Memories are not written in stone: Re-writing fear memories by means of non-invasive brain stimulation and optogenetic manipulations

Memories are not written in stone:

re-writing fear memories by means of non-invasive brain stimulation and optogenetic manipulations

Sara Borgomaneri[1,2*#], Simone Battaglia[1#], Giuseppe Sciamanna[2], Francesco Tortora[1°] & Daniela Laricchiuta[2]

[1] Centro studi e ricerche in Neuroscienze Cognitive, Dipartimento di Psicologia, Alma Mater Studiorum – Università di Bologna, Campus di Cesena, 47521 Cesena, Italy;

[2] IRCCS Fondazione Santa Lucia, 00179 Rome, Italy;

° now at: Department of Cognitive Sciences, Psychology, Education and Cultural Studies (COSPECS), University of Messina, Via Concezione 6, 98122, Messina, Italy.

# authors equally contributed

*Correspondence should be addressed to: Sara Borgomaneri, Department of Psychology, University of Bologna. Viale Berti Pichat 5, Bologna, 40127, Italy. e-mail: sara.borgomaneri@unibo.it

Keywords: Fear Conditioning, Memory Consolidation, Reconsolidation, Noninvasive Brain Stimulation, Optogenetic Manipulations

Abstract

The acquisition of fear associative memory requires brain processes of coordinated neural activity within the amygdala, prefrontal cortex (PFC), hippocampus, thalamus and brainstem. After fear consolidation, a suppression of fear memory in the absence of danger is crucial to permit adaptive coping behavior. Acquisition and maintenance of fear extinction critically depend on amygdala-PFC projections. The robust correspondence between the brain networks encompassed cortical and subcortical hubs involved into fear processing in humans and in other species underscores the potential utility of comparing the modulation of brain circuitry in humans and animals, as a crucial step to inform the comprehension of fear mechanisms and the development of treatments for fear-related disorders. The present review is aimed at providing a comprehensive description of the literature on recent clinical and experimental researches regarding the noninvasive brain stimulation and optogenetics. These innovative manipulations applied over specific hubs of fear matrix during fear acquisition, consolidation, reconsolidation and extinction allow an accurate characterization of specific brain circuits and their peculiar interaction within the specific fear processing.

1. Introduction

An aberrant fear learning process and its persistence may lead to the development of anxiety disorders, such as posttraumatic stress disorder (PTSD) (Indovina et al., 2011; Parsons and Ressler, 2013; Tortella-Feliu et al., 2019), which represents a highly debilitating psychiatric disorder affecting more than 4% of the population with genetic susceptibility (Duncan et al., 2018). Therefore, understanding how neural circuits are involved in the acquisition and consolidation of fear memories is fundamental for the development of new therapeutic protocols. To date, mechanisms of fear conditioning (FC) have been extensively investigated across different species (Haaker et al., 2019; Kim et al., 2016; Milad and Quirk, 2012; Vidal-Gonzalez et al., 2006), with the challenge of disclosing new ways to modify maladaptive fear memories. To this aim, many different methods have been explored (e.g., pharmacological or behavioral treatments) (Schiller and Phelps, 2011). However, findings from controlled laboratory studies using pharmacological manipulations of memory in humans have demonstrated that, although potentially effective, they present several limitations. They may induce side effects, or they may affect other aspects of memory, or, as in the case of direct β-adrenergic receptor agonists, they do not easily cross the blood-brain barrier. Furthermore, given that not every patient responds to pharmacological treatment, a pharmacological agent safe for human use has not been identified (Davis et al., 2010; Farach et al., 2012). On the other hand, about 28 percent of replication attempts in humans fail to demonstrate the retrieval-extinction effect (Chalkia et al., 2020; Golkar et al., 2012; Schiller et al., 2020; Soeter and Kindt, 2011). Given these premises, it is not surprising that in the last few decades, an incredible surge in interest in the neurobiology of FC has emerged (Fullana et al., 2020; Lonsdorf et al., 2019). Neural circuits underlying FC have been mapped, plastic properties of these circuits have been identified, and biochemical and genetic approaches are beginning to disentangle the molecular machinery responsible for the storage and retrieval of fear memories (Kim and Jung, 2006; Maren, 2001; Maren and Quirk, 2004). FC experiments commonly consist of a series of different experimental phases (e.g., habituation, acquisition, and extinction), and various protocols

can be applied to investigate the return of fear (e.g., reinstatement of fear; Haaker et al., 2014; Lonsdorf et al., 2017). The habituation or familiarization process is a preliminary phase before conditioned fear acquisition takes place. Habituation or familiarization phase to cue or contextual information in human FC precedes all the experimental manipulation and it may have various roles: (1) it establishing a baseline of responses, which allows the determination and correction for possible pre-conditioning differences in each participant, (2) allowing to assess a decline in responding over the first number of trials (i.e. orienting responses), (3) ensuring that participants understood the task (Lonsdorf et al., 2017). The acquisition of conditioned fear is achieved by presenting a neutral stimulus (NS) paired with an aversive event (unconditioned stimulus, US), a procedure referred to as FC. As a result of these pairings, fear learning is evidenced by an increasing conditioned response (CR) to the NS as it becomes a conditioned stimulus (CS+). While the CS+ response reflects the acquisition of conditioned fear, an additional NS, which is never paired with the US, is presented as a control stimulus (CS-). In human studies, CRs are commonly assessed as the differential response to the CS+ and CS-. This difference represents the most critical statistical index across acquisition and extinction training, as well as a measure of the return of fear (Battaglia et al., 2018; Borgomaneri et al., 2020a; Lonsdorf et al., 2017; Lonsdorf and Merz, 2017). Importantly, there are differences in the neural circuity supporting discriminative and non-discriminative FC. For example, in the case of very simple auditory stimuli (undiscriminated tones), the CS is transmitted through the auditory system to the thalamus and from there directly to the amygdala. In contrast, if an auditory discrimination is required, then the CS is transmitted from the thalamus to the auditory cortex and then to the amygdala (Phillips and LeDoux, 1992). This general scheme for auditory stimuli may be also applied to other sensory systems, especially the visual system.

Extinction learning is a well-known behavioral phenomenon that allows the organism to adapt their own behavior to a changing environment (Bouton, 2004). Extinction refers to the decrease in fear CRs that occur with repeated presentations of the CS+ that is no longer reinforced with the US. In

the past, extinction was described as a process of unlearning (i.e., the CS+ and US association is erased), but a substantial amount of evidence suggests that extinction does not destroy the original learning but instead generates new learning (for a review see Bouton, 2004). Much of the original learning has been shown to survive extinction (Battaglia et al., 2018; Borgomaneri et al., 2020a; Bouton, 2002; Delamater, 2004; Myers and Davis, 2002), thus extinction is widely thought to be a new form of inhibitory learning (Bouton, 2004). In fact, the return of fear is common following the passage of time (spontaneous recovery), when extinguished stimuli previously associated with aversive events are encountered outside the extinction context (contextual renewal) or when the US is presented in absence of the CS+ (reinstatement) (Bouton, 2004; Bouton and King, 1983; Vervliet et al., 2013). These effects provide support for the widely held view that extinction may be a new form of learning and that conditioning and extinction memories may coexist in distinct neural circuits and be independently reactivated based on environmental or situational factors (Dunsmoor et al., 2015; Kalisch et al., 2006; Milad and Quirk, 2012). Procedures that induce the return of fear in the laboratory may therefore be a fundamental control condition relevant to clinical relapse, which affects a substantial percentage of patients subjected to traumatic events (Craske, 1999). Indeed, from an evolutionary perspective, it is functional to never forget the most important life events, especially the negative ones, weakening emotional memories could be crucial to extirpate the root of many psychiatric disorders.

Many studies have demonstrated that even the most effective manipulations only eliminate fearful behavioral/psychophysiological responses (e.g., freezing in rodents and skin conductance response -SCR- changes in humans), while leaving the original fear memory trace (i.e., CS-US) intact, as demonstrated by recurrent relapse after successful extinction (Bouton, 2002). However, other studies have shown that fear memories can change when recalled, a phenomenon referred to as reconsolidation (Besnard et al., 2012; Elsey et al., 2018; Kindt et al., 2009). Reconsolidation is a process whereby previously consolidated memories can be reactivated and again made sensitive to alterations (Nader et al., 2000). Reconsolidated memories can be influenced by neurobiological

manipulations during or shortly after the memory reactivation period (Kindt et al., 2009; Tronson and Taylor, 2007).

In both animals and humans, considerable evidence indicates that blockade of the process of reconsolidation by using pharmacological manipulations produces amnesia for the original fear learning (Nader and Hardt, 2009; Sevenster et al., 2013). Among the most important studies in humans, for the first time, Kindt and colleagues (2009) tested the hypothesis that fear CRs can be weakened by disrupting the reconsolidation process of such memories and that such a disruption should permanently prevent the return of fear. Long-lasting disruptions in the reconsolidation were obtained by the administration of a β-adrenergic receptor antagonist (propranolol) prior to memory reactivation (Soeter and Kindt, 2011). Such suppression of the fear responses is in line with the idea that noradrenaline neurotransmission plays a critical role in learning and memory processes and that β-adrenergic receptor activation is important for post-retrieval stabilization of memories, as systemic injections with the β-adrenoceptor antagonist propranolol impair expression of aversive memories in rats that received reactivation (Przybyslawski et al., 1999).

The suppression of the fear responses could result from a more diffuse effect of propranolol administration by reducing the aspects of fear triggered by the aversive stimulus itself (i.e., the US rather than the CS-US association). However, the authors argued that propranolol injection specifically targeted the emotional expression of the memory (as suggested by the lack of effect of propranolol in the group without the reactivation of the fear memory to trigger the reconsolidation) and left declarative memory unchanged (Kindt et al., 2009; Soeter and Kindt, 2011). Pharmacological manipulations affect the reconsolidation process, leading to an incapacity to retrieve fear-conditioned memories, suggesting that they are erased or persistently weakened. However, these effects have not been consistently replicated (Wood et al., 2015). Cortisol has been shown to influence fear reconsolidation in men, but this effect has yet to be replicated in women (Meir Drexler et al., 2016). Unfortunately, the use of pharmacological manipulations in humans can be problematic (Schiller et al., 2010).

Currently, identifying new flexible and safe techniques in humans to target the fear memory process is of interest in the neuroscientific field (Schiller et al., 2010). On the one hand, some studies have aimed to modify fear memories by acting on the memory trace during the consolidation stage related to fear acquisition or extinction (Asthana et al., 2013; Guhn et al., 2012; Raij et al., 2018; Van 't Wout et al., 2016; Vicario et al., 2019). On the other hand, other studies have investigated the possibility of rewriting the emotional content of a memory by targeting the memory reconsolidation process (Borgomaneri et al., 2020a; Kindt et al., 2009; Mungee et al., 2016, 2014; Schiller et al., 2010). In recent years, noninvasive brain stimulation (NIBS) has established itself as an important form of therapy for neurological and psychiatric diseases (Fitzgerald et al., 2002; Kim et al., 2009; Lisanby et al., 2002) and as a crucial tool to investigate emotional processing in general (Borgomaneri et al., 2020b, 2015a; Paracampo et al., 2018, 2017; Vicario et al., 2017) and fear in particular (Borgomaneri et al., 2020b, 2017, 2015b, 2015c). Indeed, transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS) have been used to reveal the mechanisms underlying consolidation and reconsolidation of fear memories (Asthana et al., 2013; Borgomaneri et al., 2020a; Herrmann et al., 2019; Mungee et al., 2014). Based on the ability of NIBS to selectively interfere with activity in target brain regions, such techniques have been used to modulate cerebral activity during the consolidation and reconsolidation processes (Tan et al., 2019), with the ultimate goal of modulating these processes. The aim of this review is to integrate human and animal studies that have investigated the possibility of modulating fear memories by using NIBS (Table 1, Figure 1) and optogenetics to interfere with consolidation, reconsolidation, and extinction processes (Figure 2).

2. Neural bases of FC

To survive in a dynamic and challenging environment, individuals who encounter various contextual situations face imminent dangers. Prior knowledge of potential threats allows the detection of future dangers, the selection of appropriate and safe actions, and the development of

fear responses to threatening situations. In fact, during dangerous situations the associative learning increases the chances of survival by allowing individuals to anticipate a threatening event and respond preemptively, expressing species-specific fear behaviors (Blanchard and Blanchard, 1969). For example, freezing (an expression of fear, when the subject ceases all non-homeostatic motion), fleeing and fighting are common fear unconditioned responses (UR) in rodents (De Franceschi et al., 2016). For these adaptive fear responses to be developed, the brain must discriminate different sensory cues and associate relevant stimuli with aversive events (Maren and Fanselow, 1996). As previously reported, learned fear has been extensively studied using the classic FC paradigm, which has perhaps provided the most useful window for analyzing the neural and molecular basis of fear associative learning and memory formation (Davis, 2000; LeDoux, 2000). In auditory or contextual FC, a tone or a specific context (representing the CS) is associated with one or more shocks (representing the US) (Fanselow and Gale, 2003; LeDoux, 2000). The acquisition of CS-US associative memory requires brain processes of coordinated and distributed neural activity within the amygdala, medial prefrontal cortex (mPFC, encompassing in mice the anterior cingulate cortex and the rostral and ventral parts of the prefrontal pole, and in humans areas 24, 25, and 32 labelled with Brodmann's numbers), and hippocampus (Corcoran and Quirk, 2007; Etkin et al., 2011; Goshen et al., 2011; Quirk and Mueller, 2008; Zhu et al., 2014). As regard the amygdala, this almond-shape area is composed of functionally and morphologically heterogeneous subnuclei with complex interconnectivity. Specifically, the basolateral amygdala (BLA) is primarily glutamatergic (Carlsen, 1988), conversely the central amygdala (CeA), encompassing the centrolateral (CeL) and centromedial (CeM) nuclei, is mainly GABAergic (McDonald, 1982). Thus, BLA neurons could excite GABAergic CeL neurons that provide feed-forward inhibition onto CeM neurons that, in turn, contributes to mediate autonomic and behavioral fear responses via projections to the brainstem (Ressler and Maren, 2019; Tye et al., 2011). In turn, inhibitory networks within the BLA appear to play a crucial role in shaping and magnifying the difference in excitability between glutamatergic projection neurons (Krabbe et al., 2018).

Direct activation of principal neurons within the BLA is a putative mechanism by which stimuli are associated during learning (Johansen et al., 2010; Tye et al., 2011).

In animals, after conditioning, the tone alone can induce freezing in previously conditioned subjects. In humans, the crucial role of the amygdala and hippocampus has been demonstrated in brain-lesioned patients, showing that selective bilateral damage to the amygdala impeded the acquisition of CRs but not declarative memory, while selective bilateral damage to the hippocampus prevented the acquisition of declarative memory but preserves fear learning (Bechara et al., 1995). Similarly, lesions of the ventral part of the mPFC –ventromedial prefrontal cortex (vmPFC)– prevented the acquisition of fear memories (Battaglia et al., 2020).

Furthermore, FC establishes the CS as secondary incentive that can motivate avoidance behaviors. In fact, individuals can also learn instrumental responses and avoidance CR when responding to a CS to avoid a threatening event (Crawford and Masterson, 1982; Maia, 2010; Wendler et al., 2013). To know when to act in order to avoid an aversive event signaled by a cue, the subject must first learn that the specific cue predicts the aversive event and then choose the instrumental action to avoid the announced aversive event. In other words, CS can serve as a motivating factor to initiate active responses that reduce exposure to the fear arousing stimulus. There is compelling evidence that the striatum and other regions of the basal ganglia play a role in learning how to select actions that result in rewarding outcomes (Laricchiuta et al., 2020) as well as in instrumental responses to avoid aversive stimuli (Wendler et al., 2013).

As already mentioned, the fear CR to a CS+ may be gradually weakened by repeated exposure to unreinforced CS+, that is, presentation without the US (i.e., extinction procedure) (Pavlov, 1927). Extinction creates a new CS–noUS memory trace, competing with the initial fear (CS–US) memory and the recall of extinction memory (i.e., CR inhibition at later CS encounters) is facilitated by contextual cues present during extinction training (Kalisch et al., 2006). In fact, context appears to be a critical regulatory factor in the expression of this putative competition (Bouton, 2004). Additionally, the acquisition and maintenance of extinction memories critically depend on

amygdala-mPFC projections (Lacagnina et al., 2019; Tovote et al., 2015; Trouche et al., 2013). Interestingly, within the BLA populations of neurons distinct on the basis of electrophysiological or neurotransmission properties were identified to manage fear extinction responses (Herry et al., 2008; Sotres-Bayon et al., 2007). Specifically, Zimmerman and Maren (2010) showed that infusions of an N-methyl-D-aspartate (NMDA) receptor antagonist into the BLA impaired the acquisition of long-term extinction memory. Cooperating with the amygdala, the mPFC integrates information from multiple inputs to exert top-down control, allowing for appropriate responses. In fact, a widely validated mouse model suggests that the balance between expression and suppression of learned fear responses is modulated by inputs from/to the amygdala to/from two subregions of the mPFC: the prelimbic cortex (PrL), the rostral part of the mPFC considered to be a core component of the anterior cingulate cortex, which supports fear expression, and the infralimbic cortex (IL), the ventral part of the mPFC, which contributes to fear extinction (Klavir et al., 2017; Sierra-Mercado et al., 2011). Together with these areas, the hippocampus has been found to play an important role in explicit recalling extinction in humans, showing a vmPFC–hippocampal network that provides for context-dependent recall of extinction memories (Kalisch et al., 2006). In support of these findings, lesions of the hippocampus have been found to interfere with FC situations involving complex polymodal events particularly those for which spatial organization is important and involving more than one processing modality (Phillips and LeDoux, 1992). During the reconsolidation process, the same amygdala-mPFC-hippocampus brain network seems to be recruited and contributes to maintaining the delicate equilibrium between fear and safety representations. The crucial role of the amygdala during reconsolidation has been demonstrated by the administration of anisomycin, a drug that inhibits protein synthesis and activates stress-activated protein kinases and other signal transduction pathways. Anisomycin administration during the fear reactivation period resulted in intact short-term post-reactivation and impaired long-term postreactivation fear memories, suggesting a successful blockade of the reconsolidation process (Nader et al., 2000). In parallel, intrahippocampal anisomycin infusions caused amnesia for consolidated hippocampal-dependent

contextual fear memory, but only if the memory was reactivated prior to infusions, thus inducing systems-level reconsolidation (Debiec et al., 2002). Such an effect was consistently replicated even if the reactivation delay was 45 days, a time point at which contextual memory should be independent of hippocampal activation.

Finally, while previous human neuroimaging and patient studies have primarily implicated the dorsolateral part of the prefrontal cortex (dlPFC Brodmann's areas 9 and 46) in the cognitive regulation of emotional processes (Fullana et al., 2016; Ochsner et al., 2012), other studies have suggested that this brain region is also involved in some aspects of threat response reduction and fear memory modulation (Asthana et al., 2013; Mungee et al., 2014; Van 't Wout et al., 2016). Namely, the dlPFC seems crucially involved in the control of retrieval and reactivation of memory traces and their gradual consolidation (Cabeza and Nyberg, 2000; Eichenbaum, 2017; Moscovitch and Winocur, 2002; Sandrini et al., 2013; Simons and Spiers, 2003). Therefore, the neural modulation of the activity of both the vmPFC and dlPFC has been the focus of several NIBS studies.

3. NIBS to modulate fear memories

NIBS with TMS or transcranial electrical stimulation (tES) (Dayan et al., 2013; Reed and Cohen Kadosh, 2018; Rossi et al., 2009; Rossini et al., 1994) is valuable in research and has potential therapeutic applications in cognitive neuroscience, neurophysiology, psychiatry, and neurology. TMS allows neurostimulation and neuromodulation, while tES is a purely neuromodulatory application. TMS and tES allow diagnostic and interventional neurophysiology applications, and focal neuropharmacology delivery. NIBS provides a valuable tool for interventional neurophysiology applications, modulating brain activity in a specific, distributed, cortico-cortical/subcortical network (Chiappini et al., 2020; Fiori et al., 2017, 2016; Zanon et al., 2018). Thus, NIBS is considered to be a promising treatment for a variety of medical conditions (Wagner et al., 2007).

tES alters brain functions by passing an electric current through the cerebral cortex. Part of the current is absorbed by the skull, while another part penetrates the scalp and modulates cortical excitability (Reed and Cohen Kadosh, 2018). tES includes several techniques, such as tDCS and transcranial alternating current stimulation (tACS). In the tDCS technique, direct current is applied over the scalp to modulate human brain activity (George and Aston-Jones, 2010). Through this type of stimulation, feeble electric currents (1-2 mA) are conducted through two electrodes (anode and cathode), which increase or decrease neuronal activity by changing the membrane potential (Nitsche and Paulus, 2000). Although the exact functioning of tDCS is not entirely clear, it is well established that several minutes of anodic stimulation excite neurons in the stimulated area, allowing their depolarization. Conversely, cathodic stimulation has an inhibitory effect (Nitsche et al., 2008). This suggests that tDCS allows the modulation of cortical excitability (Arul-Anandam and Loo, 2009). In contrast, during tACS stimulation, a direct current is not applied, but it oscillates between the electrodes in sinusoidal waves (Reed and Cohen Kadosh, 2018). Finally, TMS is a neurostimulation and neuromodulation technique based on the principle of electromagnetic induction of an electric field in the brain. This field can be of sufficient magnitude and density to depolarize neurons, and when TMS pulses are repetitively applied, they can modulate cortical excitability. In particular, it has been shown that repetitive TMS (rTMS) can temporarily modify brain function for minutes to hours (Huang et al., 2005; Iyer et al., 2003; Jung et al., 2008). Stimulation at low (≤1 Hz) or high (≥5 Hz) frequencies can decrease or increase neuronal excitability, respectively (Klomjai et al., 2015).

Please_insert_Table_1_near_here

3.1 Changing fear memories by interfering with the consolidation process

Consolidation theory assumes that during acquisition, the memory trace is unstable and labile (i.e., susceptible to interference) for a limited time window after encoding. After this time period, memory traces become robust and resistant to interference (Alberini and LeDoux, 2013). In light of this, studies have investigated the possibility of modulating fear memories by interfering with the consolidation process by using NIBS, which is applied while memories are still in a labile state (immediately after the acquisition phase). One of the first studies investigated tDCS effectiveness on the consolidation process of fear memories (Asthana et al., 2013). The experimental design consisted of two days: in the first session (day 1), healthy participants underwent an auditory FC paradigm, in which a CS (i.e., colored square) was paired with an aversive auditory stimulus (i.e., loud auditory tone; US). Participants were randomly divided into three groups based on the tDCS protocol: anodal, cathodal and sham stimulation. On day 1, 10-20 min after fear acquisition, participants underwent brain stimulation (12 min, 1 mA). The tDCS electrodes were placed on the left dlPFC (electrode position F3, according to the electroencephalogram (EEG) electrode placement system, and the left mastoid as the reference electrode). On day 2, participants underwent an extinction procedure in which both the CS+ and CS- were presented in the absence of the US. The effects of fear acquisition and consolidation (24 hours later) were measured by means of SCRs. The authors found that cathodal stimulation of the left dlPFC, delivered few minutes after the acquisition of a fear memory, disrupted its consolidation through an inhibitory action, as indicated by a decreased SCR to the CS+ when compared to CS- during extinction learning on day 2. No changes in SCR were observed after anodal or sham stimulation. These data demonstrated the crucial role of the left dlPFC in the consolidation of fear memories.

In a subsequent study (Guhn et al., 2014), healthy volunteers underwent a FC paradigm and subsequently received an rTMS session over the mPFC to affect the consolidation process by increasing the inhibitory top-down regulation by the mPFC over amygdala activity. During day 1, participants underwent a FC protocol in which two neutral faces (CSs) were paired with a loud

auditory tone (US). On the same day, before extinction, the experimental group received rTMS (10 Hz, pulse intensity at 110% of the individual resting motor threshold—rMT) over the mPFC (Fpz electrode), while the control group received sham stimulation. Immediately after stimulation, participants underwent extinction learning. On day 2, participants underwent the extinction recall. Fear potentiated startle (FPS) responses and SCRs were used as dependent variables to assess fear responses. The authors found that participants who received rTMS showed decreased fear CRs to CS+ during extinction learning (i.e., lower FPS) and to a lesser extent, they showed decreased SCRs to the CS+, as well as altered subjective arousal ratings (i.e., the rTMS group discriminated significantly less between CS+ and CS−). The rTMS effect persisted in the recall of extinction, as demonstrated by FPS results. Conversely, the sham group showed CRs characterized by greater arousal during extinction learning (i.e., the sham group persisted in evaluating the CS+ as more arousing than the CS−). These findings demonstrated that active stimulation of the mPFC improves the retention of extinction memories. These data showed that it is possible to modulate FC by inhibiting the dlPFC immediately after the acquisition phase (Asthana et al., 2013) or by increasing mPFC activity (Guhn et al., 2014), suggesting that these two areas act in potentially opposite ways during the consolidation of fear memories.

3.2 Changing fear memories by interfering with the extinction process

It has been demonstrated that NIBS applied soon after the end of the acquisition phase is effective in manipulating the consolidation of fear memories (Asthana et al., 2013; Guhn et al., 2014). However, PTSD patients may be treated many years after the traumatic event or events have occurred; therefore, it appears to be necessary to act on a subsequent process, that is, attempting to impact the extinction process with the application of NIBS.

Van't Wout and colleagues (2016) assessed whether anodal tDCS applied over the vmPFC during extinction learning could increase extinction and subsequent recall in healthy volunteers. The experimental paradigm consisted of a 2-day crossover design. On day 1, participants underwent fear

acquisition and extinction learning, while on day 2, extinction memories were recalled. Two CS+ (i.e., colored squares) paired with a US (i.e., mild electrical stimulation) were presented during the acquisition phase, and SCR was used as a dependent variable to evaluate fear learning and extinction. The extinction phase was divided into two blocks, and in each block, only one CS+ was extinguished. Furthermore, one group of participants received anodal tDCS (10 min, 2 mA) over the vmPFC (AF3 electrode with the contralateral mastoid as a reference electrode) before and during the first extinction block, while sham stimulation was applied during the second block. The other group received the opposite stimulation protocol (sham stimulation before and during the first extinction block and anodal stimulation during the second block). The results showed that the group that selectively underwent tDCS stimulation before and during the first extinction block exhibited rapid extinction of the unextinguished CS+ compared to the other group. No effects were found in the group that underwent anodal tDCS stimulation during the second block. These results demonstrated that NIBS is selectively effective if applied immediately at the onset of the extinction process, suggesting a time-locked window of action.

In a similar attempt to increase extinction learning, anodal tDCS (20 min, 1.5 mA) was applied over the vmPFC (F7 as anode electrode with F8 as a reference electrode) (Dittert et al., 2018). This double-blind randomized study was conducted in one day: participants underwent the acquisition of FC (i.e., two neutral-looking female faces were used as CSs paired with a loud auditory tone which acts as US), which was evaluated by means of SCRs. A few minutes after fear acquisition, anodal tDCS was applied, and the stimulation lasted until the end of the extinction phase. The results showed that tDCS enhanced the extinction learning process (i.e., weaker fear response to the CS+ with a simultaneous increase in response to the CS-). Thus, it seems that stimulation could interfere with CS safety information processing, usually acquired during conditioning, which is typically mediated by the vmPFC (Battaglia et al., 2020; Fullana et al., 2018, 2016; Suarez-Jimenez et al., 2018). However, mPFC has been shown to be involved in different stages of FC (Battaglia et al., 2020; Fullana et al., 2016) but it also seems to be important for the suppression of fear reactions.

The background mechanism of these reduced fear reactions can be explained by an improvement of extinction learning, or by the simple reduction of fear expressions. Thus, authors could not definitively decide whether the tDCS in their study improved extinction learning or just suppressed fear expressions.

A further demonstration that anodal tDCS (10 min, 2 mA) during extinction learning had a facilitatory effect on extinction learning comes from a recent study in which the group that received anodal tDCS over the vmPFC (AF3 electrode with the contralateral mastoid as a reference electrode), compared to the control group, did not show significant differences between the CS+ and CS-, evaluated with SCRs (Vicario et al., 2019).

In another study, Raij and colleagues (2018) investigated the possibility of modulating fear memory extinction by means of rTMS over the vmPFC. This experimental design was divided into three days: on day 1, participants were fear-conditioned towards two different CSs (i.e., colored lights) paired with a mild electrical stimulation (US). On day 2, rTMS was paired with one of the two CSs during extinction learning ($CS+^{TMS}$ and $CS+^{noTMS}$). The rTMS protocol consisted of short trains of impulses (300 ms at 20 Hz; pulse intensity at 100% of the rMT), which started 100 ms after the onset of the CS+. rTMS was applied over two target areas in the left frontal cortex: one functionally connected with the vmPFC and the other unconnected (control stimulation site). Authors conducted a psychophysiological interaction (PPI) analysis of functional magnetic resonance imaging (fMRI) data recorded during FC to reveal surface candidate areas connected to vmPFC that can be directly stimulated by TMS. The SCR was used as an index of FC. On day 3, the authors assessed the effects of rTMS stimulation through extinction recall. The results showed that during extinction recall, fear expression was strongly reduced only for the $CS+^{TMS}$ when rTMS was selectively applied to the area connected with the vmPFC, thus ruling out the possibility that rTMS may act as an occasion setter. This result suggested that similar to tDCS, rTMS is capable of enhancing extinction learning.

Taken together, these studies provide evidence that NIBS applied in a state-dependent manner during fear extinction (i.e., rTMS or anodal tDCS over the vmPFC and cathodal tDCS over the dlPFC) facilitates fear extinction, resulting in a diminished capacity to respond to CS+ and CS- presentations as discriminative stimuli, thus weakening the strength of FC. Moreover, it has been reported a crucial role of timing of the application of the NIBS protocol, which needs to be applied at the onset of the extinction learning (Van 't Wout et al., 2016). Importantly, studies suggest that extinction learning which takes place directly after conditioning may have distinct neuronal mechanisms compared to extinction learning that is started after the completion of the consolidation of fear acquisition. Indeed, different processes seem to be at play, such as learning deleting processes for immediate extinction, whereas a new associative learning process for delayed extinction learning (Myers et al., 2006).

Although promising, none of these studies can ensure that fear memories were definitively erased, since none of them have investigated possible return of fear. Indeed, the new frontier of NIBS is the connectivity-based rTMS, which allows the indirect modulation of deep brain structures (i.e., the amygdala) by applying rTMS over a cortical node highly connected to these regions (Baeken et al., 2010; Beynel et al., 2020).

3.3 Changing fear memories by interfering with the reconsolidation process

In the previous sections, we described that NIBS techniques are able to interfere with the consolidation process, namely, affecting acquisition or, in most cases, extinction learning. This was dictated by the clinical urge to improve anxiety treatments to support existing therapies, such as exposure-based therapy, which, however, seem to not be powerful remedies (McNally, 2007). Indeed, one of the main issues associated with the treatment of PTSD is the return of fear, even after extinction learning procedures (Milad et al., 2008). This is a critical point since extinction learning does not overwrite traumatic memories but creates a new inhibitory memory trace that decays over time, allowing the return of aversive memories (Bouton, 2004). To overcome this issue, many

studies have investigated the possibility of modulating fear memories by means of modifying the original traumatic trace by interfering with the reconsolidation process (Kindt et al., 2009; Schiller et al., 2010; Schiller and Phelps, 2011).

Reconsolidation refers to the process through which consolidated memories may return to an unstable state when reactivated by a reminder (i.e., external information associated with the stored memory). These memories can be consolidated again or reconsolidated (Nader et al., 2000). Thus, reconsolidation refers to a time-locked process that can restabilize memory after reactivation (Alberini and LeDoux, 2013; Monfils et al., 2009; Nader and Hardt, 2009; Schwabe et al., 2014). During the reconsolidation time window, memories are subjected to modifications. However, reconsolidation does not occur every time a memory is reactivated. Several factors mediate the activation of this process: the strength of the memory trace (Suzuki et al., 2004), the length of the reactivation, and the generation of a prediction error during the reactivation (Milekic and Alberini, 2002; Pedreira et al., 2004). It is necessary to violate previously learned expectations to induce the extinction as well as the reconsolidation process (Merlo et al., 2014), through which memories can be updated and possibly modified (Forcato et al., 2009; Pedreira et al., 2004). The studies presented in this section shed new light on the possibility of manipulating fear memories by applying NIBS during the reconsolidation process. Compared to previous studies, these experimental designs consist of three days (Agren, 2014), and the reactivation of the acquired fear memory through the use of a reminder on day 2 is a prerequisite for the reconsolidation process (Sevenster et al., 2013).

The effects of tES on the reconsolidation of fear memories have been investigated in the study by Abend and colleagues (2016). On day 1, participants underwent acquisition of FC (photographs of a light-haired woman and a dark-haired woman displaying neutral, closed-mouthed neutral expressions were used as CSs and paired with a loud auditory tone used as US). On day 2, a single CS+ was presented together with the US as a reminder of the fear memory trace, and subsequently, the participants underwent the extinction phase. During the extinction phase on day 2, participants were randomly divided into two stimulation groups: direct current (20 min, 1.5 mA) to yield

memory-enhancing effects associated with long-term potentiation and alternating current (20 min, 1 Hz) to affect cortical oscillations in the mPFC. In the control group, sham stimulation was applied over the mPFC (anode electrode was placed centrally over the forehead). On day 3, the results showed that alternating current stimulation enhanced fear responses, while direct current stimulation led to a generalization of the fear response to nonconditioned stimuli (i.e., the response to the CS- was comparable to the response to the CS+). These data demonstrate the complex role of vmPFC function during fear extinction; for example, inducing enhanced activation during extinction learning did not directly translate into enhanced extinction retrieval. Similarly, low-frequency alternating current stimulation, which is expected to interfere with reconsolidation of fear memory, was found to enhance fear responses.

Another study that investigated the effect of NIBS on the reconsolidation process applied tDCS over the right dlPFC (Mungee et al., 2014). The authors hypothesized that anodic tDCS (20 min, 1 mA) over the right dlPFC (F4 electrode with the left supraorbital area as a reference electrode) could induce facilitatory plasticity in the cortex, which would result in stronger fear memory. On day 1, fear acquisition took place (colored squares as CSs paired with a mild electrical stimulation which acts as US), and 24 hours later (day 2), all participants were reminded of the CS+ through a single presentation without the US. According to the authors, this procedure should have induced the reconsolidation process. Subsequently, anodal tDCS was applied over the right dlPFC. On day 3, the authors tested whether the fear CR had been influenced by the stimulation through presentations of the CS+ and CS-, both without a US. The anodic tDCS-stimulated participants showed a stronger CR (i.e., mean differential SCR on day 3), indicating that anodal tDCS of the right dlPFC may have resulted in a strengthening of the memory trace encoding for conditioned fear memories.

In a subsequent study with the same paradigm, Mungee et al. (2016) demonstrated that the application of cathodal tDCS (20 min, 1 mA), aimed at decreasing neuron excitability, over the right dlPFC (F4 electrode with the left supraorbital area as a reference electrode) did not affect fear

memories, as suggested by the lack of significant differences in fear CRs between the stimulated and control (sham) groups. Furthermore, a recent study (Ganho-Ávila et al., 2019) found that cathodal tDCS (20 min, 1 mA) over the right dlPFC (F4 electrode with the contralateral deltoid as a reference electrode) affected extinction (1 to 3) months after the tDCS session. The experiment consisted of the acquisition of FC (i.e., colored squares paired with a loud auditory tone used as US) on day 1, cathodal tDCS after verbally recalling the CS+ to induce reconsolidation and extinction on day 2, and a follow-up session of reinstatement and re-extinction one to three months later. The reinstatement phase consisted of four consecutive unsignaled US presentations, and the subsequent re-extinction phase was similar to day 2. Fear responses were measured with self-report ratings on valence, arousal, contingency and expectancy, SCR, and implicit avoidance tendencies (approach-avoidance task). The results showed no effects in extinction, according to self-reports and SCR on day 2. However, one to three months after tDCS stimulation and re-extinction, there were differences between groups measured by the approach-avoidance task; in particular, the tDCS-stimulated group (but not the sham-stimulated group) showed safety behavior (a positive bias towards the CS-, which was not present in the sham group). Accordingly to Kindt et al. (2009), cathodal tDCS did not improve explicit memory-associated measures. However, the reported findings suggested that cathodal tDCS may have enhanced long-term distinctiveness between threatening and safety cues.

Finally, in a 3-day study, our group of researchers (Borgomaneri et al., 2020a) employed rTMS (1 Hz, pulse intensity at 110% of the rMT) over the dlPFC 10 min after a reminder cue that reactivated a fear memory acquired 1 day before. The day after rTMS, participants exhibited decreased physiological expressions of fear, as shown by their SCR. Similar fear reductions were observed when targeting the left and right dlPFC. In contrast, no decrease was observed in participants tested immediately after dlPFC-rTMS or in participants receiving either control rTMS (i.e., active control site and sham stimulations) or dlPFC-rTMS without the preceding fear-memory reactivation; these data showed both the site and time specificity as well as the state dependency of the rTMS

intervention. In fact, the expression of fear was reduced only when dlPFC-rTMS was administered within the reconsolidation time window. Moreover, dlPFC-rTMS prevented the subsequent return of fear after extinction training. These findings highlighted the causal role of the dlPFC in fear-memory reconsolidation and suggested that rTMS can be used in humans to prevent the return of fear.

Taken together, these data demonstrated that enhancing dlPFC activity within the reconsolidation window results in increased fear memory (Mungee et al., 2014), while its inhibition leads to a decrease in fear memory (Borgomaneri et al., 2020a; Ganho-Ávila et al., 2019).

Please_insert_Table_2_near_here

Please_insert_Colored_Figure_1_near_here

---

4. Optogenetic stimulation as a tool to reveal the causal relationship between neural firing and behavior

Many studies have assessed the role of brain circuits in fear memory acquisition, storage, extinction and reinstatement by using brain lesions, pharmacological manipulations and electrophysiological techniques (Davis, 1992; Fanselow and Poulos, 2005; LeDoux, 2000; Maren and Quirk, 2004; Pape and Pare, 2010). For example, the development of rationally designed pharmaceuticals that directly act on fear-inhibiting circuitry depends on discovering the molecular identities of neuronal populations that specifically mediate fear extinction. Thus, to dissect the function of different cell subtypes, a variety of techniques have been introduced. One of the most revolutionary techniques,

optogenetics, has offered advanced temporal precision using a combination of light presentation with genetic manipulations (Boyden et al., 2005). By injecting animals with viral vectors encoding specific rhodopsin variants (e.g., channelrhodopsin or halorhodopsin) under specific promoters, the genes are altered to change the membrane ion channels of selected subpopulations of neurons in the brain. The changes to the membrane ion channels make these specific neurons sensitive to different types of light (e.g., red, green, blue). By inserting fiber optics into the specific brain region and shining a particular type of light, action potentials are triggered in the neurons of interest (e.g., channelrhodopsin depolarizes the neuronal membrane under light stimulation) (Williams and Deisseroth, 2013). Using similar techniques, it is also possible to modify neurons so that firing is inhibited when light is presented (e.g., halorhodopsin hyperpolarizes the neuronal membrane under light stimulation) (Berndt et al., 2014). Thus, this technique provides precise control over when a neuron fires, enabling us to better understand the causal relationship between neural firing and behavior. In fact, *in vivo* optogenetic techniques allow for examination of the effects of acute and reversible neural activation or silencing on behavior in the same experimental subjects, with a high degree of temporal precision. For example, turning on BLA glutamatergic projection neurons in the CeA exerted an acute, reversible anxiolytic effect, while direct photostimulation of the BLA somata led animals to react more anxiously (Tye et al., 2011). Turning off ventral tegmental area neurons afferent to nucleus accumbens core as well as nucleus accumbens core projections to dorsolateral ventral pallidum reduced cocaine use in animals addicted to that drug (Stefanik et al., 2013). The development of optogenetics is an excellent example of how cutting-edge methods allow researchers to ask increasingly direct questions about biology and behavior in FC (Gafford and Ressler, 2016).

5. Optogenetic manipulation effects on fear acquisition and consolidation

Adopting a gain-of-function strategy to test the role of the activation and/or reactivation of fear memory ensembles in acquisition and consolidation, many optogenetic manipulations have been

performed by stimulating or inhibiting specific neurons characterized by specific types of neurotransmission in specific brain areas involved in fear learning (Beyeler et al., 2014; Hardt and Nadel, 2018). Although a complete literature review evaluating the studies that have used optogenetic manipulations to modulate fear acquisition is out of the scope of the present work (for discussions of this topic see: Belzung et al., 2014; Beyeler et al., 2014; K. M. McCullough et al., 2016), some studies are reported, for the sake of clarity.

First, it has been demonstrated that optogenetic activation of pyramidal neurons in the lateral amygdala (LA) along with the presentation of a tone in the absence of any US was sufficient to produce fear learning, but only when many training trials were used (Johansen et al., 2010) or when a beta noradrenergic receptor agonist was microinjected directly into the LA before auditory CS-photostimulation pairings (Johansen et al., 2014). The norepinephrine-enhanced effect was in line with the evidence points to the release of norepinephrine and stimulation of beta noradrenergic receptors as a key mechanism by which to increase the amygdaloid neural plasticity mediating threat conditioning (Bush et al., 2010).

Erythropoietin-producing hepatocellular (Eph) receptors, further divided into two subfamilies EphA and EphB, comprise the largest receptor tyrosine kinase family in mammals (Boyd et al., 2014), regulating important developmental processes by responding to cell-cell contacts and transmitting downstream signals into the respective cells that results in the so-called 'forward' signaling downstream (Lisabeth et al., 2013). Namely, EphB2 is brain-expressed and crucial for dendritic spine development, and synapse maintenance (Locke et al., 2017).

Activation of EphB receptors enhances glutamatergic transmission and gene expression molecular events involved in memory formation (Lamprecht and LeDoux, 2004) and long-term memory (LTM) (Takasu et al., 2002). Locke and colleagues (2017) used optogenetic techniques to induce EphB2 forward signaling by light at the highest required spatiotemporal resolution *in vivo*. The activation of EphB2 forward signaling in LA pyramidal neurons during learning, but not afterward, enhances long-term, but not short-term, auditory FC by controlling its consolidation (Alapin et al.,

2018). Moreover, EphB2 forward signaling during FC activates Ca^{2+}/cAMP-responsive element binding protein (CREB) in LA neurons.

In a recent paper (Jiang et al., 2016), BLA cholinergic fibers were concurrently photoactivated with each CS-US pairing during the training period and 24 hours posttraining; the extent of fear learning was assessed to the CS+ alone, and photostimulation was without any significant effect. Conversely, photoinhibition of cholinergic terminal fields within the BLA during training resulted in a strong and immediate inhibition of the freezing response to CS-US pairings. Measures of recall of fear learning 24 hours after training again revealed significantly increased freezing behavior in response to the CS+ in both control and photoinhibited mice. However, comparisons of the extent of recall revealed that the learned associations were decreased by photoinhibition during training. In the same phase, pairing optogenetic activation of BLA glutamatergic pyramidal neurons with CS-US presentations inhibited fear consolidation, as indicated by the attenuated freezing that the animals showed when tested the following day in the absence of optogenetic stimulation (Jasnow et al., 2013). However, inhibition of glutamatergic neurons during acquisition caused no changes in within-session freezing behavior (Kenneth M. McCullough et al., 2016). Furthermore, the BLA contains a variety of inhibitory interneurons having a role in FC (Ehrlich et al., 2009). One of the major interneuron subclasses in the BLA expresses the calcium binding protein parvalbumin and preferentially forms synapses at the perisomatic region of their target cells, thus controlling neuronal activity and spike output (McDonald and Betette, 2001; Muller et al., 2006). Conversely, other interneurons expressing somatostatin preferentially contact the distal dendrites, thus controlling the impact of inputs to their target cells (Muller et al., 2007). During the associative learning, the parvalbumin- and somatostatin-expressing inhibitory interneurons exert bidirectional control on BLA output/input by influencing perisomatic domain or dendrites of principal neurons. Specifically, pairing optogenetic activation of parvalbuminergic neurons within the BLA with entire CS-US presentations or just during US presentations resulted in decreased freezing responses during the CS+ presentations the following day when the mice were tested in the absence of

optogenetic stimulation. Interestingly, when parvalbuminergic neurons were optogenetically stimulated only during CS+ presentations, freezing levels were increased, which demonstrated the opposite roles of these neurons during CS+ and US processing (Wolff et al., 2014). Additionally, the inhibition of parvalbuminergic neurons during US presentations caused an increase in freezing the following day in the absence of optogenetic stimulation. Importantly, manipulation of somatostatin-containing neurons during CS+ presentations resulted in the opposite behavioral effects. Additionally, the effects of FC on parvalbumin-positive and somatostatin-positive pyramidal neurons in the IL-mPFC in male preadolescent, adolescent, and adult mice were evaluated using an *in vitro* optogenetic approach (Koppensteiner et al., 2019). While synaptic inhibition mediated by parvalbumin-positive pyramidal neurons did not result in age-specific or fear behavior-specific plasticity, synaptic inhibition mediated by somatostatin resulted in adolescence-specific enhanced plasticity and was suppressed by fear learning, which overlapped with reductions in calcium-permeable glutamate receptors.

In addition, the tachykinin 2 (Tac2) pathway has been shown to be necessary for the modulation of fear memories in mice (Andero et al., 2014). In fact, upregulation of Tac2 expression in the amygdala 30 min after auditory FC has been reported. In transgenic mice, *in vivo* optogenetic stimulation of CeA Tac2-expressing neurons during fear acquisition enhanced fear memory consolidation, and these effects were blocked by osanetant, a potent non-peptide antagonist of the tachykinin NK3 receptor, and that is considered to be a drug generally safe and well tolerated in humans (Malherbe et al., 2011). These findings could potentially be rapidly translated into clinical practice, unlike the findings of studies using animal models focused on modulating the original fear memory with traditionally-used chemicals that impair synapses or neurons (Nader et al., 2000; Shema et al., 2007). In fact, such compounds are not allowed for use in humans, making these approaches inappropriate for clinical purposes.

By using a c-fos-based genetic tagging system that also selectively expresses a channelrhodopsin (the light-activated cation channel) variant in neurons naturally activated during contextual FC

(Cowansage et al., 2014), the activated neuronal ensembles in the retrosplenial cortex (RSC) were stimulated at a high frequency (De Sousa et al., 2019), shown to be connected to both hippocampus and neocortical areas, and required for recent and remote contextual FC retrieval (Todd and Bucci, 2015). Posttraining stimulation of the RSC, activated during contextual FC, produced a recent memory that displayed numerous features of consolidated remote contextual FC memories, including decreased hippocampal dependence, context generalization, and greater engagement of neocortical areas during retrieval (De Sousa et al., 2019). Overall, these findings suggested that this kind of activity is able to provoke physiological changes similar to those observed during natural consolidation. Moreover, these changes were observed only when ensemble reactivation was performed during light anesthesia or natural sleep but not during active awake states. In this regard, it has been noted that without explicit external stimuli, cortical neurons exhibit spontaneous activity, which may not only be increased but even suppressed by sensory stimuli (Hromádka et al., 2008; Zhou et al., 2010). This inhibition-based modulation may contribute to stimulus-driven behaviors and associative memories of sensory stimuli (Harris and Mrsic-Flogel, 2013). To silence excitatory neurons in the auditory cortex (ACx), an adeno-associated virus vector expressing activity-regulated cytoskeleton-associated protein (Arc) under the control of the calcium/calmodulin-dependent protein kinase II (CaMKII) promoter was injected (Nomura et al., 2015). Notably, Arc protein has been particularly linked to plasticity processes and cognitive demands, and it is frequently used as a marker of cell activation, allowing each cell activated during particular behavioral outcomes to be identified (Nakamura et al., 2006; Sauvage et al., 2013). Green light illumination of Arc-expressing neurons induced an outward current and inhibited action potentials induced by depolarizing currents. Three weeks after injection of adeno-associated virus and implantation of optical fibers in the ACx, mice underwent 16 pairings of foot shocks and green light delivery to the ACx. On the next day, mice showed robust freezing during light-on periods, indicating that temporal silencing of ACx neurons can represent a CS+ in a FC paradigm.

Finally, in accordance with Baratta and colleagues (2016), who demonstrated that pharmacological and optogenetic inhibition of serotonergic dorsal raphe neurons during conditioning reduced fear in stressed animals with exaggerated fear levels, Sengupta and Holmes (2019) recently showed that the dorsal raphe/amygdala serotonergic pathway is engaged during fear memory formation and retrieval and that the activity of these projections facilitates fear and impairs extinction. These data provided a way to overcome a critical barrier in the successful treatment of stress-induced trauma disorders. Thus, the benchmark for any successful treatment of these disorders should not be the elimination of fear but simply its reduction to normal, adaptive levels. In fact, administration of a serotonergic receptor antagonist, such as agomelatine, which is already approved by the Food and Drug Administration for human use, might prevent or treat PTSD by reducing the consolidation or reconsolidation of traumatic memories (Baratta et al., 2016).

6.1 Optogenetic manipulation effects on fear extinction and reconsolidation

Inhibition of glutamatergic neurons during 15 or 30 CS+ presentations during fear extinction sessions increased freezing time throughout the session, suggesting that inhibition of BLA glutamatergic neurons enhanced fear expression and blunted fear extinction consolidation (Kenneth M. McCullough et al., 2016). Once a fearful association was extinguished, the inhibition of BLA glutamatergic neurons during the final extinction session was not sufficient for reinstatement and did not drive spontaneous fear expression.

In this framework, does attenuation of BLA-mPFC synaptic transmission lead to changes in the representation of cued fear in mPFC neurons? To answer this question, Klavir and colleagues (2017) injected mice with viral vectors encoding the fast channelrhodopsin variant $ChETA_{TC}$ under a CaMKIIα promoter into the BLA and implanted them with fixed multielectrode arrays targeting the ipsilateral mPFC. On the day following the conditioning to fear, mice were tested in a different context by exposing them to CS+ presentations, followed by sham or high-frequency optogenetic stimulations that evoked action potentials with high temporal precision and spike fidelity. In

optogenetically stimulated mice, the magnitude of cue-evoked responses was significantly reduced after the stimulation schedule was initiated, consistent with the recruitment of the BLA-mPFC network in transmitting learned CS-US associations to the mPFC (Senn et al., 2014; Sotres-Bayon et al., 2012). To elucidate whether the information carried by the projections from the BLA to the PrL was necessary for fear learning, other mice were bilaterally injected with adeno-associated virus vectors into the BLA and implanted with optical fibers above the PrL subregion (Klavir et al., 2017). The mice underwent high-frequency optogenetic stimulation, followed by FC in context A. On the following day, mice underwent extinction training in context B, and those that received optogenetic stimulation exhibited reduced freezing during fear recall. In particular, the reduction appeared during early extinction training but not during the later stages, suggesting that modified synaptic transmission in the BLA-PrL network during fear acquisition interfered with long-term consolidation of fear but not with extinction. This finding was also confirmed by applying optogenetic stimulation immediately before extinction training and the day after fear acquisition.

Klavir and colleagues (2017) repeated the experiments by implanting fibers in the IL subregion of the mPFC to examine how this region contributes to fear extinction and maintenance (Sotres-Bayon and Quirk, 2010). When optogenetic stimulation was applied to BLA-IL projections before fear acquisition, mice showed reduced freezing responses during extinction training. When optogenetic stimulation was applied immediately before extinction training, mice showed reduced cue-associated freezing, both during extinction learning and extinction retrieval testing.

Similarly, Kim and coworkers (2016) used mice in which adeno-associated virus expressing channelrhodopsin-2 under the CaMKII promoter was injected into the right IL cortex. These mice were submitted to auditory FC and subsequent extinction. In the extinction retrieval test, optogenetic stimulation delivered to the IL during the first four trial blocks of CS+ presentations induced a strong reduction in freezing behavior. Furthermore, the same authors determined the effect of IL optogenetic inhibition on the expression of extinction memories, by using mice in which adeno-associated virus expressing halorhodopsin (eNpHR3.0), a light-activated chloride

pump driven by the neuron-specific human synapsin (hSyn) promoter, was injected into the IL cortex. These virus-injected mice were subjected to FC with extinction training and testing (Kim et al., 2016). The expression of fear extinction memories was impaired by IL photoinhibition at the time of retrieval. This impairment was reversible and specific: freezing returned to control levels in virus-injected mice in the absence of photoinhibition. Furthermore, the impairment of extinction retrieval was specific to silencing the activities of both glutamatergic and GABAergic neurons. Kim and coworkers (2016) also reported no effect of IL inactivation on the expression of conditioned fear that did not undergo extinction and simply enhanced the freezing evoked by CS+ presentations. Thus, IL activity is specifically involved in the expression of fear extinction but not the expression of fear memory *per se*, consistent with previous findings (Sierra-Mercado et al., 2011). However, it must be noted that the preceding studies reported that electrical stimulation of IL reduced conditioned freezing in animals that did not undergo extinction (Milad et al., 2004; Milad and Quirk, 2002; Vidal-Gonzalez et al., 2006). Thus, artificially activated IL neurons alone were sufficient to induce extinction-like inhibition of conditioned fear expression, even without extinction training.

By using *ex vivo* electrophysiology combined with optogenetic techniques, it was shown that fear extinction had decreased the efficacy of glutamatergic synaptic transmission in projections from the mPFC to the BLA, whereas inhibitory responses were not altered (Cho et al., 2013). In parallel, BLA projection neurons targeting the PrL subdivision of the mPFC were active during states of high fear, whereas those targeting the IL subdivision were recruited and exhibited cell-type-specific plasticity during fear extinction (Senn et al., 2014). Thus, pathway-specific optogenetic manipulations have demonstrated that the balanced activity between the BLA and mPFC is causally involved in fear extinction. By using transgenic mice expressing channelrhodopsin in pyramidal neurons, very recently Laricchiuta et al., 2021 showed that the optogenetic activation of PrL pyramidal neurons in fear-conditioned transgenic mice induces fear extinction deficits, reflected in an increase of cellular excitability, excitatory neurotransmission, and

spinogenesis of PrL pyramidal neurons, and associated to strong modifications of the transcriptome of amygdala pyramidal neurons.

In another study, BLA pyramidal neurons received optogenetic stimulations coincident with presentations of the CS+ alone during fear extinction training. On the following day, during unstimulated extinction retention test conditions, memory for extinction was enhanced (Jasnow et al., 2013). Additionally, the selective photoactivation of GABAergic neurons in the anterior insular cortex, a region anatomically and functionally connected to the amygdala, promoted cued fear extinction (Shi et al., 2020).

Regarding the retrieval of extinction, mice exposed to optogenetic stimulation of the cholinergic terminal fields in the BLA during the initial training were more resistant to extinction learning than controls (Jiang et al., 2016). Interestingly, a form of muscarinic acetylcholine receptor (mAChR)-dependent long-term depression in the PFC is involved in appropriate fear responses (Walker et al., 2015) and could serve to reduce cortical hyperactivity following stress. By using optogenetic manipulations with extracellular and whole-cell electrophysiology, the effect of mAChR activation on the synaptic strength of PFC inputs was assessed, and by using selective pharmacological tools, the involvement of M1 mAChRs in conditioned fear extinction was evaluated in control and stressed mice, which represent an enhanced fear-learning model (Maksymetz et al., 2019). In stressed mice, while systemic treatment with an M1 mAChR antagonist impaired contextual fear extinction, treatment with an M1-positive allosteric modulator enhanced contextual fear extinction consolidation. In parallel, M1 mAChR activation induced long-term depression in the pathway from the ventral hippocampus (VH) and BLA to the PFC (Maksymetz et al., 2019). Through its direct excitatory projections, the VH may regulate the neural activity of the mPFC and amygdala, which is necessary for the recall of contextual fear memory (Corcoran and Quirk, 2007; Goshen et al., 2011; Zhu et al., 2014). Based on evidence that the inhibition of hippocampal activity interferes with extinction learning and the context dependency of extinction retrieval (Corcoran and Maren, 2004, 2001; Hobin et al., 2006), how the activity of

hippocampal circuits may regulate the expression of extinction memory has been investigated. In particular, by using an *in vivo* optogenetic approach, the role of the VH in the recall of fear memories after contextual FC was investigated (Kim and Cho, 2017a). To silence neural activity, adeno-associated virus encoding the eArch3 gene was bilaterally injected into the VH (Chow et al., 2010; Mattis et al., 2012), and eArch3 was expressed in CA1 pyramidal neurons of the VH. After contextual FC, mice were tested for freezing behavior once per day for 3 successive days. Freezing behavior in optogenetically stimulated eArch3 mice was strongly reduced, suggesting that VH activity is necessary for the recall of contextual fear memory.

To elucidate the specific contribution of the hippocampal CA1 to the reinstatement of fear memories and to evaluate the long-term effects of destabilizing memory traces on neuronal activity, Lux and colleagues (2017) interfered with the reinstatement of a contextual FC memory trace by infecting CA1 pyramidal cells with a light-driven outward proton pump that allowed optogenetic inhibition of cell firing in the CA1 region shortly after the retrieval of the contextual fear memory. Subsequently, the same authors investigated memory performance and activation patterns in hippocampal CA1 and CA3 regions and the BLA during memory retrieval by using high-resolution molecular imaging, a technique based on the detection of the immediate early gene Arc. Light-controlled inhibition of cell firing in the CA1 region led to reduced strength of the US-CS association, that is, the memory trace was depotentiated. One day after optogenetic inhibition of hippocampal CA1, Arc RNA levels in the CA1, CA3 and BLA of light-stimulated animals were lower than those in control animals. Furthermore, inhibition of cell firing in the CA1 region also reduced the percentage of Arc-positive cells in the BLA and CA3. These findings suggested that the decreased Arc levels observed in the optogenetically stimulated mice, in which the memory trace was depotentiated, were likely associated with a failure to recall this memory.

Continuing the examination of hippocampal involvement in the fear process, the dentate gyrus (DG) appears to also play a significant role in the acquisition of contextual fear memory, which in fact activates a sparse ensemble of DG granule fear engram cells, whose reactivation is

necessary (Denny et al., 2014) and sufficient (Liu et al., 2012; Ramirez et al., 2013; Redondo et al., 2014; Ryan et al., 2015) for the expression of contextual conditioned fear. Furthermore, in accordance with the hippocampal re-engagement upon remote memory recall (Gräff et al., 2014), the reactivation of recall-induced neurons in the DG not only accompanied behavioral attenuation of a 4-week-old fear memory, but the continued activity of recall-induced neurons is necessary for memory attenuation (Khalaf et al., 2018). When optogenetically stimulated, DG engram cells retain their ability to evoke fear at remote time points (Kitamura et al., 2017) and after amnestic treatments (Roy et al., 2016; Ryan et al., 2015). Bernier and colleagues (2017) evaluated the effects of transient optogenetic inhibition of the dorsal DG during contextual FC, recall, generalization, and extinction. DG inhibition during training impaired contextual fear acquisition, while the same inhibition during recall did not impair fear expression in the training context unless mice had to distinguish between feared and neutral contexts. Furthermore, DG inhibition increased generalization of fear to an unfamiliar context that was similar to a feared one, impaired fear expression in the conditioned context when it was similar to a neutral one, and impaired fear extinction.

Lacagnina and colleagues in 2019 used an activity-dependent neuronal tagging transgenic mouse model (Denny et al., 2014) to permanently label and manipulate dorsal DG granule cells that were active during either contextual fear acquisition or extinction. Namely, in these ArcCreER^{T2}-channelrhodopsin transgenic mice, the activity of the immediate early gene Arc drives expression of tamoxifen-dependent CreER^{T2} recombinase. An injection of 4-hydroxytamoxifen (4-OHT) transiently activates recombinase activity, thereby permanently tagging Arc-expressing neurons with a reporter. The optogenetic stimulation of the neurons tagged during fear acquisition increased fear, while the optogenetic silencing of the neurons tagged during fear acquisition decreased fear, and the silencing of the neurons tagged during extinction training increased fear after extinction.

Additionally, Mendez and coworkers (2018) used an activity-dependent neuronal tagging transgenic mouse model to permanently label and manipulate dorsal DG granule cells active during

contextual fear acquisition (Guenthner et al., 2013) and showed that mice that received optogenetic stimulation in the DG during extinction training displayed reduced freezing levels compared to control littermates. These results suggested that homeostatic synaptic adaptations induced by spike trains provoked by optogenetics during recall facilitated long-term fear memory extinction. Interestingly, freezing levels during the retention test in control and optogenetically stimulated mice were undistinguishable when spikes were delivered in anesthetized animals, indicating that simultaneous fear memory recall is required for optogenetic facilitation of extinction.

Applying a very similar methodology, Kim and Cho (2017b) demonstrated that postsynaptically expressed long-term potentiation is selectively induced in the ACx and in the medial geniculate nucleus (MGN), a pathway conveying CS information to the amygdala in discriminative fear learning. In this study, low-frequency photostimulations *in vivo* were applied to induce depotentiation in the CS-specific ACx/MGN-amygdala pathways, which were potentiated after FC. The adeno-associated virus encoding channelrhodopsin and fluorescent protein gene in a double inverse open reading frame was injected into the ACx and MGN in activity-dependent neuronal tagging transgenic mice, and in the same mice, the contralateral amygdala was excitotoxically lesioned. Then, mice received behavioral training, thereby labeling ACx/MGN neurons responding to the auditory CS and fear conditioned with US-CS pairings. Mice then received photostimulations and displayed reduced freezing behavior in response to the CS+.

As previously reported, Nomura and coworkers (2015) injected adeno-associated virus expressing Arc under the control of CaMKII promoter and implanted optical fibers into the ACx. Mice underwent pairings of foot shock with light delivery to the ACx. They showed robust freezing during light-on periods, even 30 days after FC.

Regarding the examination of the involvement of the thalamus in the fear process, recent studies have revealed the critical role of the limbic part of this structure in the persistent attenuation of fear by using pharmacological and optogenetic manipulations (Do-Monte et al., 2015) and in fear extinction by using genetic (Lee et al., 2012) and chemogenetic (Ramanathan et al., 2018)

manipulations. The thalamic reticular nucleus (TRN), a shell of GABAergic neurons surrounding the anterior and lateral parts of the thalamus, provides monosynaptic inhibitory inputs to the dorsal thalamus (Pinault and Deschênes, 1998), And an anatomical study in primates showed that the limbic sector of the TRN receives input from the amygdala (Zikopoulos and Barbas, 2012). Lee and colleagues in 2019 found that TRN neurons were activated during extinction learning, suppressing the spiking activity of dorsal midline thalamus (dMT) neurons to the CeA, in turn promoting fear extinction. These results suggest a novel neural hub underlying fear extinction.

Finally, Salinas-Hernández and colleagues (2018) attempted to elucidate the neuronal mechanisms that initiate extinction learning. By using single-unit electrophysiology and cell-type specific fiber photometry (dopamine neuron-specific calcium recordings), they showed that dopamine neurons in the ventral tegmental area were activated by the omission of the aversive US during fear extinction. The dopamine signal occurred specifically during the beginning of extinction, when US omission was unexpected, and correlated strongly with extinction learning. Furthermore, temporally specific optogenetic inhibition or excitation of dopamine neurons at the time of US omission revealed that this dopaminergic signal is both necessary for and sufficient to accelerate normal fear extinction learning. These results identified a prediction error-like neuronal signal necessary to initiate fear extinction and revealed a crucial role of dopamine neurons in this form of safety learning.

5.2 Optogenetic manipulation effects on long-lasting fear attenuation and fear renewal

To explore the role of the PrL in extinction recall, Kim and colleagues (2016) injected adeno-associated virus carrying halorhodopsin (eNpHR3.0), which hyperpolarizes the neuronal membrane under light stimulation, specifically into the PrL area of the mPFC, and mice were then trained in an auditory FC paradigm, followed by extinction training. During extinction retrieval tests, mice in the virus-injected and control groups displayed similar and normal expression of fear extinction. Furthermore, while the expression of extinction has been associated with reactivation of neurons

that were active during extinction training, the expression of fear during spontaneous recovery has been associated with the reactivation of hippocampal DG neurons that were active during fear acquisition (Lacagnina et al., 2019). To achieve better temporal resolution in the fear renewal experiment, an optogenetic inhibition technique was implemented. Namely, mice injected with adeno-associated virus carrying halorhodopsin and control littermates were bilaterally implanted with optical fibers targeting the dorsal DG. After 2-4 weeks, mice were subjected to contextual FC training followed immediately by an injection of 4-OHT, which permits the expression of halorhodopsin only in neurons active during fear acquisition, and then a course of extinction training. Contextual fear decreased across the 10 days of extinction. Five days after the final extinction session, mice were returned to the context for an extinction retrieval test, and silencing the fear acquisition-tagged neurons had no effect on freezing. When mice were returned to the conditioning context for a spontaneous recovery test 28 days later, silencing the fear acquisition-tagged neurons reduced contextual fear, demonstrating that the neurons active during fear acquisition are not required for extinction retrieval but are necessary for spontaneous fear recovery. Similarly, adeno-associated virus carrying halorhodopsin was injected 3 weeks before behavioral training into the posterior parietal cortex (PPC) (Joo et al., 2020), which receives diverse sensory and cognitive inputs (Andersen and Buneo, 2002; Morcos and Harvey, 2016; Raposo et al., 2014) and integrates multisensory signals (Andersen and Buneo, 2002; Najafi and Churchland, 2018; Song et al., 2017). Sensory information is processed by the PPC and transformed into behavioral outcomes. After extinction training, the mice were exposed to a novel context (context C), in which neither FC (context A) nor extinction (context B) had occurred (Joo et al., 2020). The relapse of fear was tested in the novel context (context C). Mice with optogenetic inactivation of the PPC did not display significant fear renewal relative to the last CS+ of extinction. However, optogenetic inactivation of the PPC did not impair fear renewal in a familiar context (ABA renewal).

Among the current clinical treatments that produce a long-lasting attenuation of fear, several effective psychotherapeutic methods use visual stimulation, eye movements or attentional control of

cognitive processes (Badura-Brack et al., 2015; Shapiro, 2001). In eye movement desensitization and reprocessing (EMDR), for example, patients are instructed to recall a traumatic memory and simultaneously to orient to alternating bilateral sensory stimulation (ABS) (Shapiro, 2001; Wurtz et al., 2016). Given that modulation of visual-attentional processes is a common component in treatment regimens for PTSD, a neuronal pathway driven by the superior colliculus (SC) has been reported to mediate the persistent attenuation of fear and its long-lasting effects (Baek et al., 2019). Optogenetic manipulations have revealed that the SC-mediodorsal thalamus (MD) circuit was necessary and sufficient to prevent the return of fear. Given that the MD is important for fear extinction and subsequent fear recovery (Herry and Garcia, 2002; Lee et al., 2012), optogenetic silencing of the MD-BLA pathway completely blocked the fear-attenuating effect of ABS (Baek et al., 2019).

6. A general overview of fear processing across species

The robust correspondences between the networks associated with fear processing in humans and in other species underscore the potential utility of analyzing the modulation of brain circuitry in animal models as a crucial step to inform the comprehension of physiological processes underlying fear and the development of treatments for fear-related disorders in humans (Schiller and Delgado, 2010; Suarez-Jimenez et al., 2020). Since the resulting physiological and cellular mechanisms are quite elaborate, a comprehensive description of the fear mechanisms has not been provided to date. The development of new clinical and experimental tools, such as NIBS and optogenetics, partially overcomes this gap, allowing a more accurate characterization of specific circuits and their particular interactions within the overall fear processing network.

Both in humans and animals, learned fear has been extensively studied using the FC paradigm, in which a CS is associated with a US. The acquisition of CS-US associative memories requires brain processes of coordinated and distributed neural activity within the amygdala, PFC, hippocampus, thalamus and brainstem (Corcoran and Quirk, 2007; Goshen et al., 2011; Zhu et al., 2014). After

conditioning, suppression of fear memory in the absence of danger is crucial to permit other survival functions, and impairments in such coping mechanisms to attenuate fear memories may lead to maladaptive behaviors. Acquisition and maintenance of extinction memories critically depend on amygdala-mPFC projections (Lacagnina et al., 2019; Tovote et al., 2015; Trouche et al., 2013). Thus, it should be noted that fear expression, fear extinction and extinction retention are overlapping processes, depending on the balance of signaling processes, rather than unitary elements acting independently (Figure 2).

In the "fear matrix" of the brain, control centers, integrative sites and effector sites can be distinguished. Specifically, during fear conditioning, reconsolidation, and extinction phases, the PrL and IL subregions of the control center represented by the mPFC receive from and project back to integrative sites in the BLA. This region in turn directly projects to the CeA or indirectly projects via inhibitory intercalated cells (ITCs), a scattered group of GABAergic neurons mainly located around the BLA area. Finally, from the output neurons of the CeA, information is relayed to the effector sites in the periaqueductal gray and premotor structures that mediate fear CRs. In other words, the BLA is the station receiving thalamic and cortical information about the CS and US that is sent in turn to the CeA, which targets such information to the midbrain and hypothalamus nuclei to mediate motor and autonomic responses to fear. The BLA-CeA pathway is currently considered the main route in the fear-processing network (Herry et al., 2008).

During fear memory consolidation, thalamic and cortical inputs conveying CS and US sensory information cause a marked increase in evoked activity in the BLA due to local long-term potentiation of excitatory synapses. The integrated information increases the firing activity of the "*fear neurons*" that stimulate the CeA, causing a strong increase in output from the entire amygdala and triggering fear behavior. Moreover, mutual connections between the BLA and cortical PrL area are able to stimulate the activity of *fear neurons*, causing further increases in CeA activity.

During fear extinction, inhibitory signaling in the amygdala reduces whole BLA excitability and elicits a general downregulation of CeA output, thus becoming a crucial element in extinguishing

memories (Lee et al., 2013). Notably, the glutamatergic input from the cortical IL suppresses the activity of BLA *fear neurons* by acting on BLA intrinsic inhibitory interneurons and inhibits CeA activity through activation of the ITC. The BLA intrinsic inhibitory circuit further reduces amygdala activity, thereby suppressing the fear response.

Overall, consistent with a serial model of amygdala information processing, FC strengthens BLA excitatory synapses, increases excitatory transmission onto CeA, and changes the responsiveness of intra-CeA cell populations, ultimately promoting the fear expression (Li et al., 2013). However, it has to be underlined that the described serial processing in the amygdala fear nuclei does not represent the unique view, and an alternative parallel processing is possible (Figure 2). Interesting evidence supports that the CeA has many of the same characteristics that originally implicated the BLA as a critical site for fear learning and it may participate in both the acquisition and storage of conditioned fear memories (Fadok et al., 2018; Wilensky et al., 2006). CS-evoked neural responses of subpopulation of cells within the CeA undergo learning dependent modifications, similarly to what occurs in the BLA (Haubensak et al., 2010; Penzo et al., 2014). Very importantly, activity of protein kinase C δ expressing cells in the lateral division of the CeA is necessary for FC induced synaptic strengthening in the LA, just playing an important role in conveying information about the US to the LA (Yu et al., 2017). These findings suggest that BLA and CeA may not be organized only in series but in parallel and that both subnuclei encode the same type of CS-US association (Balleine and Killcross, 2006; Ressler and Maren, 2019). The serial and parallel models emphasize the importance of coordinated or distributed amygdaloid plasticity, respectively, but future experiments will be necessary to determine the precise roles and interactions of the various intra-amygdala regions in the fear learning, reconsolidation, and extinction processes.

As a final note, it has to be reported that in detecting and responding to fearful stimuli, the amygdala (and not only this brain area) might work in terms of a non-conscious defensive survival circuit than a conscious fear one (LeDoux, 2012). Facing dangers, sensory systems detect threatening stimuli and defensive responses and feelings of fear may co-occur. Sensory system

connections to the amygdala trigger fear behavioral responses, while sensory system connections to the PFC permit the integration of information arising the feeling of fear (LeDoux, 2020).

Findings in humans have demonstrated that interfering with the consolidation process, that is, inhibitory stimulation of the dlPFC, disrupted fear memory consolidation (Asthana et al., 2013). During reconsolidation, it is possible to modulate fear responses in humans by administering drugs (Kindt et al., 2009) and behavioral procedures (Schiller et al., 2010) or delivering inhibitory rTMS over the dlPFC (Borgomaneri et al., 2020a). Such state-dependent effects appear to be critical for consideration in psychiatric populations, such as PTSD patients, in which the optimal conditions to induce reconsolidation may be to recall traumatic events while rTMS is applied rather than by applying rTMS *per sè*, which was found to be ineffective in healthy participants (Borgomaneri et al., 2020a). Again, human findings have demonstrated that interfering with the extinction process, e.g., excitatory stimulation applied over the vmPFC and inhibitory stimulation over the dlPFC, increased extinction (Dittert et al., 2018; Raij et al., 2018; Van ’t Wout et al., 2016; Vicario et al., 2019).

Within this framework, to initiate extinction learning, the absence of the expected aversive outcome must be detected and signaled to the brain regions mediating fear extinction and reconsolidation. New extinction learning is initiated, and reconsolidation is possible when outcomes violate expectations (Rescorla and Wagner, 1972). Such violations are thought to cause ‘prediction error’ signals that initiate neural processes that ultimately lead to changes in behavior (Den Ouden et al., 2012; Friston, 2012; Garofalo et al., 2017). During fear extinction, the absence of the US is an unexpected event and likely generates a prediction error signal that initiates extinction learning. More specifically, the omission of the aversive US can be conceptualized as a better-than-expected outcome. It is well established that the activity of midbrain dopamine neurons represents the degree to which outcomes are better or worse than expected (Bayer and Glimcher, 2005; Eshel et al., 2016, 2015; Schultz et al., 1997; Schultz and Dickinson, 2000).

Although a better understanding of fear processing has been achieved, innovative technical tools such as NIBS and optogenetics will allow a more specific identification and manipulation of the neurobiology of fear, leading to the identification of a possible therapeutic target for pathological fear states caused by trauma, stress and anxiety (Borgomaneri et al., 2021).

Please_insert_Colored_Figure_2_near_here

Please_insert_Colored_Box_1_near_here

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgments

This work was supported by grants from Ministero della Salute, Italy [GR-2018-12365733] awarded to D.L., S.Borgomaneri and G.S.

References

Abend, R., Jalon, I., Gurevitch, G., Sar-el, R., Shechner, T., Pine, D.S., Hendler, T., Bar-Haim, Y., 2016. Modulation of fear extinction processes using transcranial electrical stimulation. Transl. Psychiatry 6, e913. https://doi.org/10.1038/tp.2016.197

Agren, T., 2014. Human reconsolidation: A reactivation and update. Brain Res. Bull. 105, 70–82. https://doi.org/10.1016/j.brainresbull.2013.12.010

Alapin, J.M., Dines, M., Vassiliev, M., Tamir, T., Ram, A., Locke, C., Yu, J., Lamprecht, R., 2018. Activation of EphB2 forward signaling enhances memory consolidation. Cell Rep. 23, 2014–2025. https://doi.org/10.1016/j.celrep.2018.04.042

Alberini, C.M., LeDoux, J.E., 2013. Memory reconsolidation. Curr. Biol. 23, R746–R750. https://doi.org/10.1016/j.cub.2013.06.046

Andero, R., Dias, B.G., Ressler, K.J., 2014. A role for Tac2, NkB, and Nk3 receptor in normal and dysregulated fear memory consolidation. Neuron 83, 444–454. https://doi.org/10.1016/j.neuron.2014.05.028

Andersen, R.A., Buneo, C.A., 2002. Intentional maps in posterior parietal cortex. Annu. Rev. Neurosci. 25, 189–220. https://doi.org/10.1146/annurev.neuro.25.112701.142922

Arul-Anandam, A.P., Loo, C., 2009. Transcranial direct current stimulation: A new tool for the treatment of depression? J. Affect. Disord. 117, 137–145. https://doi.org/10.1016/j.jad.2009.01.016

Asthana, M., Nueckel, K., Mühlberger, A., Neueder, D., Polak, T., Domschke, K., Deckert, J., Herrmann, M.J., 2013. Effects of transcranial direct current stimulation on consolidation of fear memory. Front. Psychiatry 4, 107. https://doi.org/10.3389/fpsyt.2013.00107

Badura-Brack, A.S., Naim, R., Ryan, T.J., Levy, O., Abend, R., Khanna, M.M., McDermott, T.J., Pine, D.S., Bar-Haim, Y., 2015. Effect of attention training on attention bias variability and PTSD symptoms: Randomized controlled trials in Israeli and U.S. Combat Veterans. Am. J. Psychiatry 172, 1233–1241. https://doi.org/10.1176/appi.ajp.2015.14121578

Baek, J., Lee, S., Cho, T., Kim, S.-W., Kim, M., Yoon, Y., Kim, K.K., Byun, J., Kim, S.J., Jeong, J., Shin, H.-S., 2019. Neural circuits underlying a psychotherapeutic regimen for fear disorders. Nature 566, 339–343. https://doi.org/10.1038/s41586-019-0931-y

Baeken, C., De Raedt, R., Van Schuerbeek, P., Vanderhasselt, M.A.A., De Mey, J., Bossuyt, A., Luypaert, R., 2010. Right prefrontal HF-rTMS attenuates right amygdala processing of negatively valenced emotional stimuli in healthy females. Behav. Brain Res. 214, 450–455.

Balleine, B.W., Killcross, S., 2006. Parallel incentive processing: an integrated view of amygdala function. Trends Neurosci. 29, 272–279. https://doi.org/10.1016/j.tins.2006.03.002

Baratta, M. V., Kodandaramaiah, S.B., Monahan, P.E., Yao, J., Weber, M.D., Lin, P.A., Gisabella, B., Petrossian, N., Amat, J., Kim, K., Yang, A., Forest, C.R., Boyden, E.S., Goosens, K.A., 2016. Stress enables reinforcement-elicited serotonergic consolidation of fear memory. Biol. Psychiatry 79, 814–822. https://doi.org/10.1016/j.biopsych.2015.06.025

Battaglia, S., Garofalo, S., di Pellegrino, G., 2018. Context-dependent extinction of threat memories: influences of healthy aging. Sci. Rep. 8, 12592. https://doi.org/10.1038/s41598-018-31000-9

Battaglia, S., Garofalo, S., di Pellegrino, G., Starita, F., 2020. Revaluing the role of vmPFC in the acquisition of Pavlovian threat conditioning in humans. J. Neurosci. 40, 8491–8500. https://doi.org/10.1523/jneurosci.0304-20.2020

Bayer, H.M., Glimcher, P.W., 2005. Midbrain dopamine neurons encode a quantitative reward prediction error signal. Neuron 47, 129–141. https://doi.org/10.1016/j.neuron.2005.05.020

Bechara, A., Tranel, D., Damasio, H., Adolphs, R., Rockland, C., Damasio, A.R., 1995. Double dissociation of conditioning and declarative knowledge relative to the amygdala and hippocampus in humans. Science 269, 1115–1118. https://doi.org/10.1126/science.7652558

Belzung, C., Turiault, M., Griebel, G., 2014. Optogenetics to study the circuits of fear- and depression-like behaviors: A critical analysis. Pharmacol. Biochem. Behav. 122, 144–157. https://doi.org/10.1016/j.pbb.2014.04.002

Berndt, A., Lee, S.Y., Ramakrishnan, C., Deisseroth, K., 2014. Structure-guided transformation of channelrhodopsin into a light-activated chloride channel. Science 344, 420–424. https://doi.org/10.1126/science.1252367

Bernier, B.E., Lacagnina, A.F., Ayoub, A., Shue, F., Zemelman, B. V., Krasne, F.B., Drew, M.R., 2017. Dentate gyrus contributes to retrieval as well as encoding: Evidence from context fear conditioning, recall, and extinction. J. Neurosci. 37, 6359–6371. https://doi.org/10.1523/JNEUROSCI.3029-16.2017

Besnard, A., Caboche, J., Laroche, S., 2012. Reconsolidation of memory: A decade of debate. Prog. Neurobiol. 99, 61–80. https://doi.org/10.1016/j.pneurobio.2012.07.002

Beyeler, A., Eckhardt, C.A., Tye, K.M., 2014. Deciphering memory function with optogenetics. Prog. Mol. Biol. Transl. Sci. 122, 341–390. https://doi.org/10.1016/B978-0-12-420170-5.00012-X

Beynel, L., Powers, J.P., Appelbaum, L.G., 2020. Effects of repetitive transcranial magnetic stimulation on resting-state connectivity: A systematic review. Neuroimage 211, 116596. https://doi.org/10.1016/j.neuroimage.2020.116596

Blanchard, R.J., Blanchard, D.C., 1969. Crouching as an index of fear. J. Comp. Physiol. Psychol. 67, 370–375. https://doi.org/10.1037/h0026779

Borgomaneri, S., Battaglia, S., Avenanti, A., di Pellegrino, G., 2021. Don't Hurt Me No More: State-dependent Transcranial Magnetic Stimulation for the treatment of specific phobia. J. Affect. Disord. 286, 78–79. https://doi.org/10.1016/j.jad.2021.02.076

Borgomaneri, S., Battaglia, S., Garofalo, S., Tortora, F., Avenanti, A., di Pellegrino, G., 2020a. State-dependent TMS over prefrontal cortex disrupts fear-memory reconsolidation and prevents the return of fear. Curr. Biol. 30, 3672–3679. https://doi.org/10.1016/j.cub.2020.06.091

Borgomaneri, S., Gazzola, V., Avenanti, A., 2015a. Transcranial magnetic stimulation reveals two functionally distinct stages of motor cortex involvement during perception of emotional body language. Brain Struct. Funct. 220, 2765–2781. https://doi.org/10.1007/s00429-014-0825-

Borgomaneri, S., Vitale, F., Avenanti, A., 2020b. Early motor reactivity to observed human body postures is affected by body expression, not gender. Neuropsychologia 146, 107541. https://doi.org/10.1016/j.neuropsychologia.2020.107541

Borgomaneri, S., Vitale, F., Avenanti, A., 2017. Behavioral inhibition system sensitivity enhances motor cortex suppression when watching fearful body expressions. Brain Struct. Funct. 222, 3267–3282. https://doi.org/10.1007/s00429-017-1403-5

Borgomaneri, S., Vitale, F., Avenanti, A., 2015b. Early changes in corticospinal excitability when seeing fearful body expressions. Sci. Rep. 5, 14122. https://doi.org/10.1038/srep14122

Borgomaneri, S., Vitale, F., Gazzola, V., Avenanti, A., 2015c. Seeing fearful body language rapidly freezes the observer's motor cortex. Cortex 65, 232–245. https://doi.org/10.1016/j.cortex.2015.01.014

Bouton, M.E., 2004. Context and behavioral processes in extinction. Learn. Mem. 11, 485–494. https://doi.org/10.1101/lm.78804

Bouton, M.E., 2002. Context, ambiguity, and unlearning: sources of relapse after behavioral extinction. Biol Psychiatry 52, 976–986. https://doi.org/10.1016/s0006-3223(02)01546-9.

Bouton, M.E., King, D.A., 1983. Contextual control of the extinction of conditioned fear: Tests for the associative value of the context. J. Exp. Psychol. Anim. Behav. Process. 9, 248–265. https://doi.org/10.1037/0097-7403.9.3.248

Boyd, A.W., Bartlett, P.F., Lackmann, M., 2014. Therapeutic targeting of EPH receptors and their ligands. Nat. Rev. Drug Discov. https://doi.org/10.1038/nrd4175

Boyden, E.S., Zhang, F., Bamberg, E., Nagel, G., Deisseroth, K., 2005. Millisecond-timescale, genetically targeted optical control of neural activity. Nat. Neurosci. 8, 1263–1268. https://doi.org/10.1038/nn1525

Bush, D.E.A., Caparosa, E.M., Gekker, A., LeDoux, J., 2010. Beta-adrenergic receptors in the lateral nucleus of the amygdala contribute to the acquisition but not the consolidation of

auditory fear conditioning. Front. Behav. Neurosci. 4, 154.
https://doi.org/10.3389/fnbeh.2010.00154

Cabeza, R., Nyberg, L., 2000. Neural bases of learning and memory: Functional neuroimaging evidence. Curr. Opin. Neurol. 13, 415–421. https://doi.org/10.1097/00019052-200008000-00008

Carlsen, J., 1988. Immunocytochemical localization of glutamate decarboxylase in the rat basolateral amygdaloid nucleus, with special reference to GABAergic innervation of amygdalostriatal projection neurons. J. Comp. Neurol. 273, 513–526. https://doi.org/10.1002/cne.902730407

Chalkia, A., Van Oudenhove, L., Beckers, T., 2020. Preventing the return of fear in humans using reconsolidation update mechanisms: A verification report of Schiller et al. (2010). Cortex 129, 510–525. https://doi.org/10.1016/j.cortex.2020.03.031

Chiappini, E., Borgomaneri, S., Marangon, M., Turrini, S., Romei, V., Avenanti, A., 2020. Driving associative plasticity in premotor-motor connections through a novel paired associative stimulation based on long-latency cortico-cortical interactions. Brain Stimul. 13, 1461–1463. https://doi.org/10.1016/j.brs.2020.08.003

Cho, J.H., Deisseroth, K., Bolshakov, V.Y., 2013. Synaptic encoding of fear extinction in mPFC-amygdala circuits. Neuron 80, 1491–1507. https://doi.org/10.1016/j.neuron.2013.09.025

Chow, B.Y., Han, X., Dobry, A.S., Qian, X., Chuong, A.S., Li, M., Henninger, M.A., Belfort, G.M., Lin, Y., Monahan, P.E., Boyden, E.S., 2010. High-performance genetically targetable optical neural silencing by light-driven proton pumps. Nature 463, 98–102. https://doi.org/10.1038/nature08652

Corcoran, K.A., Maren, S., 2004. Factors regulating the effects of hippocampal inactivation on renewal of conditional fear after extinction. Learn. Mem. 11, 598–603. https://doi.org/10.1101/lm.78704

Corcoran, K.A., Maren, S., 2001. Hippocampal inactivation disrupts contextual retrieval of fear

memory after extinction. J. Neurosci. 21, 1720–1726. https://doi.org/10.1523/jneurosci.21-05-01720.2001

Corcoran, K.A., Quirk, G.J., 2007. Recalling safety: Cooperative functions of the ventromedial prefrontal cortex and the hippocampus in extinction. CNS Spectr. 12, 200–206. https://doi.org/10.1017/S1092852900020915

Cowansage, K.K., Shuman, T., Dillingham, B.C., Chang, A., Golshani, P., Mayford, M., 2014. Direct reactivation of a coherent neocortical memory of context. Neuron 84, 432–441. https://doi.org/10.1016/j.neuron.2014.09.022

Craske, M.G., 1999. Anxiety disorders: psychological approaches to theory and treatment. Westview Press.

Crawford, M., Masterson, F.A., 1982. Species-specific defense reactions and avoidance learning - An evaluative review. Pavlov. J. Biol. Sci. 17, 204–214. https://doi.org/10.1007/BF03001275

Davis, M., 2000. The role of the amygdala in conditioned and unconditioned fear and anxiety., in: Aggleton, J.P. (Ed.), The Amygdala: A Functional Analysis, Second Edition. Oxford University Press, New York, pp. 213–287.

Davis, M., 1992. The role of the amygdala in fear and anxiety. Annu. Rev. Neurosci. 15, 353–375. https://doi.org/10.1146/annurev.ne.15.030192.002033

Davis, M., Myers, K.M., Chhatwal, J., Ressler, K.J., 2010. Pharmacological treatments that facilitate extinction of fear: Relevance to psychotherapy. NeuroRx 3, 82–96. https://doi.org/10.1016/j.nurx.2005.12.008

Dayan, E., Censor, N., Buch, E.R., Sandrini, M., Cohen, L.G., 2013. Noninvasive brain stimulation: From physiology to network dynamics and back. Nat. Neurosci. 16, 838–844. https://doi.org/10.1038/nn.3422

De Franceschi, G., Vivattanasarn, T., Saleem, A.B., Solomon, S.G., 2016. Vision guides selection of freeze or flight defense strategies in mice. Curr. Biol. 26, 2150–2154. https://doi.org/10.1016/j.cub.2016.06.006

De Sousa, A.F., Cowansage, K.K., Zutshi, I., Cardozo, L.M., Yoo, E.J., Leutgeb, S., Mayford, M., 2019. Optogenetic reactivation of memory ensembles in the retrosplenial cortex induces systems consolidation. Proc. Natl. Acad. Sci. U. S. A. 116, 8576–8581. https://doi.org/10.1073/pnas.1818432116

Debiec, J., LeDoux, J.E., Nader, K., 2002. Cellular and systems reconsolidation in the hippocampus. Neuron 36, 527–538. https://doi.org/10.1016/S0896-6273(02)01001-2

Delamater, A.R., 2004. Experimental extinction in Pavlovian conditioning: Behavioural and neuroscience perspectives. Q. J. Exp. Psychol. Sect. B 57, 97–132. https://doi.org/10.1080/02724990344000097

Den Ouden, H.E.M., Kok, P., de Lange, F.P., 2012. How prediction errors shape perception, attention, and motivation. Front. Psychol. 3, 548. https://doi.org/10.3389/fpsyg.2012.00548

Denny, C.A., Kheirbek, M.A., Alba, E.L., Tanaka, K.F., Brachman, R.A., Laughman, K.B., Tomm, N.K., Turi, G.F., Losonczy, A., Hen, R., 2014. Hippocampal memory traces are differentially modulated by experience, time, and adult neurogenesis. Neuron 83, 189–201. https://doi.org/10.1016/j.neuron.2014.05.018

Dittert, N., Hüttner, S., Polak, T., Herrmann, M.J., 2018. Augmentation of fear extinction by transcranial direct current stimulation (tDCS). Front. Behav. Neurosci. 12, 76. https://doi.org/10.3389/fnbeh.2018.00076

Do-Monte, F.H., Manzano-Nieves, G., Quiñones-Laracuente, K., Ramos-Medina, L., Quirk, G.J., 2015. Revisiting the role of infralimbic cortex in fear extinction with optogenetics. J. Neurosci. 35, 3607–3615. https://doi.org/10.1523/JNEUROSCI.3137-14.2015

Duncan, L.E., Cooper, B.N., Shen, H., 2018. Robust findings from 25 Years of PSTD genetics research. Curr. Psychiatry Rep. 20, 115. https://doi.org/10.1007/s11920-018-0980-1

Dunsmoor, J.E., Niv, Y., Daw, N., Phelps, E.A., 2015. Rethinking extinction. Neuron 88, 47–63. https://doi.org/10.1016/j.neuron.2015.09.028.

Ehrlich, I., Humeau, Y., Grenier, F., Ciocchi, S., Herry, C., Lüthi, A., 2009. Amygdala

inhibitory circuits and the control of fear memory. Neuron 62, 757–771. https://doi.org/10.1016/j.neuron.2009.05.026

Eichenbaum, H., 2017. Prefrontal-hippocampal interactions in episodic memory. Nat. Rev. Neurosci. 18, 547–558. https://doi.org/10.1038/nrn.2017.74

Elsey, J.W.B., Van Ast, V.A., Kindt, M., 2018. Human memory reconsolidation: A guiding framework and critical review of the evidence. Psychol. Bull. 144, 797–848. https://doi.org/10.1037/bul0000152

Eshel, N., Bukwich, M., Rao, V., Hemmelder, V., Tian, J., Uchida, N., 2015. Arithmetic and local circuitry underlying dopamine prediction errors. Nature 525, 243–246. https://doi.org/10.1038/nature14855

Eshel, N., Tian, J., Bukwich, M., Uchida, N., 2016. Dopamine neurons share common response function for reward prediction error. Nat. Neurosci. 19, 479–486. https://doi.org/10.1038/nn.4239

Etkin, A., Egner, T., Kalisch, R., 2011. Emotional processing in anterior cingulate and medial prefrontal cortex. Trends Cogn. Sci. 15, 85–93. https://doi.org/10.1016/j.tics.2010.11.004.

Fadok, J.P., Markovic, M., Tovote, P., Lüthi, A., 2018. New perspectives on central amygdala function. Curr. Opin. Neurobiol. 49, 141–147. https://doi.org/10.1016/j.conb.2018.02.009

Fanselow, M.S., Gale, G.D., 2003. The amygdala, fear, and memory, in: Annals of the New York Academy of Sciences. New York Academy of Sciences, pp. 125–134. https://doi.org/10.1111/j.1749-6632.2003.tb07077.x

Fanselow, M.S., Poulos, A.M., 2005. The neuroscience of mammalian associative learning. Annu. Rev. Psychol. 56, 207–234. https://doi.org/10.1146/annurev.psych.56.091103.070213

Farach, F.J., Pruitt, L.D., Jun, J.J., Jerud, A.B., Zoellner, L.A., Roy-Byrne, P.P., 2012. Pharmacological treatment of anxiety disorders: Current treatments and future directions. J. Anxiety Disord. 26, 833–843. https://doi.org/10.1016/j.janxdis.2012.07.009

Fiori, F., Chiappini, E., Candidi, M., Romei, V., Borgomaneri, S., Avenanti, A., 2017. Long-

latency interhemispheric interactions between motor-related areas and the primary motor cortex: A dual site TMS study. Sci. Rep. 7, 14936. https://doi.org/10.1038/s41598-017-13708-2

Fiori, F., Chiappini, E., Soriano, M., Paracampo, R., Romei, V., Borgomaneri, S., Avenanti, A., 2016. Long-latency modulation of motor cortex excitability by ipsilateral posterior inferior frontal gyrus and pre-supplementary motor area. Sci. Rep. 6, 38396. https://doi.org/10.1038/srep38396

Fitzgerald, P.B., Brown, T.L., Daskalakis, Z.J., 2002. The application of transcranial magnetic stimulation in psychiatry and neurosciences research. Acta Psychiatr. Scand. 105, 324–340. https://doi.org/10.1034/j.1600-0447.2002.1r179.x

Forcato, C., Argibay, P.F., Pedreira, M.E., Maldonado, H., 2009. Human reconsolidation does not always occur when a memory is retrieved: The relevance of the reminder structure. Neurobiol. Learn. Mem. 91, 50–57. https://doi.org/10.1016/j.nlm.2008.09.011

Friston, K., 2012. Prediction, perception and agency. Int. J. Psychophysiol. 83, 248–252. https://doi.org/10.1016/j.ijpsycho.2011.11.014

Fullana, M.A., Albajes-Eizagirre, A., Soriano-Mas, C., Vervliet, B., Cardoner, N., Benet, O., Radua, J., Harrison, B.J., 2018. Fear extinction in the human brain: A meta-analysis of fMRI studies in healthy participants. Neurosci. Biobehav. Rev. 88, 16–25. https://doi.org/10.1016/j.neubiorev.2018.03.002

Fullana, M.A., Dunsmoor, J.E., Schruers, K.R.J., Savage, H.S., Bach, D.R., Harrison, B.J., 2020. Human fear conditioning: From neuroscience to the clinic. Behav. Res. Ther. 124, 103528. https://doi.org/10.1016/j.brat.2019.103528

Fullana, M.A., Harrison, B.J., Soriano-Mas, C., Vervliet, B., Cardoner, N., Àvila-Parcet, A., Radua, J., 2016. Neural signatures of human fear conditioning: An updated and extended meta-analysis of fMRI studies. Mol. Psychiatry 21, 500–508. https://doi.org/10.1038/mp.2015.88

Gafford, G.M., Ressler, K.J., 2016. Mouse models of fear-related disorders: Cell-type-specific manipulations in amygdala. Neuroscience 321, 108–120.

https://doi.org/10.1016/j.neuroscience.2015.06.019

Ganho-Ávila, A., Gonçalves, Ó.F., Guiomar, R., Boggio, P.S., Asthana, M.K., Krypotos, A.M., Almeida, J., 2019. The effect of cathodal tDCS on fear extinction: A cross-measures study. PLoS One 14, e0221282. https://doi.org/10.1371/journal.pone.0221282

Garofalo, S., Timmermann, C., Battaglia, S., Maier, M.E., di Pellegrino, G., 2017. Mediofrontal negativity signals unexpected timing of salient. J. Cogn. Neurosci. 29, 718–727. https://doi.org/10.1162/jocn_a_01074

George, M.S., Aston-Jones, G., 2010. Noninvasive techniques for probing neurocircuitry and treating illness: Vagus nerve stimulation (VNS), transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS). Neuropsychopharmacology 35, 301–316. https://doi.org/10.1038/npp.2009.87

Golkar, A., Bellander, M., Olsson, A., Öhman, A., 2012. Are fear memories erasable? - reconsolidation of learned fear with fear relevant and fearirrelevant stimuli. Front. Behav. Neurosci. 6, 80. https://doi.org/10.3389/fnbeh.2012.00080

Goshen, I., Brodsky, M., Prakash, R., Wallace, J., Gradinaru, V., Ramakrishnan, C., Deisseroth, K., 2011. Dynamics of retrieval strategies for remote memories. Cell 147, 678–689. https://doi.org/10.1016/j.cell.2011.09.033

Gräff, J., Joseph, N.F., Horn, M.E., Samiei, A., Meng, J., Seo, J., Rei, D., Bero, A.W., Phan, T.X., Wagner, F., Holson, E., Xu, J., Sun, J., Neve, R.L., Mach, R.H., Haggarty, S.J., Tsai, L.H., 2014. Epigenetic priming of memory updating during reconsolidation to attenuate remote fear memories. Cell 156, 261–276. https://doi.org/10.1016/j.cell.2013.12.020

Guenthner, C.J., Miyamichi, K., Yang, H.H., Heller, H.C., Luo, L., 2013. Permanent genetic access to transiently active neurons via TRAP: Targeted recombination in active populations. Neuron 78, 773–784. https://doi.org/10.1016/j.neuron.2013.03.025

Guhn, A., Dresler, T., Andreatta, M., Müller, L.D., Hahn, T., Tupak, S. V, Polak, T., Deckert, J., Herrmann, M.J., 2014. Medial prefrontal cortex stimulation modulates the processing of

conditioned fear. Front. Behav. Neurosci. 8, 44. https://doi.org/10.3389/fnbeh.2014.00044

Guhn, A., Dresler, T., Hahn, T., Mühlberger, A., Ströhle, A., Deckert, J., Herrmann, M.J., 2012. Medial prefrontal cortex activity during the extinction of conditioned fear: An investigation using functional near-infrared spectroscopy. Neuropsychobiology 65, 173–182. https://doi.org/10.1159/000337002

Haaker, J., Golkar, A., Hermans, D., Lonsdorf, T.B., 2014. A review on human reinstatement studies: An overview and methodological challenges. Learn. Mem. 21, 424–440. https://doi.org/10.1101/lm.036053.114

Haaker, J., Maren, S., Andreatta, M., Merz, C.J., Richter, J., Richter, S.H., Meir Drexler, S., Lange, M.D., Jüngling, K., Nees, F., Seidenbecher, T., Fullana, M.A., Wotjak, C.T., Lonsdorf, T.B., 2019. Making translation work: Harmonizing cross-species methodology in the behavioural neuroscience of Pavlovian fear conditioning. Neurosci. Biobehav. Rev. 107, 329–345. https://doi.org/10.1016/j.neubiorev.2019.09.020

Hardt, O., Nadel, L., 2018. Systems consolidation revisited, but not revised: The promise and limits of optogenetics in the study of memory. Neurosci. Lett. 680, 54–59. https://doi.org/10.1016/j.neulet.2017.11.062

Harris, K.D., Mrsic-Flogel, T.D., 2013. Cortical connectivity and sensory coding. Nature 503, 51–58. https://doi.org/10.1038/nature12654

Haubensak, W., Kunwar, P.S., Cai, H., Ciocchi, S., Wall, N.R., Ponnusamy, R., Biag, J., Dong, H.W., Deisseroth, K., Callaway, E.M., Fanselow, M.S., Lüthi, A., Anderson, D.J., 2010. Genetic dissection of an amygdala microcircuit that gates conditioned fear. Nature 468, 270–276. https://doi.org/10.1038/nature09553

Herrmann, M.J., Mühlberger, A., Ehlis, A.C., Deckert, J., Polak, T., 2019. Non-invasive brain stimulation and fear extinction. Nervenheilkunde 38, 537–541. https://doi.org/10.1055/a-0916-1334

Herry, C., Ciocchi, S., Senn, V., Demmou, L., Müller, C., Lüthi, A., 2008. Switching on and off

fear by distinct neuronal circuits. Nature 454, 600–606. https://doi.org/10.1038/nature07166

Herry, C., Garcia, R., 2002. Prefrontal cortex long-term potentiation, but not long-term depression, is associated with the maintenance of extinction of learned fear in mice. J. Neurosci. 22, 577–583. https://doi.org/10.1523/jneurosci.22-02-00577.2002

Hobin, J.A., Ji, J., Maren, S., 2006. Ventral hippocampal muscimol disrupts context-specific fear memory retrieval after extinction in rats. Hippocampus 16, 174–182. https://doi.org/10.1002/hipo.20144

Hromádka, T., DeWeese, M.R., Zador, A.M., 2008. Sparse representation of sounds in the unanesthetized auditory cortex. PLoS Biol. 6, e16. https://doi.org/10.1371/journal.pbio.0060016

Huang, Y.Z., Edwards, M.J., Rounis, E., Bhatia, K.P., Rothwell, J.C., 2005. Theta burst stimulation of the human motor cortex. Neuron 45, 201–216. https://doi.org/10.1016/j.neuron.2004.12.033

Indovina, I., Robbins, T.W., Núñez-Elizalde, A.O., Dunn, B.D., Bishop, S.J., 2011. Fear-conditioning mechanisms associated with trait vulnerability to anxiety in humans. Neuron 69, 563–571. https://doi.org/10.1016/j.neuron.2010.12.034

Iyer, M.B., Schleper, N., Wassermann, E.M., 2003. Priming stimulation enhances the depressant effect of low-frequency repetitive transcranial magnetic stimulation. J. Neurosci. 23, 10867–10872. https://doi.org/10.1523/jneurosci.23-34-10867.2003

Jasnow, A.M., Ehrlich, D.E., Choi, D.C., Dabrowska, J., Bowers, M.E., McCullough, K.M., Rainnie, D.G., Ressler, K.J., 2013. Thy1-expressing neurons in the basolateral amygdala may mediate fear inhibition. J. Neurosci. 33, 10396–10404. https://doi.org/10.1523/JNEUROSCI.5539-12.2013

Jiang, L., Kundu, S., Lederman, J.D., López-Hernández, G.Y., Ballinger, E.C., Wang, S., Talmage, D.A., Role, L.W., 2016. Cholinergic signaling controls conditioned fear behaviors and enhances plasticity of cortical-amygdala circuits. Neuron 90, 1057–1070. https://doi.org/10.1016/j.neuron.2016.04.028

Johansen, J.P., Diaz-Mataix, L., Hamanaka, H., Ozawa, T., Ycu, E., Koivumaa, J., Kumar, A., Hou, M., Deisseroth, K., Boyden, E.S., LeDoux, J.E., 2014. Hebbian and neuromodulatory mechanisms interact to trigger associative memory formation. Proc. Natl. Acad. Sci. U. S. A. 111, E5584–E5592. https://doi.org/10.1073/pnas.1421304111

Johansen, J.P., Hamanaka, H., Monfils, M.H., Behnia, R., Deisseroth, K., Blair, H.T., LeDoux, J.E., 2010. Optical activation of lateral amygdala pyramidal cells instructs associative fear learning. Proc. Natl. Acad. Sci. U. S. A. 107, 12692–12697. https://doi.org/10.1073/pnas.1002418107

Joo, B., Koo, J.W., Lee, S., 2020. Posterior parietal cortex mediates fear renewal in a novel context. Mol. Brain 13, 1–11. https://doi.org/10.1186/s13041-020-0556-y

Jung, S.H., Shin, J.E., Jeong, Y.S., Shin, H.I., 2008. Changes in motor cortical excitability induced by high-frequency repetitive transcranial magnetic stimulation of different stimulation durations. Clin. Neurophysiol. 119, 71–79. https://doi.org/10.1016/j.clinph.2007.09.124

Kalisch, R., Korenfeld, E., Stephan, K.E., Weiskopf, N., Seymour, B., Dolan, R.J., 2006. Context-dependent human extinction memory is mediated by a ventromedial prefrontal and hippocampal network. J. Neurosci. 26, 9503–9511. https://doi.org/10.1523/JNEUROSCI.2021-06.2006

Khalaf, O., Resch, S., Dixsaut, L., Gorden, V., Glauser, L., Gräff, J., 2018. Reactivation of recall-induced neurons contributes to remote fear memory attenuation. Science 360, 1239–1242. https://doi.org/10.1126/science.aas9875

Kim, D.R., Pesiridou, A., O'Reardon, J.P., 2009. Transcranial magnetic stimulation in the treatment of psychiatric disorders. Curr. Psychiatry Rep. 11, 447–452. https://doi.org/10.1007/s11920-009-0068-z

Kim, H.S., Cho, H.Y., Augustine, G.J., Han, J.H., 2016. Selective control of fear expression by optogenetic manipulation of infralimbic cortex after extinction. Neuropsychopharmacology 41, 1261–1273. https://doi.org/10.1038/npp.2015.276

Kim, J.J., Jung, M.W., 2006. Neural circuits and mechanisms involved in Pavlovian fear conditioning: a critical review. Neurosci. Biobehav. Rev. 30, 188–202. https://doi.org/10.1016/j.neubiorev.2005.06.005

Kim, W.B., Cho, J.H., 2017a. Synaptic targeting of double-projecting ventral CA1 hippocampal neurons to the medial prefrontal cortex and basal amygdala. J. Neurosci. 37, 4868–4882. https://doi.org/10.1523/JNEUROSCI.3579-16.2017

Kim, W.B., Cho, J.H., 2017b. Encoding of discriminative fear memory by input-specific LTP in the amygdala. Neuron 95, 1129-1146.e5. https://doi.org/10.1016/j.neuron.2017.08.004

Kindt, M., Soeter, M., Vervliet, B., 2009. Beyond extinction: Erasing human fear responses and preventing the return of fear. Nat. Neurosci. 12, 256–258. https://doi.org/10.1038/nn.2271

Kitamura, T., Ogawa, S.K., Roy, D.S., Okuyama, T., Morrissey, M.D., Smith, L.M., Redondo, R.L., Tonegawa, S., 2017. Engrams and circuits crucial for systems consolidation of a memory. Science 356, 73–78. https://doi.org/10.1126/science.aam6808

Klavir, O., Prigge, M., Sarel, A., Paz, R., Yizhar, O., 2017. Manipulating fear associations via optogenetic modulation of amygdala inputs to prefrontal cortex. Nat. Neurosci. 20, 836–844. https://doi.org/10.1038/nn.4523

Klomjai, W., Katz, R., Lackmy-Vallée, A., 2015. Basic principles of transcranial magnetic stimulation (TMS) and repetitive TMS (rTMS). Ann. Phys. Rehabil. Med. 58, 208–213. https://doi.org/10.1016/j.rehab.2015.05.005

Koessler, L., Maillard, L., Benhadid, A., Vignal, J.P., Felblinger, J., Vespignani, H., Braun, M., 2009. Automated cortical projection of EEG sensors: Anatomical correlation via the international 10-10 system. Neuroimage 46, 64–72. https://doi.org/10.1016/j.neuroimage.2009.02.006

Koppensteiner, P., Von Itter, R., Melani, R., Galvin, C., Lee, F.S., Ninan, I., 2019. Diminished fear extinction in adolescents is associated with an altered somatostatin interneuron–mediated inhibition in the infralimbic cortex. Biol. Psychiatry 86, 682–692.

https://doi.org/10.1016/j.biopsych.2019.04.035

Krabbe, S., Gründemann, J., Lüthi, A., 2018. Amygdala Inhibitory Circuits Regulate Associative Fear Conditioning. Biol. Psychiatry. https://doi.org/10.1016/j.biopsych.2017.10.006

Lacadie, C.M., Fulbright, R.K., Rajeevan, N., Constable, R.T., Papademetris, X., 2008. More accurate Talairach coordinates for neuroImaging using nonlinear registration. Neuroimage 42, 717–725. https://doi.org/10.1016/j.neuroimage.2008.04.240

Lacagnina, A.F., Brockway, E.T., Crovetti, C.R., Shue, F., McCarty, M.J., Sattler, K.P., Lim, S.C., Santos, S.L., Denny, C.A., Drew, M.R., 2019. Distinct hippocampal engrams control extinction and relapse of fear memory. Nat. Neurosci. 22, 753–761. https://doi.org/10.1038/s41593-019-0361-z

Lamprecht, R., LeDoux, J., 2004. Structural plasticity and memory. Nat. Rev. Neurosci. 5, 45–54. https://doi.org/10.1038/nrn1301

Laricchiuta, D., Balsamo, F., Fabrizio, C., Panuccio, A., Termine, A., Petrosini, L., 2020. CB1 activity drives the selection of navigational strategies: A behavioral and c-fos immunoreactivity study. Int. J. Mol. Sci. 21, 1072. https://doi.org/10.3390/ijms21031072

Laricchiuta, D., Sciamanna, G., Gimenez, J., Termine, A., Fabrizio, C., Caioli, S., Balsamo, F., Panuccio, A., De Bardi, M., Saba, L., Passarello, N., Cutuli, D., Mattioni, A., Zona, C., Orlando, V., Petrosini, L., 2021. Optogenetic stimulation of prelimbic pyramidal neurons maintains fear memories and modulates amygdala pyramidal neuron transcriptome. Int. J. Mol. Sci. 22, 1–29. https://doi.org/10.3390/ijms22020810

LeDoux, J., 2012. Rethinking the emotional brain. Neuron 73, 653–76. https://doi.org/10.1016/j.neuron.2012.02.004

LeDoux, J.E., 2020. Thoughtful feelings. Curr. Biol. 30, R619–R623. https://doi.org/10.1016/j.cub.2020.04.012

LeDoux, J.E., 2000. Emotion circuits in the brain. Annu. Rev. Neurosci. 23, 155–184. https://doi.org/10.1146/annurev.neuro.23.1.155

Lee, J.H., Latchoumane, C.F. V., Park, J., Kim, J., Jeong, J., Lee, K.H., Shin, H.S., 2019. The rostroventral part of the thalamic reticular nucleus modulates fear extinction. Nat. Commun. 10, 4637. https://doi.org/10.1038/s41467-019-12496-9

Lee, S., Ahmed, T., Lee, S., Kim, H., Choi, S., Kim, D.S., Kim, S.J., Cho, J., Shin, H.S., 2012. Bidirectional modulation of fear extinction by mediodorsal thalamic firing in mice. Nat. Neurosci. 15, 308–314. https://doi.org/10.1038/nn.2999

Lee, S., Kim, S.-J., Kwon, O.-B., Lee, J.H., Kim, J.-H., 2013. Inhibitory networks of the amygdala for emotional memory 7, 129. https://doi.org/10.3389/fncir.2013.00129

Li, H., Penzo, M.A., Taniguchi, H., Kopec, C.D., Huang, Z.J., Li, B., 2013. Experience-dependent modification of a central amygdala fear circuit. Nat. Neurosci. 16, 332–339. https://doi.org/10.1038/nn.3322

Lisabeth, E.M., Falivelli, G., Pasquale, E.B., 2013. Eph receptor signaling and ephrins. Cold Spring Harb. Perspect. Biol. 5, a009159. https://doi.org/10.1101/cshperspect.a009159

Lisanby, S.H., Kinnunen, L.H., Crupain, M.J., 2002. Applications of TMS to therapy in psychiatry. J. Clin. Neurophysiol. 19, 344–360. https://doi.org/10.1097/00004691-200208000-00007

Liu, X., Ramirez, S., Pang, P.T., Puryear, C.B., Govindarajan, A., Deisseroth, K., Tonegawa, S., 2012. Optogenetic stimulation of a hippocampal engram activates fear memory recall. Nature 484, 381–385. https://doi.org/10.1038/nature11028

Locke, C., Machida, K., Tucker, C.L., Wu, Y., Yu, J., 2017. Optogenetic activation of EphB2 receptor in dendrites induced actin polymerization by activating Arg kinase. Biol. Open 6, 1820–1830. https://doi.org/10.1242/bio.029900

Lonsdorf, T.B., Menz, M.M., Andreatta, M., Fullana, M.A., Golkar, A., Haaker, J., Heitland, I., Hermann, A., Kuhn, M., Kruse, O., Meir Drexler, S., Meulders, A., Nees, F., Pittig, A., Richter, J., Römer, S., Shiban, Y., Schmitz, A., Straube, B., Vervliet, B., Wendt, J., Baas, J.M.P., Merz, C.J., 2017. Don't fear 'fear conditioning': Methodological considerations for the design and

analysis of studies on human fear acquisition, extinction, and return of fear. Neurosci. Biobehav. Rev. 77, 247–285. https://doi.org/10.1016/j.neubiorev.2017.02.026

Lonsdorf, T.B., Merz, C.J., 2017. More than just noise: Inter-individual differences in fear acquisition, extinction and return of fear in humans - Biological, experiential, temperamental factors, and methodological pitfalls. Neurosci. Biobehav. Rev. 80, 703–728. https://doi.org/10.1016/j.neubiorev.2017.07.007

Lonsdorf, T.B., Merz, C.J., Fullana, M.A., 2019. Fear extinction retention: Is it what we think it is? Biol. Psychiatry 85, 1074–1082. https://doi.org/10.1016/j.biopsych.2019.02.011

Lux, V., Masseck, O.A., Herlitze, S., Sauvage, M.M., 2017. Optogenetic destabilization of the memory trace in CA1: Insights into reconsolidation and retrieval processes. Cereb. cortex 27, 841–851. https://doi.org/10.1093/cercor/bhv282

Maia, T. V., 2010. Two-factor theory, the actor-critic model, and conditioned avoidance. Learn. Behav. 38, 50–67. https://doi.org/10.3758/LB.38.1.50

Maksymetz, J., Joffe, M.E., Moran, S.P., Stansley, B.J., Li, B., Temple, K., Engers, D.W., Lawrence, J.J., Lindsley, C.W., Conn, P.J., 2019. M1 muscarinic receptors modulate fear-related inputs to the prefrontal cortex: Implications for novel treatments of Posttraumatic Stress Disorder. Biol. Psychiatry 85, 989–1000. https://doi.org/10.1016/j.biopsych.2019.02.020

Malherbe, P., Knoflach, F., Hernandez, M.C., Hoffmann, T., Schnider, P., Porter, R.H., Wettstein, J.G., Ballard, T.M., Spooren, W., Steward, L., 2011. Characterization of RO4583298 as a novel potent, dual antagonist with in vivo activity at tachykinin NK 1 and NK 3 receptors. Br. J. Pharmacol. 162, 929–946. https://doi.org/10.1111/j.1476-5381.2010.01096.x

Maren, S., 2001. Neurobiology of Pavlovian fear conditioning. Annu. Rev. Neurosci. 897–931. https://doi.org/10.1146/annurev.neuro.24.1.897.

Maren, S., Fanselow, M.S., 1996. The amygdala and fear conditioning: Has the nut been cracked? Neuron 16, 237–240. https://doi.org/10.1016/S0896-6273(00)80041-0

Maren, S., Quirk, G.J., 2004. Neuronal signalling of fear memory. Nat. Rev. Neurosci. 5, 844–

852. https://doi.org/10.1038/nrn1535

Mattis, J., Tye, K.M., Ferenczi, E.A., Ramakrishnan, C., O'Shea, D.J., Prakash, R., Gunaydin, L.A., Hyun, M., Fenno, L.E., Gradinaru, V., Yizhar, O., Deisseroth, K., 2012. Principles for applying optogenetic tools derived from direct comparative analysis of microbial opsins. Nat. Methods 9, 159–172. https://doi.org/10.1038/nmeth.1808

McCullough, Kenneth M., Choi, D., Guo, J., Zimmerman, K., Walton, J., Rainnie, D.G., Ressler, K.J., 2016. Molecular characterization of Thy1 expressing fear-inhibiting neurons within the basolateral amygdala. Nat. Commun. 7, 13149. https://doi.org/10.1038/ncomms13149

McCullough, K. M., Morrison, F.G., Ressler, K.J., 2016. Bridging the Gap: Towards a cell-type specific understanding of neural circuits underlying fear behaviors. Neurobiol. Learn. Mem. 135, 27–39. https://doi.org/10.1016/j.nlm.2016.07.025

McDonald, A.J., 1982. Cytoarchitecture of the central amygdaloid nucleus of the rat. J. Comp. Neurol. 208, 401–418. https://doi.org/10.1002/cne.902080409

McDonald, A.J., Betette, R.L., 2001. Parvalbumin-containing neurons in the rat basolateral amygdala: Morphology and co-localization of Calbindin-D28k. Neuroscience 102, 413–425. https://doi.org/10.1016/S0306-4522(00)00481-4

McNally, R.J., 2007. Mechanisms of exposure therapy: How neuroscience can improve psychological treatments for anxiety disorders. Clin. Psychol. Rev. 27, 750–759. https://doi.org/10.1016/j.cpr.2007.01.003

Meir Drexler, S., Merz, C.J., Hamacher-Dang, T.C., Wolf, O.T., 2016. Cortisol effects on fear memory reconsolidation in women. Psychopharmacology (Berl). 233, 2687–2697. https://doi.org/10.1007/s00213-016-4314-x

Mendez, P., Stefanelli, T., Flores, C.E., Muller, D., Lüscher, C., 2018. Homeostatic plasticity in the hippocampus facilitates memory extinction. Cell Rep. 22, 1451–1461. https://doi.org/10.1016/j.celrep.2018.01.025

Merlo, E., Milton, A.L., Goozée, Z.Y., Theobald, D.E., Everitt, B.J., 2014. Reconsolidation and extinction are dissociable and mutually exclusive processes: Behavioral and molecular evidence. J Neurosci 34, 2422–2431. https://doi.org/10.1523/JNEUROSCI.4001-13.2014

Milad, M.R., Orr, S.P., Lasko, N.B., Chang, Y., Rauch, S.L., Pitman, R.K., 2008. Presence and acquired origin of reduced recall for fear extinction in PTSD: Results of a twin study. J. Psychiatr. Res. 42, 515–520. https://doi.org/10.1016/j.jpsychires.2008.01.017

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. https://doi.org/10.1146/annurev.psych.121208.131631

Milad, M.R., Quirk, G.J., 2002. Neurons in medial prefrontal cortex signal memory for fear extinction. Nature 420, 70–74. https://doi.org/10.1038/nature01138

Milad, M.R., Vidal-Gonzalez, I., Quirk, G.J., 2004. Electrical stimulation of medial prefrontal cortex reduces conditioned fear in a temporally specific manner. Behav. Neurosci. 118, 389–394. https://doi.org/10.1037/0735-7044.118.2.389

Milekic, M.H., Alberini, C.M., 2002. Temporally graded requirement for protein synthesis following memory reactivation. Neuron 36, 521–525. https://doi.org/10.1016/S0896-6273(02)00976-5

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. https://doi.org/10.1126/science.1167975

Morcos, A.S., Harvey, C.D., 2016. History-dependent variability in population dynamics during evidence accumulation in cortex. Nat. Neurosci. 19, 1672–1681. https://doi.org/10.1038/nn.4403

Moscovitch, M., Winocur, G., 2002. The frontal cortex and working with memory, in: Principles of Frontal Lobe Function. Oxford University Press, pp. 188–209. https://doi.org/10.1093/acprof:oso/9780195134971.003.0012

Muller, J.F., Mascagni, F., McDonald, A.J., 2007. Postsynaptic targets of somatostatin-containing interneurons in the rat basolateral amygdala. J. Comp. Neurol. 500, 513–529. https://doi.org/10.1002/cne.21185

Muller, J.F., Mascagni, F., McDonald, A.J., 2006. Pyramidal cells of the rat basolateral amygdala: Synaptology and innervation by parvalbumin-immunoreactive interneurons. J. Comp. Neurol. 494, 635–650. https://doi.org/10.1002/cne.20832

Mungee, A., Burger, M., Bajbouj, M., 2016. No effect of cathodal transcranial direct current stimulation on fear memory in healthy human subjects. Brain Sci. 6, 55. https://doi.org/10.3390/brainsci6040055

Mungee, A., Kazzer, P., Feeser, M., Nitsche, M.A., Schiller, D., Bajbouj, M., 2014. Transcranial direct current stimulation of the prefrontal cortex: A means to modulate fear memories. Neuroreport 25, 480–484. https://doi.org/10.1097/WNR.0000000000000119

Myers, K.M., Davis, M., 2002. Behavioral and neural analysis of extinction. Neuron 36, 567–584. https://doi.org/10.1016/S0896-6273(02)01064-4

Myers, K.M., Ressler, K.J., Davis, M., 2006. Different mechanisms of fear extinction dependent on length of time since fear acquisition. Learn. Mem. 13, 216–223. https://doi.org/10.1101/lm.119806

Nader, K., Hardt, O., 2009. A single standard for memory: The case for reconsolidation. Nat. Rev. Neurosci. 10, 224–234. https://doi.org/10.1038/nrn2590

Nader, K., Schafe, G.E., Le Doux, J.E., 2000. Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. Nature 406, 722–726. https://doi.org/10.1038/35021052

Najafi, F., Churchland, A.K., 2018. Perceptual decision-making: A field in the midst of a transformation. Neuron 100, 453–462. https://doi.org/10.1016/j.neuron.2018.10.017

Nakamura, K., Hara, N., Kouider, S., Takayama, Y., Hanajima, R., Sakai, K., Ugawa, Y., 2006. Task-guided selection of the dual neural pathways for reading. Neuron 52, 557–564.

https://doi.org/10.1016/j.neuron.2006.09.030

Nitsche, M.A., Cohen, L.G., Wassermann, E.M., Priori, A., Lang, N., Antal, A., Paulus, W., Hummel, F., Boggio, P.S., Fregni, F., Pascual-Leone, A., 2008. Transcranial direct current stimulation: State of the art 2008. Brain Stimul. 1, 206–223. https://doi.org/10.1016/j.brs.2008.06.004

Nitsche, M.A., Paulus, W., 2000. Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. J. Physiol. 527, 633–639. https://doi.org/10.1111/j.1469-7793.2000.t01-1-00633.x

Nomura, H., Hara, K., Abe, R., Hitora-Imamura, N., Nakayama, R., Sasaki, T., Matsuki, N., Ikegaya, Y., 2015. Memory formation and retrieval of neuronal silencing in the auditory cortex. Proc. Natl. Acad. Sci. U. S. A. 112, 9740–9744. https://doi.org/10.1073/pnas.1500869112

Ochsner, K.N., Silvers, J.A., Buhle, J.T., 2012. Functional imaging studies of emotion regulation: a synthetic review and evolving model of the cognitive control of emotion. Ann. N. Y. Acad. Sci. 1251, E1–E24. https://doi.org/10.1111/j.1749-6632.2012.06751.x

Pape, H.C., Pare, D., 2010. Plastic synaptic networks of the amygdala for the acquisition, expression, and extinction of conditioned fear. Physiol. Rev. 90, 419–463. https://doi.org/10.1152/physrev.00037.2009

Paracampo, R., Pirruccio, M., Costa, M., Borgomaneri, S., Avenanti, A., 2018. Visual, sensorimotor and cognitive routes to understanding others' enjoyment: An individual differences rTMS approach to empathic accuracy. Neuropsychologia 116, 86–98. https://doi.org/10.1016/j.neuropsychologia.2018.01.043

Paracampo, R., Tidoni, E., Borgomaneri, S., di Pellegrino, G., Avenanti, A., 2017. Sensorimotor network crucial for inferring amusement from smiles. Cereb. Cortex 27, 5116–5129. https://doi.org/10.1093/cercor/bhw294

Parsons, R.G., Ressler, K.J., 2013. Implications of memory modulation for post-traumatic stress and fear disorders. Nat. Neurosci. 16, 146–153. https://doi.org/10.1038/nn.3296

Pavlov, I.P., 1927. Conditioned reflexes: an investigation of the physiological activity of the cerebral cortex., Annals of neurosciences. Oxford Univ. Press. https://doi.org/10.5214/ans.0972-7531.1017309

Pedreira, M.E., Pérez-Cuesta, L.M., Maldonado, H., 2004. Mismatch between what is expected and what actually occurs triggers memory reconsolidation or extinction. Learn. Mem. 11, 579–585. https://doi.org/10.1101/lm.76904

Penzo, M.A., Robert, V., Li, B., 2014. Fear conditioning potentiates synaptic transmission onto long-range projection neurons in the lateral subdivision of central amygdala. J. Neurosci. 34, 2432–2437. https://doi.org/10.1523/JNEUROSCI.4166-13.2014

Phillips, R.G., LeDoux, J.E., 1992. Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. Behav. Neurosci. 106, 274–285. https://doi.org/10.1037/0735-7044.106.2.274

Pinault, D., Deschênes, M., 1998. Projection and innervation patterns of individual thalamic reticular axons in the thalamus of the adult rat: A three-dimensional, graphic, and morphometric analysis. J. Comp. Neurol. 391, 180–203. https://doi.org/10.1002/(SICI)1096-9861(19980209)391:2<180::AID-CNE3>3.0.CO;2-Z

Przybyslawski, J., Roullet, P., Sara, S.J., 1999. Attenuation of emotional and nonemotional memories after their reactivation: Role of β adrenergic receptors. J. Neurosci. 19, 6623–6628. https://doi.org/10.1523/jneurosci.19-15-06623.1999

Quirk, G.J., Mueller, D., 2008. Neural mechanisms of extinction learning and retrieval. Neuropsychopharmacology 33, 56–72. https://doi.org/10.1038/sj.npp.1301555

Raij, T., Nummenmaa, A., Marin, M.F., Porter, D., Furtak, S., Setsompop, K., Milad, M.R., 2018. Prefrontal cortex stimulation enhances fear extinction memory in humans. Biol. Psychiatry 84, 129–137. https://doi.org/10.1016/j.biopsych.2017.10.022

Ramanathan, K.R., Jin, J., Giustino, T.F., Payne, M.R., Maren, S., 2018. Prefrontal projections to the thalamic nucleus reuniens mediate fear extinction. Nat. Commun. 9, 4527.

https://doi.org/10.1038/s41467-018-06970-z

Ramirez, S., Liu, X., Lin, P.A., Suh, J., Pignatelli, M., Redondo, R.L., Ryan, T.J., Tonegawa, S., 2013. Creating a false memory in the hippocampus. Science 341, 387–391. https://doi.org/10.1126/science.1239073

Raposo, D., Kaufman, M.T., Churchland, A.K., 2014. A category-free neural population supports evolving demands during decision-making. Nat. Neurosci. 17, 1784–1792. https://doi.org/10.1038/nn.3865

Redondo, R.L., Kim, J., Arons, A.L., Ramirez, S., Liu, X., Tonegawa, S., 2014. Bidirectional switch of the valence associated with a hippocampal contextual memory engram. Nature 513, 426–430. https://doi.org/10.1038/nature13725

Reed, T., Cohen Kadosh, R., 2018. Transcranial electrical stimulation (tES) mechanisms and its effects on cortical excitability and connectivity. J. Inherit. Metab. Dis. 41, 1123–1130. https://doi.org/10.1007/s10545-018-0181-4

Rescorla, R.A., Wagner, A.R., 1972. A theory of Pavlovian conditioning: Variations in the effectiveness of reinforcement and nonreinforcement. Class. Cond. II Curr. Res. theory 2, 64–99.

Ressler, R.L., Maren, S., 2019. Synaptic encoding of fear memories in the amygdala. Curr. Opin. Neurobiol. 54, 54–59. https://doi.org/10.1016/j.conb.2018.08.012

Rossi, S., Hallett, M., Rossini, P.M., Pascual-Leone, A., Nasreldin, M., Nakatsuka, M., Koganemaru, S., Fawi, G., Group, T.S. of T.C., 2009. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. Clin. Neurophysiol. 120, 2008–2039. https://doi.org/10.1016/j.clinph.2009.08.016

Rossini, P.M., Barker, A.T., Berardelli, A., Caramia, M.D., Caruso, G., Cracco, R.Q., Dimitrijević, M.R., Hallett, M., Katayama, Y., Lücking, C.H., Maertens de Noordhout, A., Marsden, C.D., Murray, N.M.F., Rothwell, J.C., Swash, M., Tomberg, C., 1994. Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and

procedures for routine clinical application. Report of an IFCN committee. Electroencephalogr. Clin. Neurophysiol. 91, 79–92. https://doi.org/10.1016/0013-4694(94)90029-9

Roy, D.S., Arons, A., Mitchell, T.I., Pignatelli, M., Ryan, T.J., Tonegawa, S., 2016. Memory retrieval by activating engram cells in mouse models of early Alzheimer's disease. Nature 531, 508–512. https://doi.org/10.1038/nature17172

Ryan, T.J., Roy, D.S., Pignatelli, M., Arons, A., Tonegawa, S., 2015. Engram cells retain memory under retrograde amnesia. Science 348, 1007–1013. https://doi.org/10.1126/science.aaa5542

Salinas-Hernández, X.I., Vogel, P., Betz, S., Kalisch, R., Sigurdsson, T., Duvarci, S., 2018. Dopamine neurons drive fear extinction learning by signaling the omission of expected aversive outcomes. Elife 7, e38818. https://doi.org/10.7554/eLife.38818

Sandrini, M., Censor, N., Mishoe, J., Cohen, L.G., 2013. Causal role of prefrontal cortex in strengthening of episodic memories through reconsolidation. Curr. Biol. 23, 2181–2184. https://doi.org/10.1016/j.cub.2013.08.045

Sauvage, M.M., Nakamura, N.H., Beer, Z., 2013. Mapping memory function in the medial temporal lobe with the immediate-early gene Arc. Behav. Brain Res. 254, 22–33. https://doi.org/10.1016/j.bbr.2013.04.048

Schiller, D., Delgado, M.R., 2010. Overlapping neural systems mediating extinction, reversal and regulation of fear. Trends Cogn. Sci. 14, 268–276. https://doi.org/10.1016/j.tics.2010.04.002

Schiller, D., LeDoux, J.E., Phelps, E.A., 2020. Reply to Beckers, McIntosh and Chambers on the verification of 'preventing the return of fear using retrieval-extinction in humans.' PsyArXiv. https://doi.org/10.31234/osf.io/jn6uw

Schiller, D., Monfils, M.-H., Raio, C.M., Johnson, D.C., Ledoux, J.E., Phelps, E.A., 2010. Preventing the return of fear in humans using reconsolidation update mechanisms. Nature 463, 49–53. https://doi.org/10.1038/nature08637

Schiller, D., Phelps, E.A., 2011. Does reconsolidation occur in humans? Front. Behav. Neurosci. 5, 24. https://doi.org/10.3389/fnbeh.2011.00024

Schultz, W., Dayan, P., Montague, P.R., 1997. A neural substrate of prediction and reward. Science 275, 1593–1599. https://doi.org/10.1126/science.275.5306.1593

Schultz, W., Dickinson, A., 2000. Neuronal coding of prediction errors. Annu. Rev. Neurosci. 23, 473–500. https://doi.org/10.1146/annurev.neuro.23.1.473

Schwabe, L., Nader, K., Pruessner, J.C., 2014. Reconsolidation of human memory: Brain mechanisms and clinical relevance. Biol. Psychiatry 76, 274–280. https://doi.org/10.1016/j.biopsych.2014.03.008

Sengupta, A., Holmes, A., 2019. A discrete dorsal raphe to basal amygdala 5-HT circuit calibrates aversive memory. Neuron 103, 489-505.e7. https://doi.org/10.1016/j.neuron.2019.05.029

Senn, V., Wolff, S.B.E., Herry, C., Grenier, F., Ehrlich, I., Gründemann, J., Fadok, J.P., Müller, C., Letzkus, J.J., Lüthi, A., 2014. Long-range connectivity defines behavioral specificity of amygdala neurons. Neuron 81, 428–437. https://doi.org/10.1016/j.neuron.2013.11.006

Sevenster, D., Beckers, T., Kindt, M., 2013. Prediction error governs pharmacologically induced amnesia for learned fear. Science 339, 830–833. https://doi.org/10.1126/science.1231357

Shapiro, F., 2001. Eye Movement Desensitization and Reprocessing (EMDR) Therapy: Third Edition: Basic Principles, Protocols, and Procedures, New York: ed.

Shema, R., Sacktor, T.C., Dudai, Y., 2007. Rapid erasure of long-term memory associations in the cortex by an inhibitor of PKMζ. Science 317, 951–953. https://doi.org/10.1126/science.1144334

Shi, T., Feng, S., Wei, M., Zhou, W., 2020. Role of the anterior agranular insular cortex in the modulation of fear and anxiety. Brain Res. Bull. 155, 174–183. https://doi.org/10.1016/j.brainresbull.2019.12.003

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. https://doi.org/10.1038/npp.2010.184

Simons, J.S., Spiers, H.J., 2003. Prefrontal and medial temporal lobe interactions in long-term memory. Nat. Rev. Neurosci. 4, 637–648. https://doi.org/10.1038/nrn1178

Soeter, M., Kindt, M., 2011. Disrupting reconsolidation: Pharmacological and behavioral manipulations. Learn. Mem. 18, 357–366. https://doi.org/10.1101/lm.2148511

Song, Y.H., Kim, J.H., Jeong, H.W., Choi, I., Jeong, D., Kim, K., Lee, S.H., 2017. A neural circuit for auditory dominance over visual perception. Neuron 93, 940-954.e6. https://doi.org/10.1016/j.neuron.2017.01.006

Sotres-Bayon, F., Bush, D.E.A., LeDoux, J.E., 2007. Acquisition of fear extinction requires activation of NR2B-containing NMDA receptors in the lateral amygdala. Neuropsychopharmacology 32, 1929–1940. https://doi.org/10.1038/sj.npp.1301316

Sotres-Bayon, F., Quirk, G.J., 2010. Prefrontal control of fear: More than just extinction. Curr. Opin. Neurobiol. 20, 231–235. https://doi.org/10.1016/j.conb.2010.02.005

Sotres-Bayon, F., Sierra-Mercado, D., Pardilla-Delgado, E., Quirk, G.J., 2012. Gating of fear in prelimbic cortex by hippocampal and amygdala inputs. Neuron 76, 804–812. https://doi.org/10.1016/j.neuron.2012.09.028

Stefanik, M.T., Kupchik, Y.M., Brown, R.M., Kalivas, P.W., 2013. Optogenetic evidence that pallidal projections, not nigral projections, from the nucleus accumbens core are necessary for reinstating cocaine seeking. J. Neurosci. 33, 13654–13662. https://doi.org/10.1523/JNEUROSCI.1570-13.2013

Suarez-Jimenez, B., Albajes-Eizagirre, A., Lazarov, A., Zhu, X., Harrison, B.J., Radua, J., Neria, Y., Fullana, M.A., 2020. Neural signatures of conditioning, extinction learning, and extinction recall in posttraumatic stress disorder: A meta-analysis of functional magnetic

resonance imaging studies. Psychol. Med. 50, 1442–1451. https://doi.org/10.1017/S0033291719001387

Suarez-Jimenez, B., Bisby, J.A., Horner, A.J., King, J.A., Pine, D.S., Burgess, N., 2018. Linked networks for learning and expressing location-specific threat. Proc. Natl. Acad. Sci. U. S. A. 115, E1032–E1040. https://doi.org/10.1073/pnas.1714691115

Suzuki, A., Josselyn, S.A., Frankland, P.W., Masushige, S., Silva, A.J., Kida, S., 2004. Memory reconsolidation and extinction have distinct temporal and biochemical signatures. J. Neurosci. 24, 4787–4795. https://doi.org/10.1523/JNEUROSCI.5491-03.2004

Takasu, M.A., Dalva, M.B., Zigmond, R.E., Greenberg, M.E., 2002. Modulation of NMDA receptor - Dependent calcium influx and gene expression through EphB receptors. Science 295, 491–495. https://doi.org/10.1126/science.1065983

Tan, S.Z.K., Sheng, V., Chan, Y.S., Lim, L.W., 2019. Eternal sunshine of the neuromodulated mind: Altering fear memories through neuromodulation. Exp. Neurol. 314, 9–19. https://doi.org/10.1016/j.expneurol.2019.01.004

Todd, T.P., Bucci, D.J., 2015. Retrosplenial cortex and long-term memory: Molecules to behavior. Neural Plast. 2015, 414173. https://doi.org/10.1155/2015/414173

Tortella-Feliu, M., Fullana, M.A., Pérez-Vigil, A., Torres, X., Chamorro, J., Littarelli, S.A., Solanes, A., Ramella-Cravaro, V., Vilar, A., González-Parra, J.A., Andero, R., Reichenberg, A., Mataix-Cols, D., Vieta, E., Fusar-Poli, P., Ioannidis, J.P.A., Stein, M.B., Radua, J., Fernández de la Cruz, L., 2019. Risk factors for posttraumatic stress disorder: An umbrella review of systematic reviews and meta-analyses. Neurosci. Biobehav. Rev. 107, 154–165. https://doi.org/10.1016/j.neubiorev.2019.09.013

Tovote, P., Fadok, J.P., Lüthi, A., 2015. Neuronal circuits for fear and anxiety. Nat. Rev. Neurosci. 16, 317–331. https://doi.org/10.1038/nrn3945

Tronson, N.C., Taylor, J.R., 2007. Molecular mechanisms of memory reconsolidation. Nat. Rev. Neurosci. 8, 262–275. https://doi.org/10.1038/nrn2090

Trouche, S., Sasaki, J.M., Tu, T., Reijmers, L.G., 2013. Fear extinction causes target-specific remodeling of perisomatic inhibitory synapses. Neuron 80, 1054–1065. https://doi.org/10.1016/j.neuron.2013.07.047

Tye, K.M., Prakash, R., Kim, S.Y., Fenno, L.E., Grosenick, L., Zarabi, H., Thompson, K.R., Gradinaru, V., Ramakrishnan, C., Deisseroth, K., 2011. Amygdala circuitry mediating reversible and bidirectional control of anxiety. Nature 471, 358–362. https://doi.org/10.1038/nature09820

Van ’t Wout, M., Mariano, T.Y., Garnaat, S.L., Reddy, M.K., Rasmussen, S.A., Greenberg, B.D., 2016. Can transcranial direct current stimulation augment extinction of conditioned fear? Brain Stimul. 9, 529–536. https://doi.org/10.1016/j.brs.2016.03.004

Vervliet, B., Baeyens, F., Van den Bergh, O., Hermans, D., 2013. Extinction, generalization, and return of fear: A critical review of renewal research in humans. Biol. Psychol. 92, 51–58. https://doi.org/10.1016/j.biopsycho.2012.01.006

Vicario, C.M., Nitsche, M.A., Hoysted, I., Yavari, F., Avenanti, A., Salehinejad, M.A., Felmingham, K.L., 2019. Anodal transcranial direct current stimulation over the ventromedial prefrontal cortex enhances fear extinction in healthy humans: A single blind sham-controlled study. Brain Stimul. 10–12. https://doi.org/10.1016/j.brs.2019.12.022

Vicario, C.M., Rafal, R.D., Borgomaneri, S., Paracampo, R., Kritikos, A., Avenanti, A., 2017. Pictures of disgusting foods and disgusted facial expressions suppress the tongue motor cortex. Soc. Cogn. Affect. Neurosci. 12, 352–362. https://doi.org/10.1093/scan/nsw129

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. https://doi.org/10.1101/lm.306106

Wagner, T., Valero-Cabre, A., Pascual-Leone, A., 2007. Noninvasive human brain stimulation. Annu. Rev. Biomed. Eng. 9, 527–565. https://doi.org/10.1146/annurev.bioeng.9.061206.133100

Walker, A.G., Wenthur, C.J., Xiang, Z., Rook, J.M., Emmitte, K.A., Niswender, C.M.,

Lindsley, C.W., Conn, P.J., 2015. Metabotropic glutamate receptor 3 activation is required for long-term depression in medial prefrontal cortex and fear extinction. Proc. Natl. Acad. Sci. U. S. A. 112, 1196–1201. https://doi.org/10.1073/pnas.1416196112

Wendler, E., Gaspar, J.C.C., Ferreira, T.L., Barbiero, J.K., Andreatini, R., Vital, M.A.B.F., Blaha, C.D., Winn, P., Da Cunha, C., 2013. The roles of the nucleus accumbens core, dorsomedial striatum, and dorsolateral striatum in learning: Performance and extinction of Pavlovian fear-conditioned responses and instrumental avoidance responses. Neurobiol. Learn. Mem. 109, 27–36. https://doi.org/10.1016/j.nlm.2013.11.009

Wilensky, A.E., Schafe, G.E., Kristensen, M.P., LeDoux, J.E., 2006. Rethinking the fear circuit: The central nucleus of the amygdala is required for the acquisition, consolidation, and expression of pavlovian fear conditioning. J. Neurosci. 26, 12387–12396. https://doi.org/10.1523/JNEUROSCI.4316-06.2006

Williams, S.C.P., Deisseroth, K., 2013. Optogenetics. Proc. Natl. Acad. Sci. U. S. A. 110, 16287. https://doi.org/10.1073/pnas.1317033110

Wolff, S.B.E., Gründemann, J., Tovote, P., Krabbe, S., Jacobson, G.A., Müller, C., Herry, C., Ehrlich, I., Friedrich, R.W., Letzkus, J.J., Lüthi, A., 2014. Amygdala interneuron subtypes control fear learning through disinhibition. Nature 509, 453–458. https://doi.org/10.1038/nature13258

Wood, N.E., Rosasco, M.L., Suris, A.M., Spring, J.D., Marin, M.-F., Lasko, N.B., Goetz, J.M., Fischer, A.M., Orr, S.P., Pitman, R.K., 2015. Pharmacological blockade of memory reconsolidation in posttraumatic stress disorder: Three negative psychophysiological studies. Psychiatry Res. 225, 31–39. https://doi.org/10.1016/j.psychres.2014.09.005

Wurtz, H., El-Khoury-Malhame, M., Wilhelm, F.H., Michael, T., Beetz, E.M., Roques, J., Reynaud, E., Courtin, J., Khalfa, S., Herry, C., 2016. Preventing long-lasting fear recovery using bilateral alternating sensory stimulation: A translational study. Neuroscience 321, 222–235. https://doi.org/10.1016/j.neuroscience.2015.06.012

Yu, K., Ahrens, S., Zhang, X., Schiff, H., Ramakrishnan, C., Fenno, L., Deisseroth, K., Zhao, F., Luo, M.H., Gong, L., He, M., Zhou, P., Paninski, L., Li, B., 2017. The central amygdala controls learning in the lateral amygdala. Nat. Neurosci. 20, 1680–1685. https://doi.org/10.1038/s41593-017-0009-9

Zanon, M., Borgomaneri, S., Avenanti, A., 2018. Action-related dynamic changes in inferior frontal cortex effective connectivity: a TMS/EEG coregistration study. Cortex 108, 193–209. https://doi.org/10.1016/j.cortex.2018.08.004

Zhou, Y., Liu, B., Wu, G.K., Kim, Y.-J., Xiao, Z., Tao, H.W., Zhang, L.I., 2010. Preceding inhibition silences layer 6 neurons in auditory cortex. Neuron 65, 706–717. https://doi.org/10.1016/j.neuron.2010.02.021

Zhu, H., Pleil, K.E., Urban, D.J., Moy, S.S., Kash, T.L., Roth, B.L., 2014. Chemogenetic inactivation of ventral hippocampal glutamatergic neurons disrupts consolidation of contextual fear memory. Neuropsychopharmacology 39, 1880–1892. https://doi.org/10.1038/npp.2014.35

Zikopoulos, B., Barbas, H., 2012. Pathways for emotions and attention converge on the thalamic reticular nucleus in primates. J. Neurosci. 32, 5338–5350. https://doi.org/10.1523/jneurosci.4793-11.2012

Zimmerman, J.M., Maren, S., 2010. NMDA receptor antagonism in the basolateral but not central amygdala blocks the extinction of Pavlovian fear conditioning in rats 31, 1664–1670. https://doi.org/10.1111/j.1460-9568.2010.07223.x.NMDA

Figure Legend

Figure 1: Talairach coordinates of the targeted cortical sites in the rTMS and electrodes placement for stimulation in the tDCS/tES studies were converted in Talairach coordinates (Koessler et al., 2009). The targeted cortical sites were reconstructed using Surf Ice (https://www.nitrc.org/projects/surfice).

Figure 2: A) During fear conditioning, thalamic and cortical afferents convey conditioned stimulus (CS) and unconditioned stimulus (US) sensory information into the baso-lateral amygdala (BLA), causing a marked potentiation of excitatory synapses by long-term synaptic plasticity (LTP). This phenomenon causes indeed an increase of firing activity of a specific class of neurons, called "*fear neurons*". *Fear neurons* stimulate central amygdala (CeA) (*serial processing*) causing a strong increase of the whole amygdala output triggering both motor and autonomic fear behaviour by involving periaqueductal gray matter (PAG) and hypothalamus. Connection between cortical prelimbic (PrL) and BLA neurons is able to stimulate the activity of BLA *fear neurons* causing a further increase in CeA activity. BLA neurons activity is also potentiated by a backward excitatory pathway originating from CeA. CS-US association induces in CeA neurons similar learning-dependent modifications occurring in BLA (*parallel processing)*. From BLA, CS-US information is also redirected to Basal Ganglia which support the instrumental response.

B) During the extinction phase**,** the input from cortical infralimbic (IL) area suppresses the BLA *fear neurons* activity by the interplay with intrinsic inhibitory interneurons. Moreover, IL area directly inhibits the CeA trough activation of intercalated cells (ITCs), a scattered group of GABAergic neurons mainly located around the BLA area. The local inhibitory circuit further reduces the neural activity of *fear neurons* of BLA neurons and the subsequent BLA-CeA pathway, thereby suppressing the fear response.

Box 1: Schematic overview representing the overall effect in fear processing triggered by optogenetic stimulation together with the receptorial modulation.

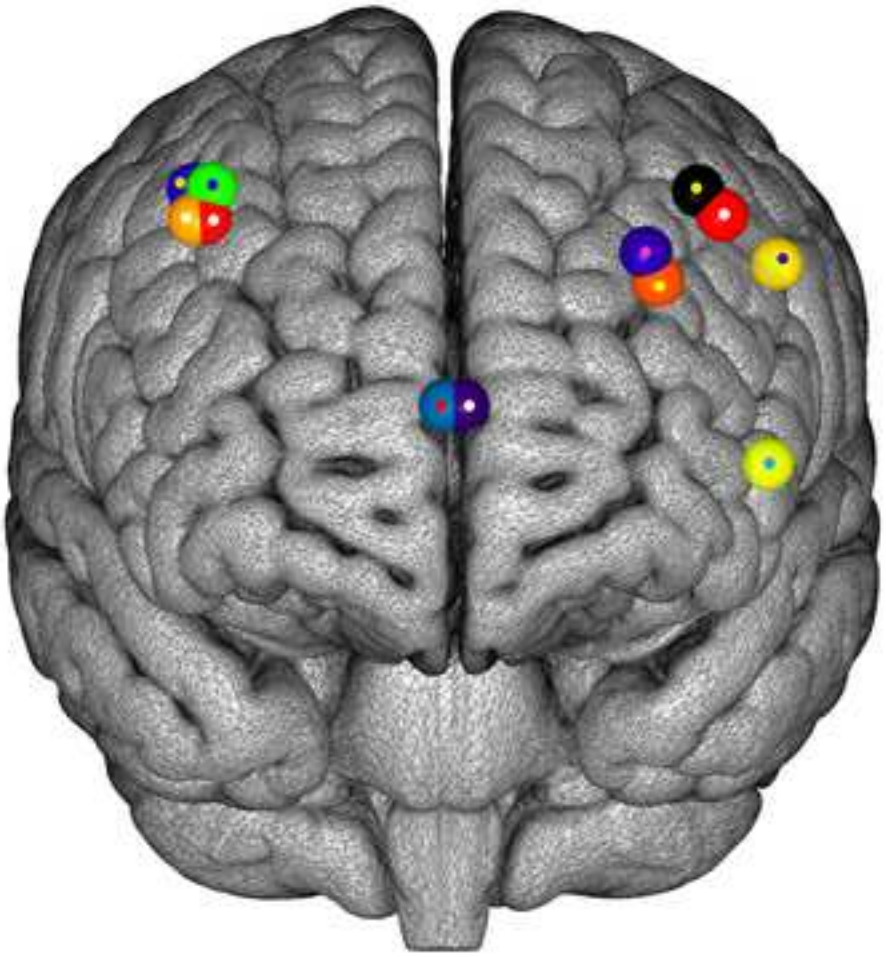
Abbreviations, FC: Fear Conditioning, ChR2: Channelrhodopsin2, eNpHR: Halorhodopsin, eArch3: Activity-regulated cytoskeleton-associated protein, EphB: Erythropoietin-producing hepatocellular-B, Tac2: Tachykinin 2, PV^{+}: Parvalbuminergic positive neurons, M1-ACh: Muscarinic 1 acetylcholine receptors, DA: Dopaminergic neurons, LA: Lateral Amygdala, BLA: Basolateral Amygdala, CeA: Central Amygdala, VH: Ventral Hippocampus, VTA: Ventral Tegmental Area, PFC: Prefrontal Cortex

Tables

Table 1. Summary of NIBS methods used in fear conditioning studies.

Table 2: Summary of NIBS findings in fear conditioning. Studies that reported coordinates based on the Montreal Neurological Institute were converted into Talairach coordinates through the application of the Yale BioImage Suite Package (Lacadie et al., 2008).

Figure1



Memory Consolidation

- Asthana et al., 2013 *[dlPFC]*
- Guhn et al., 2014 *[mPFC]*

Memory Extinction

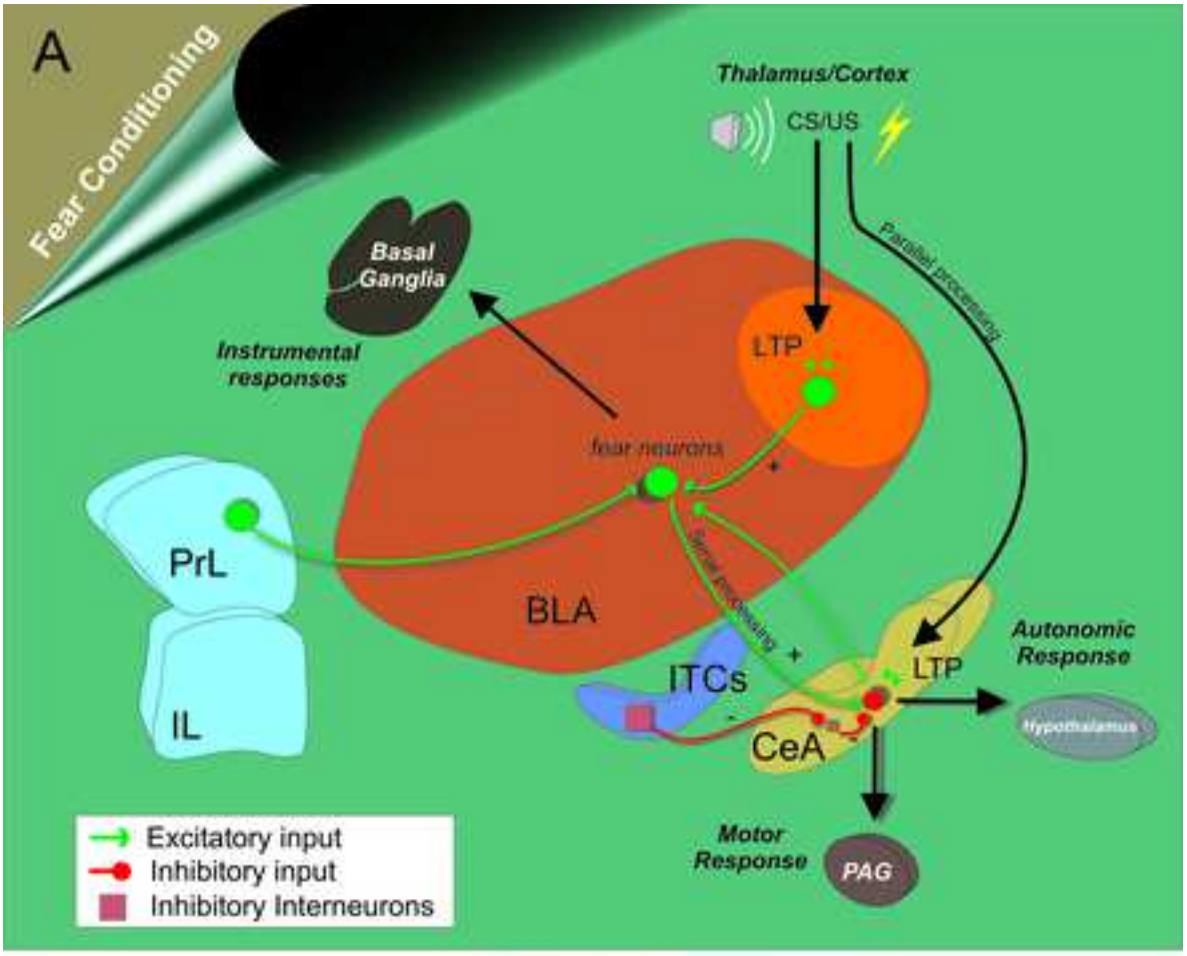
- Van 't Wout et al., 2016 *[PFC]*
- Dittert et al., 2018 *[PFC]*
- Vicario et al., 2019 *[PFC]*
- Raij et al., 2018 *[PFC]*

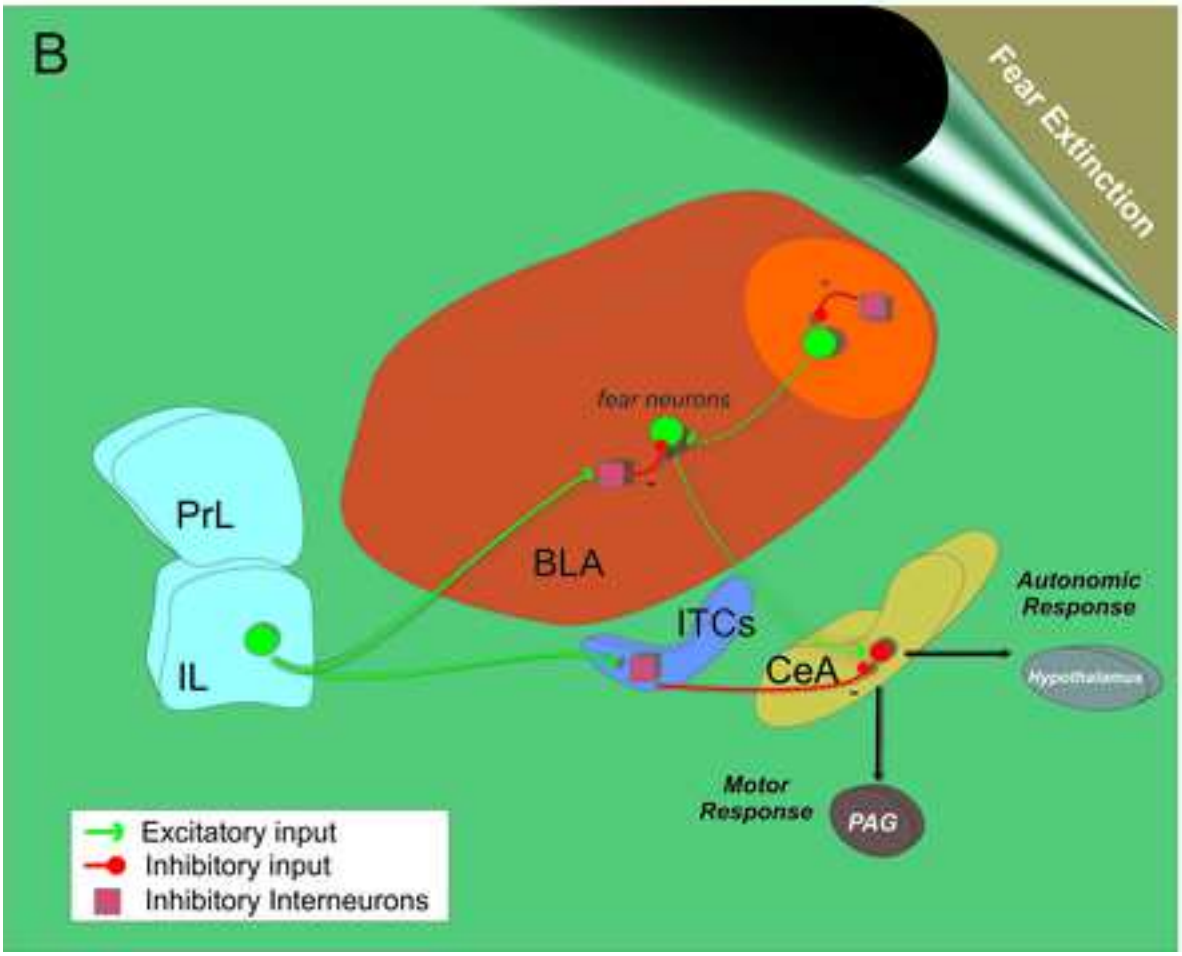
Memory Reconsolidation

- Abend et al., 2016 *[mPFC]*
- Mungee et al., 2014 *[dlPFC]*
- Mungee et al., 2016 *[dlPFC]*
- Ganho-Ávila et al., 2019 *[dlPFC]*
- Borgomaneri et al., 2020 *[dlPFC]*

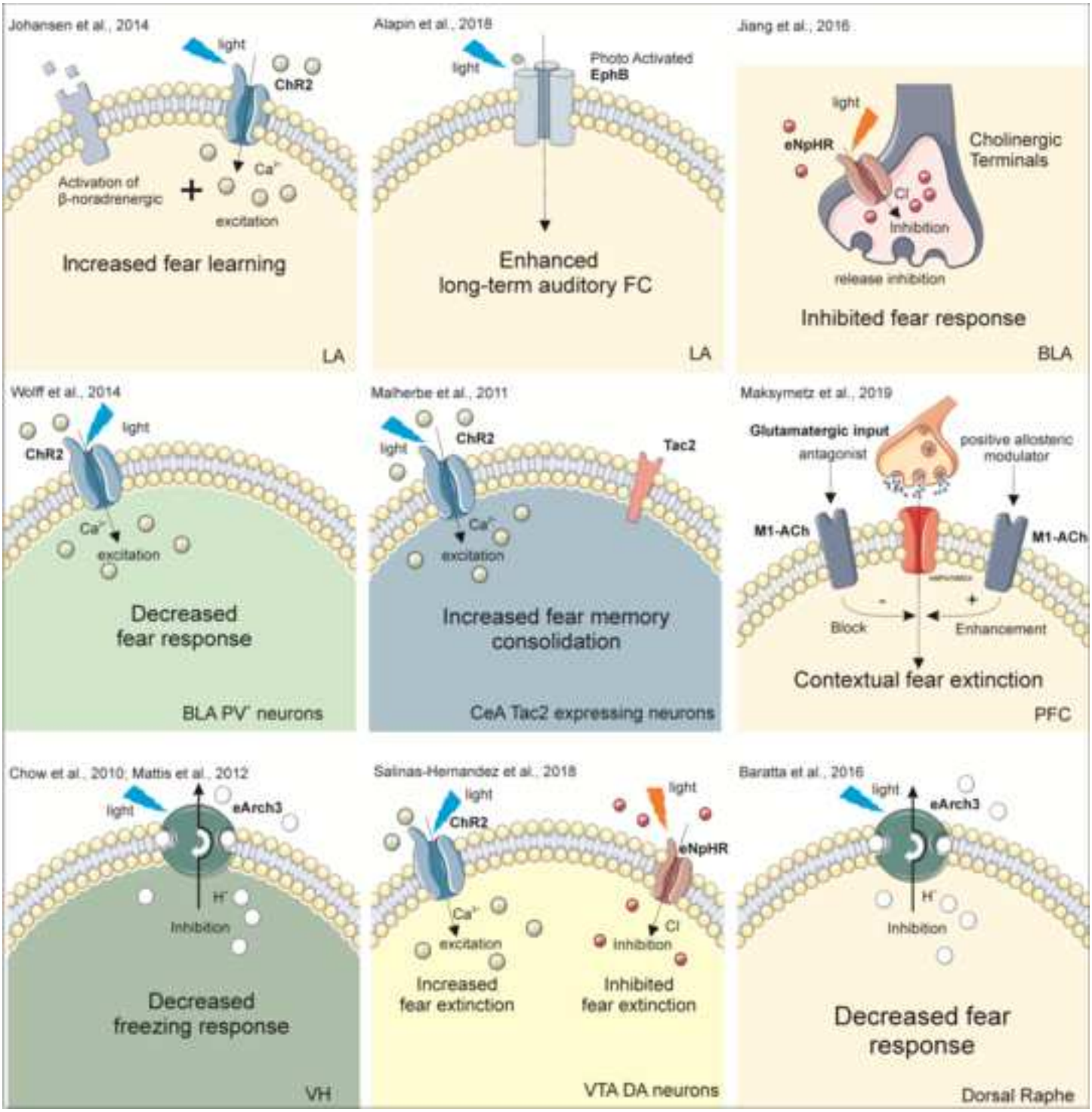
Figure2

Click here to access/download;Figure;Figure2_R1.tif





Box1 Click here to access/download;Figure;Box1.tif



Method		Excitatory Mode	Inhibitory Mode
rTMS		High frequency [> 5 Hz]	Low frequency [0.2 – 1 Hz]
tES	*tDCS*	Anodal [1 – 2 mA]	Cathodal [1 – 2 mA]
	tACS		

Table2

Study	*Experimental Paradigm*	*Physiological index*	*N*	*Stimulation*					*Main Findings*
				Method	*Talairach Coordinates (x, y, z) or Electrode*	*Duration*	*Intensity / Frequency*	*Online/ Offline*	
Memory Consolidation									
Asthana et al., 2013	2-days	SCR	69	Anodal/Cathodal tDCS	F3	12 min	1.0 mA	Offline	Cathodal tDCS disrupts fear memory
Guhn et al., 2014	2-days	FPS and SCR	45	rTMS	FPz	20 min (1560 pulses)	10 Hz 110% rMT	Offline	Enhances extinction
Memory Extinction									
Van 't Wout et al., 2016	2-days	SCR	44	Anodal tDCS	AF3	10 min	2.0 mA	Online/ Offline	Enhances extinction
Dittert et al., 2018	1-days	SCR	84	Anodal tDCS	F7	20 min	1.5 mA	Online	Enhances extinction
Vicario et al., 2019	2-days	SCR	32	Anodal tDCS	AF3	10 min	2.0 mA	Online	Enhances extinction
Raij et al., 2018	3-days	SCR	28	rTMS	[-54, 2, 38]	300ms after CS (28 pulses)	20 Hz 100% rMT	Online	Enhances extinction
Memory Reconsolidation									
Abend et al., 2016	3-days	SCR	45	tACS/tDCS	FPz	20 min	tACS: 1 Hz tDCS: 1.5 mA	Online	Anodal tDCS disrupts fear memory
Mungee et al., 2014	3-days	SCR	74	Anodal tDCS	F4	20 min	1.0 mA	Offline	Anodal tDCS enhances fear memory
Mungee et al., 2016	3-days	SCR	17	Cathodal tDCS	F4	20 min	1.0 mA	Offline	No effects

Ganho-Ávila et al., 2019		2-days	SCR	41	Cathodal tDCS	F4	20 min	1.0 mA	Offline	No effects
Borgomaneri et al., 2020		3-days	SCR	84	rTMS	F3	15 min (900 pulses)	1 Hz 120% rMT	Offline	Disrupt fear memory before and after reinstatement