region in mediating the extinction of conditioned fear. There is some evidence that the metabotropic glutamate receptor 7 (mGluR7) may be involved in the mediation or modulation of extinction. The aim of present study was to assess the potential role of mGluR7 in the mPFC in the extinction of conditioned fear in mice by local injections of AMN082, a positive allosteric modulator of mGluR7. Furthermore, for comparison we injected the GABA-A receptor agonist muscimol, which should lead to a temporary inactivation of mPFC. We found impaired between-session extinction of conditioned fear for the muscimol group as well as a decrease in fear expression. However, local injections of AMN082 into the mPFC had no effects. Overall, the results of the experiment add to a growing body of evidence that mPFC, especially the infralimbic region, is crucial in the extinction of fear memory.

Key words: AMN082, extinction, fear conditioning, freezing, metabotropic glutamate receptor, mGluR7, prefrontal cortex.

INTRODUCTION

During fear conditioning, humans or animals learn to associate a formerly neutral cue (e.g. a tone stimulus) with an aversive event (e.g. mild electric stimulus). After several pairings of neutral cues with aversive events, an increase in fear level can be observed, as the now conditioned cue is presented alone. A prominent fear response in rodents is freezing, which is defined as a cessation of all bodily movements except those for respiration (Fendt and Fanselow, 1999). Extinction occurs when, as a result of presentation of the conditioned stimulus (CS) alone, a new memory trace forms, signalling that the CS is no longer a threat (Ehrlich et al., 2009). Extinction is an important model of inhibitory learning, which, in contrast to excitatory learning, results in decreased response amplitude to certain stimuli. Extinction processes might be disturbed in anxiety disorders such as post-traumatic stress disorder or panic disorders (Garakani et al., 2006; Milad et al., 2009).

Different brain areas have been implicated in the expression and extinction of fear memory. A body of evidence points towards the amygdala as being the brain area responsible for fear expression (Ehrlich et al., 2009; Kim and Jung, 2006; Likhtik et al., 2005; Sierra-Mercado et al., 2011). The amygdala receives input from multiple brain areas, including the medial prefrontal cortex (mPFC). Inactivation or lesion of the mPFC, especially of the infralimbic cortex (IL), as well as blockade of protein synthesis, blocks extinction of conditioned fear (Lebron et al., 2004; Morgan et al., 1993; Morgan and LeDoux, 1995; Morgan and LeDoux, 1999; Santini et al., 2004; Sierra-Mercado et al., 2011). Furthermore, neurons within the IL signal the memory for extinction, i.e. activity of these neurons is correlated with the behavioural expression of extinction (Milad and Quirk, 2002). Last, stimulation of the IL results in a response similar to extinction, i.e. reduced freezing (Milad and Quirk, 2002).

Recent research presents metabotropic glutamate receptor 7 (mGluR7) as a promising therapeutic target, as activation of the receptor has been shown to increase extinction of conditioned fear in rodent models of fear and anxiety (Fendt et al., 2008; Goddyn et al., 2008; Ugolini et al., 2008). mGluR7 is located presynaptically in primary afferent neurons, both excitatory and inhibitory ones (Ohishi et al., 1995), and their activation can decrease the release of both glutamate and GABA (Schoepp, 2001). Downregulation of mGluR7 blocks the extinction of conditioned fear in mice (Fendt et al., 2008). Oral administration of AMN082, a specific allosteric modulator of mGluR7 (Mitsukawa et al., 2005), has been shown to prevent the acquisition of conditioned fear in the fear-potentiated startle paradigm, as well as facilitate extinction (Fendt et al., 2008).

The aim of the experiments performed in the present study was to assess the function of mGluR7 in the mPFC in extinction of conditioned fear, using local injections of AMN082 into the mPFC of mice. In addition, we also injected the GABA agonist muscimol, which should lead to a temporary inactivation of the mPFC. The muscimol-injected mice served as a positive control group.

MATERIALS AND METHODS

Animals

Eighty-three male C57BL6/J mice (Charles River Laboratories, France), weighing 20–25 g, were kept three to four in a cage equipped with plastic nest boxes, wooden chew toys and nesting materials in humidity- and temperature-controlled conditions (45% humidity, 22°C) under a 12 h:12 h light:dark photoperiod (lights on at 06:00 h). Water and food were available *ad libitum.* All experiments were conducted according to international guidelines for the care and use of laboratory animals and with respect to national laws on animal use. The local ethics committee (Kantonales Veterinaramt Basel, Basel, Switzerland) approved all experiments.

Apparatus

A computerised fear conditioning system (TSE Systems, Bad Homburg, Germany) was used, consisting of four sound-attenuating chambers, equipped with loudspeakers for acoustic stimuli delivery, light sources and a ventilation fan. The delivery of the stimuli was controlled by a pre-programmed computer. The movement of the animals was detected by infrared beams spaced 14 mm apart. Freezing, defined as time spent with no infrared beams crossed for more than 1 s, was automatically recorded. Freezing measured by this method is highly correlated with human scoring of freezing (Endres et al., 2007; Misane et al., 2005).

For conditioning, four transparent, bottomless Plexiglas® (46×46×32 cm) boxes were used, with flooring consisting of stainless steel grids (bars: 4 mm diameter, distance apart: 8.9 mm), which were connected to a shock unit and able to deliver foot shocks of defined duration and intensity. In this context (context A), a ventilation fan was used as background noise and light intensity was set to a low illumination level (ca. 10 lx). For both extinction training and testing, a different context was used (context B), consisting of four additional, black Plexiglas® boxes (46×46×32 cm), with no ventilation fan and light intensity set to a slightly higher illumination level (ca. 50 lx).

Surgery

All mice were implanted with one intracranial guide cannula (outer diameter: 0.65 mm, length: 6 mm; Eisenhut-Vet AG, Allschwil, Switzerland) into the mPFC for local microinjections. The surgery was performed using standard aseptic and stereotaxic techniques, with intraperitoneal ketamine/xylazine (10:1; 110 mg/10 ml kg^{-1}) for anaesthesia and intraperitoneal temgesic (0.05 mg/10 ml kg^{-1}) used for analgesia (animals received analgesic injections up to 2 days after the surgery). The stereotaxic coordinates for the mPFC were as follows: +1.6 mm anterior to Bregma, and 2.1 mm ventral to the skull surface (Paxinos and Franklin, 2001). The guide cannula was fixed to the skull with two stainless steel skull screws (Bossard, Zug, Switzerland) and dental acrylic cement. Animals were allowed to recover in freshly prepared cages for 3–5 days after the surgery, prior to behavioural testing.

Experimental procedure

After an acclimatisation period of 1 min, the mice were fear conditioned with six pairings of 0.4 mA scrambled foot shocks [unconditioned stimulus (US)] paired with 30 s, 75 dB, 8 kHz tones (CS) in context A at an interstimulus interval (ISI) of 30 s. On the second day, the context was changed to context B. The mice were randomly allocated to one of the three treatment groups, and microinjections with phosphate buffered saline, AMN082 (1 µmol l^{-1}; synthesised at Novartis; dissolved in 0.1% methanol + H_2O) or muscimol (0.25 µg/0.3 µl; Sigma-Aldrich, St Louis, MO, USA) were performed. The injection volume was 0.3 µl, the injection speed was 0.1 µl min^{-1}, and the injection cannula was left in for an additional 1 min to allow the drug to diffuse. All doses were determined in accordance with previous studies (Siegl et al., 2008; Sierra-Mercado et al., 2011), as well as previous microinjection studies done in house. Furthermore, it was demonstrated previously that 0.1% methanol in H_2O can be used a neutral vehicle (Fendt et al., 2008; Siegl et al., 2008). Approximately 30 min after the microinjections, the animals were put into context B and extinction training was performed with 20 CS presentations (5 s ISI). On the third day, the mice were presented in the same context with further 10 CS presentations (5 s ISI) to test between-session extinction memory.

Histology

To verify the injection site and the integrity of the mPFC, the animals were euthanised after the experiment. The brains were removed and immersion-fixed in 8% paraformaldehyde/30% sucrose. The brains were then cut with a freezing microtome in 100 µm thick slices. The slices were fixed to previously gelatinised slides and stained using a standard Nissl staining procedure. The brain slices were analysed under a microscope to determine the injection sites. The brain areas were identified by comparison with a mouse brain atlas (Paxinos and Franklin, 2001).

Statistical analyses

The data were separately analysed for muscimol and AMN082 injections (comparison with vehicle) with Student's *t*-tests or two-way ANOVAs. For latter, treatment group was used as the between-subject factor and trial number (i.e. time) as the within-subject factor. *Post hoc* comparisons were performed with Bonferroni tests.

RESULTS

Histology

Five animals were excluded from final analysis, as they expressed no fear behaviour (freezing) during the fear conditioning. The histological examination further enabled rejection of some subjects from the final analysis due to lesions (N=22) and misplaced injections (N=20; e.g. in the septum or nucleus accumbens), leaving the final number of subjects at 36 (Fig. 1). Injections into the mPFC, including the IL, the cingulate cortex and the dorsal peduncular area, were classified as hits (see Fig. 2 for a representative example).

Behaviour

As described above, conditioning was done without treatment. There were no differences between the groups (F<1.00, P>0.84; Fig. 3A). However, significant effects of the factor trial number (F>13.83, P<0.001) indicate that fear conditioning was successful.

On day 2 (Fig. 3B), injections of vehicle, muscimol or AMN082 were performed. Again, the factor trial number was significant for all treatments (F>10.00, P<0.001), demonstrating within-session extinction in the different treatment groups. For muscimol, there was a significant main effect of treatment ($F_{1,456}$=5.503, P=0.0276) and a significant interaction between the factors treatment and trial number ($F_{19,456}$=1.645, P=0.0425). This indicates that muscimol injections into the mPFC increase fear expression and/or affect within-session extinction. Notably, muscimol injections also significantly increased freezing during the pre-CS period (t_{24}=4.55, P<0.001), suggesting that the effect of muscimol on fear expression is not specific to conditioned fear induced by the CS. In contrast to muscimol injections, mPFC injections of AMN082 did not affect freezing on day 2. There were neither main treatment effects ($F_{1,399}$=0.35, P=0.56) nor a significant interaction between treatment and trial number ($F_{19,399}$=0.80, P=0.71).

On the third test day (without treatment; Fig. 3C) we tested for effects on between-session extinction. Again, statistical analyses revealed the presence of within-session extinction (trial number, F>18.48, P<0.001). For the animals receiving muscimol injections on the day before, there was no main treatment effect if all CS presentations were included in the analysis ($F_{1,216}$=0.002, P=0.97). However, a significant interaction between treatment and trial number on this test day indicates some subtle effects of muscimol ($F_{9,216}$=2.59, P=0.008). More detailed analysis of the freezing response to the first CS presentation revealed a significant increase

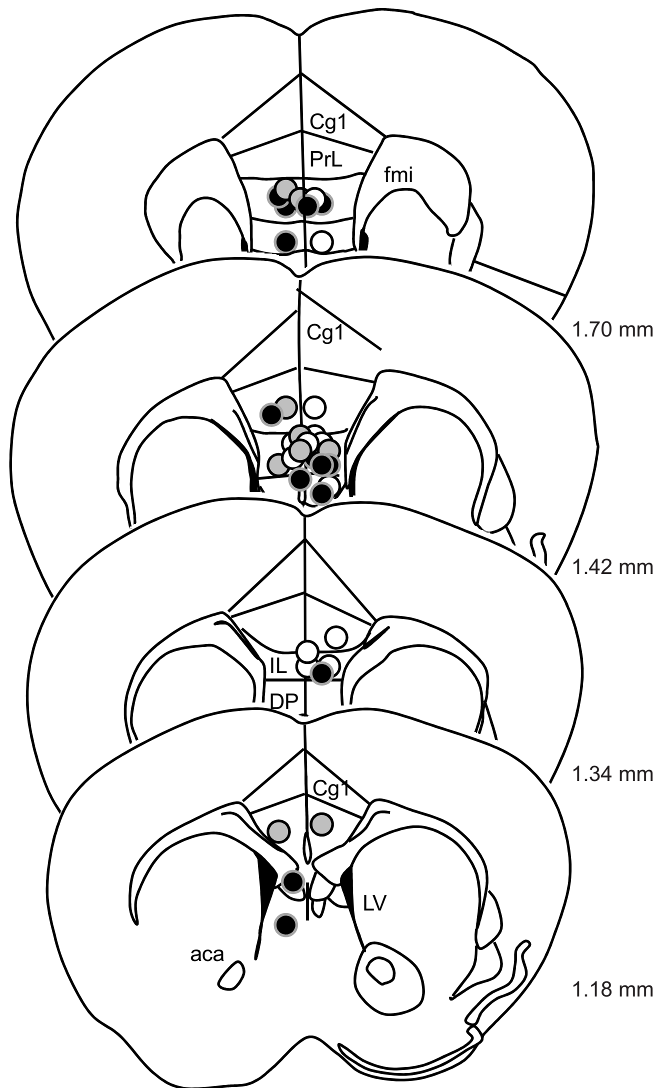

Fig. 1. Frontal sections of the mouse prefrontal cortex depicting the injection sites of vehicle (white circles), AMN082 (grey circles) and muscimol (black circles). Measurements indicate the distance to Bregma. Aca, anterior commissure, anterior part; cc, corpus callosum; Cg1, cingulate cortex 1; DP, dorsal peduncular area; fmi, forceps minor corpus callosum; IL, infralimbic cortex; LV, lateral ventricle; PrL, prelimbic cortex.

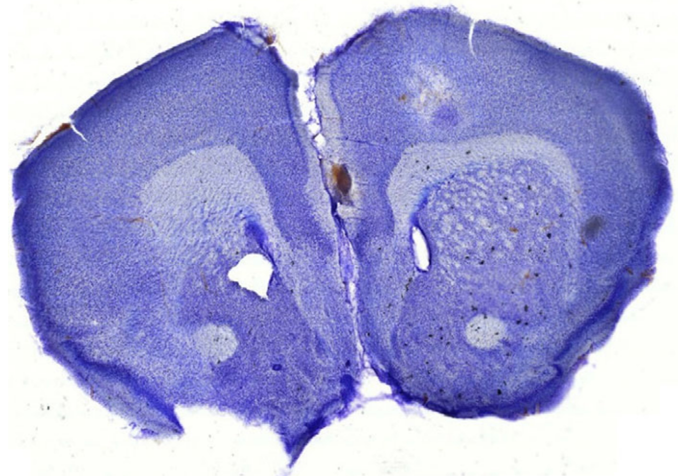

Fig. 2. Photomicrograph showing a representative brain section of a medial prefrontal cortex (mPFC) injection in the mouse.

of freezing in the muscimol group (t_{24}=2.102, P=0.046). This indicates an effect of muscimol on between-session extinction (cf. Sierra-Mercado et al., 2011). Again, there were no effects in the AMN082 group (treatment, $F_{1,189}$=0.08, P=0.78; interaction, $F_{9,189}$=0.63, P=0.77), suggesting that between-session extinction was also not affected by AMN082 injections. Behaviour in the pre-CS period was not affected in either group.

Misplaced injections of muscimol or AMN082 (mainly localized in the lateral septum and the nucleus accumbens) did not affect freezing behaviour on the second or third test day (data not shown; treatment, F<0.61, P>0.45; treatment × trial number interaction, F<1.42, P>0.12).

DISCUSSION

In the present study we examined the effects of muscimol and AMN082 injections into the mPFC on fear expression and extinction in mice. Previous studies have demonstrated effects on extinction after pharmacological IL inactivation in rats (e.g. Sierra-Mercado et al., 2011); however, similar studies have never been performed in mice. In the present study, muscimol infusions into the mPFC resulted in increased freezing in the extinction training session and impairment of between-session extinction. AMN082 infusions did not yield any significant effects.

Our study demonstrates that temporary inactivation of the mPFC during extinction training impairs the acquisition of (between-session) extinction memory in conditioned mice. This result is in accordance with the literature, where inactivation of the mPFC, especially the IL region, has been shown to result in weaker extinction memory in fear-conditioned rats (Sierra-Mercado et al., 2011), and stimulation of the IL has been shown to facilitate acquisition and recall of extinction memory in rats (Vidal-Gonzalez et al., 2006). Furthermore, stimulation of the IL at the CS onset (Milad and Quirk, 2002), but not prior or after the CS (Vidal-Gonzalez et al., 2006) has been shown to result in extinction of fear memory, suggesting its role in the gating of downstream structures involved in fear memory. The IL is thought to inhibit the amygdala, especially its basolateral complex (BLA) (Grace and Rosenkranz, 2002), and the effect of the IL on suppression of the BLA is mediated by inhibitory interneurons (Amir et al., 2011; Pare and Smith, 1993).

Infusions of muscimol into the mPFC also resulted in higher general fear expression, measured by freezing behaviour in the pre-CS period and throughout the extinction session. Within-session extinction was also affected, resulting in a dextral shift of the extinction curve to the right. This may imply disrupted acquisition of extinction memory, supporting the mPFC's role in the initial step of extinction in mice (Vidal-Gonzalez et al., 2006). However, the high baseline fear level points to disinhibition of the BLA, which leads to increased fear expression even before the first CS presentation. The effect of muscimol infusions on between-session extinction was smaller than expected, as freezing in response to the first cue presentation was the only response that was affected the subsequent day (cf. Sierra-Mercado et al., 2011). This effect may be due to inactivation of a brain area larger than just the IL, as reports show that the prelimbic cortex might exhibit effects opposite to those of the IL (Burgos-Robles et al., 2009; Vidal-Gonzalez et al., 2006), thus leading to a decrease in fear in the case of muscimol infusions into this brain area (Sierra-Mercado et al., 2011).

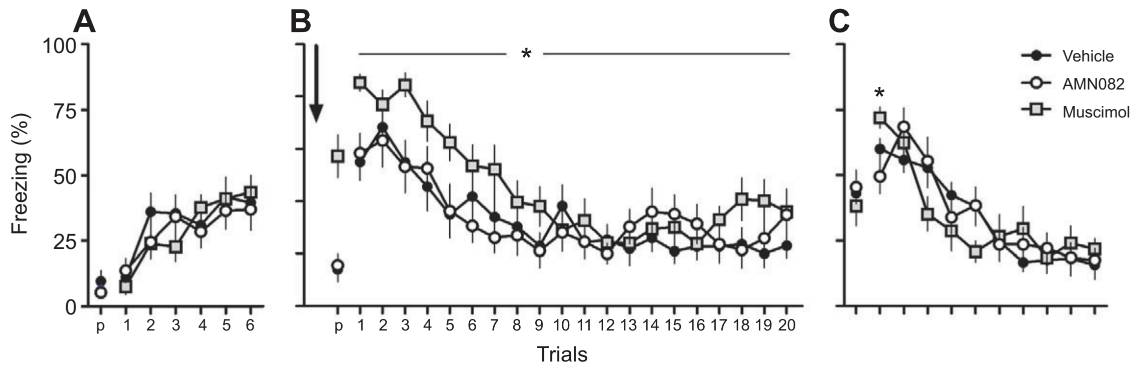

Fig. 3. Mean (±s.e.m.) percent freezing of mice during (A) fear conditioning, (B) a retention test and extinction training and (C) a test on extinction memory. Groups received intra-mPFC injections (arrow) of vehicle (black circles), AMN082 (white circles) and muscimol (grey squares) prior to the extinction training. Asterisks indicate significant differences between the treatment groups comparison vehicle and muscimol (*P<0.05; **P<0.01). p, pre-CS period.

As in previous studies (e.g. Santini et al., 2004), we only implanted a single cannula into the mPFC. Most of the injection sites were not precisely localised on the midline. However, with the used injection volume of 0.3 μl, an injection diffusion radius of ca. 0.5 mm can be assumed (Edeline et al., 2002; Martin, 1991), meaning that the entire mPFC is flooded by the injected volume and unilateral effects are rather unlikely, as all of our injections sites were closer than 0.5 mm to the midline.

In contrast to muscimol injections, AMN082 injections into the mPFC had no effect on fear expression or fear extinction – despite several studies that have demonstrated that mGluR7 is involved in the modulation of the extinction phenomenon. For example, mGluR7-deficient mice expressed impaired acquisition and extinction of conditioned fear (Goddyn et al., 2008), and systemic injections of AMN082 facilitated between-session extinction of conditioned fear in rats (Fendt et al., 2008). There are several possibilities explaining the lack of effect of AMN082 on extinction of conditioned fear in our study. First, our injections may affect not only the IL but also other brain areas close to the IL. We tried to hit the IL, but because of the small size of the mouse brain we also hit neighbouring brain areas, such as the prelimbic cortex, the cingulate cortex and the dorsal peduncular cortex. However, even injections localized within the IL may diffuse to neighbouring brain areas and effects in these areas may interfere with effects within the IL. For example, the prelimbic cortex been identified to be involved in the expression of fear behaviour (Sierra-Mercado et al., 2011). Second, the AMN082 dose used in the present study could be too low to modulate extinction. However, similar doses of AMN082 led to clear behavioural effects after injections into the BLA of rats (Siegl et al., 2008). Third, the effects of systemic AMN082 administration on extinction could be mediated by other brain areas. Potential candidates for this are the amygdala or the hippocampus, as well as other brain areas involved in extinction processes.

Without doubt, further experimental approaches with higher area specificity should be explored. Using microiontophoresis instead of single-cannula microinjections may be a way forward, as such injections are much more precise (e.g. Herry et al., 2008). Furthermore, angled cannulas can be used to avoid cortex destruction in the regions dorsal to the IL, which could mask the potential effects on extinction (Sierra-Mercado et al., 2011; Vidal-Gonzalez et al., 2006). Insertion of two cannulas instead of one could lead to more prominent effects, as the compound could affect a greater proportion of the desired area. However, there would also be a greater chance for diffusion of drugs into neighbouring areas. Last, the species of choice for performing such experiments could be the rat, rather than the mouse, as the area of interest in rats is bigger than in mice.

In conclusion, temporary inactivation of mPFC with the GABA-A agonist muscimol increases fear expression and impairs extinction, supporting the view that the mPFC, especially the IL, is a key region responsible for the extinction of conditioned fear in mice. AMN082 microinjections in the mPFC did not have any effect on extinction of conditioned fear, suggesting that mGluR7 in the mPFC region may not be involved in fear extinction. Further studies involving more precise microinjections are needed to replicate the systemic effects of AMN082 and identify the brain area that is mediating these effects.

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REFERENCES

Amir, A., Amano, T. and Pare, D. (2011). Physiological identification and infralimbic responsiveness of rat intercalated amygdala neurons. *J. Neurophysiol.* **105**, 3054-3066.

Burgos-Robles, A., Vidal-Gonzalez, I. and Quirk, G. J. (2009). Sustained conditioned responses in prelimbic prefrontal neurons are correlated with fear expression and extinction failure. *J. Neurosci.* **29**, 8474-8482.

Edeline, J.-M., Hars, B., Hennevin, E. and Cotillon, N. (2002). Muscimol diffusion after intracerebral microinjections: a reevaluation based on electrophysiological and autoradiographic quantifications. *Neurobiol. Learn. Mem.* **78**, 100-124.

Ehrlich, I., Humeau, Y., Grenier, F., Ciocchi, S., Herry, C. and Luthi, A. (2009). Amygdala inhibitory circuits and the control of fear memory. *Neuron* **62**, 757-771.

Endres, T., Widmann, K. and Fendt, M. (2007). Are rats predisposed to learn 22 kHz calls as danger-predicting signals? *Behav. Brain Res.* **185**, 69-75.

Fendt, M. and Fanselow, M. S. (1999). The neuroanatomical and neurochemical basis of conditioned fear. *Neurosci. Biobehav. Rev.* **23**, 743-760.

Fendt, M., Schmid, S., Thakker, D. R., Jacobson, L. H., Yamamoto, R., Mitsukawa, K., Maier, R., Natt, F., Hüsken, D., Kelly, P. H. et al. (2008). mGluR7 facilitates extinction of aversive memories and controls amygdala plasticity. *Mol. Psychiatry* **13**, 970-979.

Garakani, A., Mathew, S. J. and Charney, D. S. (2006). Neurobiology of anxiety disorders and implications for treatment. *Mt. Sinai J. Med.* **73**, 941-949.

Goddyn, H., Callaerts-Vegh, Z., Stroobants, S., Dirikx, T., Vansteenwegen, D., Hermans, D., van der Putten, H. and D'Hooge, R. (2008). Deficits in acquisition and extinction of conditioned responses in mGluR7 knockout mice. *Neurobiol. Learn. Mem.* **90**, 103-111.

Grace, A. A. and Rosenkranz, J. A. (2002). Regulation of conditioned responses of basolateral amygdala neurons. *Physiol. Behav.* **77**, 489-493.

Herry, C., Ciocchi, S., Senn, V., Demmou, L., Muller, C. and Luthi, A. (2008). Switching on and off fear by distinct neuronal circuits. *Nature* **454**, 600-606.

Kim, J. J. and Jung, M. W. (2006). Neural circuits and mechanisms involved in Pavlovian fear conditioning: a critical review. *Neurosci. Biobehav. Rev.* **30**, 188-202.

Lebron, K., Milad, M. R. and Quirk, G. J. (2004). Delayed recall of fear extinction in rats with lesions of ventral medial prefrontal cortex. *Learn. Mem.* **11**, 544-548.
Likhtik, E., Pelletier, J. G., Paz, R. and Pare, D. (2005). Prefrontal control of the amygdala. *J. Neurosci.* **25**, 7429-7437.
Martin, J. H. (1991). Autoradiographic estimation of the extent of reversible inactivation produced by microinjection of lidocaine and muscimol in the rat. *Neurosci. Lett.* **127**, 160-164.
Milad, M. R. and Quirk, G. J. (2002). Neurons in medial prefrontal cortex signal memory for fear extinction. *Nature* **420**, 70-74.
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. and Rauch, S. L. (2009). Neurobiological basis of failure to recall extinction memory in posttraumatic stress disorder. *Biol. Psychiatry* **66**, 1075-1082.
Misane, I., Tovote, P., Meyer, M., Spiess, J., Ogren, S. O. and Stiedl, O. (2005). Time-dependent involvement of the dorsal hippocampus in trace fear conditioning in mice. *Hippocampus* **15**, 418-426.
Mitsukawa, K., Yamamoto, R., Ofner, S., Nozulak, J., Pescott, O., Lukic, S., Stoehr, N., Mombereau, C., Kuhn, R., McAllister, K. H. et al. (2005). A selective metabotropic glutamate receptor 7 agonist: activation of receptor signaling *via* an allosteric site modulates stress parameters *in vivo*. *Proc. Natl. Acad. Sci. USA* **102**, 18712-18717.
Morgan, M. A. and LeDoux, J. E. (1995). Differential contribution of dorsal and ventral medial prefrontal cortex to the acquisition and extinction of conditioned fear in rats. *Behav. Neurosci.* **109**, 681-688.
Morgan, M. A. and LeDoux, J. E. (1999). Contribution of ventrolateral prefrontal cortex to acquisition and extinction of conditioned fear in rats. *Neurobiol. Learn. Mem.* **72**, 244-251.
Morgan, M. A., Romanski, L. B. and LeDoux, J. E. (1993). Extinction of emotional learning: contribution of medial prefrontal cortex. *Neurosci. Lett.* **163**, 109-113.
Ohishi, H., Nomura, S., Ding, Y. Q., Shigemoto, R., Wada, E., Kinoshita, A., Li, J. L., Neki, A., Nakanishi, S. and Mizuno, N. (1995). Presynaptic localization of a metabotropic glutamate receptor, mGluR7, in the primary afferent neurons: an immunohistochemical study in the rat. *Neurosci. Lett.* **202**, 85-88.
Pare, D. and Smith, Y. (1993). The intercalated cell masses project to the central and medial nuclei of the amygdala in cats. *Neuroscience* **57**, 1077-1090.
Paxinos, G. and Franklin, K. B. J. (2001). *The Mouse Brain in Stereotaxic Coordinates*. San Diego, CA: Academic Press.
Santini, E., Ge, H., Ren, K., Peña de Ortiz, S. and Quirk, G. J. (2004). Consolidation of fear extinction requires protein synthesis in the medial prefrontal cortex. *J. Neurosci.* **24**, 5704-5710.
Schoepp, D. D. (2001). Unveiling the functions of presynaptic metabotropic glutamate receptors in the central nervous system. *J. Pharmacol. Exp. Ther.* **299**, 12-20.
Siegl, S., Flor, P. J. and Fendt, M. (2008). Amygdaloid metabotropic glutamate receptor subtype 7 is involved in the acquisition of conditioned fear. *Neuroreport* **19**, 1147-1150.
Sierra-Mercado, D., Padilla-Coreano, N. and 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.
Ugolini, A., Large, C. H. and Corsi, M. (2008). AMN082, an allosteric mGluR(7) agonist that inhibits afferent glutamatergic transmission in rat basolateral amygdala. *Neuropharmacology* **55**, 532-536.
Vidal-Gonzalez, I., Vidal-Gonzalez, B., Rauch, S. L. and Quirk, G. J. (2006). Microstimulation reveals opposing influences of prelimbic and infralimbic cortex on the expression of conditioned fear. *Learn. Mem.* **13**, 728-733.