Spatiotemporally precise targeting of memory processes to prevent and ameliorate maladaptive fear responses

Lizeth Katherine Pedraza Correa

PhD Thesis

Supervisor:

Dr. Antal Berényi

Szeged, 2023

University of Szeged

Albert Szent-Györgyi Medical School

Doctoral School of Theoretical Medicine

Spatiotemporally precise targeting of memory processes to prevent and ameliorate maladaptive fear responses

PhD Thesis

Lizeth Katherine Pedraza Correa

Supervisor:

Dr. Antal Berényi

Szeged, 2023

LIST OF PUBLICATIONS

PUBLICATIONS RELATED TO THE SUBJECT OF THE THESIS

I. **Sierra, R. O.** [*]**, Pedraza, L. K.**[*]**,** Barcsai, L., Pejin, A., Li, Q., Kozák, G., Takeuchi, Y., Nagy, A. J., Lőrincz, M. L., Devinsky, O., Buzsáki, G., & Berényi, A. (2023). Closed-loop brain stimulation augments fear extinction in male rats. **Nature communications**, 14(1), 3972.
https://doi.org/10.1038/s41467-023-39546-7
[*] Equal contribution

II. **Pedraza, L. K.**, Sierra, R. O., Giachero, M., Nunes-Souza, W., Lotz, F. N., & de Oliveira Alvares, L. (2019). Chronic fluoxetine prevents fear memory generalization and enhances subsequent extinction by remodeling hippocampal dendritic spines and slowing down systems consolidation. **Translational psychiatry**, 9(1), 53.
https://doi.org/10.1038/s41398-019-0371-3

III. **Pedraza, L. K.**, Sierra, R. O., & de Oliveira Alvares, L. (2022). Systems consolidation and fear memory generalisation as a potential target for trauma-related disorders. **The world journal of biological psychiatry**, 23(9), 653–665.
https://doi.org/10.1080/15622975.2022.2027010

OTHER PUBLICATIONS

I. Li, Q., Takeuchi, Y., Wang, J., Gellért, L., Barcsai, L., **Pedraza, L. K.**, Nagy, A. J., Kozák, G., Nakai, S., Kato, S., Kobayashi, K., Ohsawa, M., Horváth, G., Kékesi, G., Lőrincz, M. L., Devinsky, O., Buzsáki, G., & Berényi, A. (2023). Reinstating olfactory bulb-derived limbic gamma oscillations alleviates depression-like behavioral deficits in rodents. **Neuron**, 111(13),2065–2075.e5.
https://doi.org/10.1016/j.neuron.2023.04.013

II. Takeuchi, Y., Harangozó, M., **Pedraza, L. K.**, Földi, T., Kozák, G., Li, Q., & Berényi, A. (2021). Closed-loop stimulation of the medial septum terminates epileptic seizures. **Brain**, 144(3), 885–908.
https://doi.org/10.1093/brain/awaa450

1. TABLE OF CONTENTS

2. LIST OF ABBREVIATIONS

ACC: Anterior cingulate cortex

AP: Anteroposterior

APA: American Psychiatric Association

BLA: Basolateral amygdala

CAPS-5: Clinician-Administered PTSD Scale for DSM-5

CL: Closed-loop

CEA: Central amygdala

CS: Conditioned stimulus

DBS: Deep-brain stimulation

DH: Dorsal hippocampus

dlPFC: Dorsolateral prefrontal cortex

dmPFC: Dorsomedial prefrontal cortex

DSM-5: Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition

DV: Dorsoventral

EEG: Electroencephalography

FDA: Food and Drug Administration

fMRI: Functional magnetic resonance imaging

IL: Infralimbic cortex

LFP: local field potential

MDMA: 3,4-Methylenedioxy methamphetamine

MFB: Medial-forebrain bundle

ML: Mediolateral

Nac: Nucleus accumbens

NIMH: National Institute of Mental Health

NMDARs: N-methyl-D-aspartate receptor

non-REM sleep: non-rapid eye-movement sleep

OL: Open loop

PFC: Prefrontal cortex

PL: Prelimbic cortex

PTSD: Post-traumatic stress disorder

RDoC: Research Domain Criteria

SNRIs: Selective norepinephrine reuptake inhibitors

SSRIs: Selective serotonin reuptake inhibitors

SWRs: Sharp-wave ripples

tDCS: Transcranial direct current stimulation

US: Unconditioned stimulus

VHC: Ventral hippocampal commissure

vmPFC: Ventromedial prefrontal cortex

VTA: Ventral tegmental area

3. INTRODUCTION

Posttraumatic stress disorder (PTSD) is an incapacitating chronic disorder that results from direct or indirect exposure to stressful events, threats, or life-threatening events that are perceived to compromise physical or mental safety[1-3] Symptoms include severe sensations of baseless fear, episodes of panic, anxiety; intrusive memories of fear during consciousness or nightmares; broadened fear response; and evasion of analogous but non-threatening stimuli[4,5]. PTSD exhibits significant resistance to both psychotherapy and pharmacotherapy [6,7], Remission rates following conventional treatments are low, with approximately 20-30% of patients with PTSD being classified as treatment-resistant. [8].

Fear conditioning is considered a valid animal model for studying some of the core aspects of PTSD in human patients, including hyperarousal, avoidance behaviors, and long-lasting, persistent, and generalized fear expression [9-11].

PTSD is postulated as a memory-based disorder. Mnemonic alterations include involuntary hypermnesia or explicit amnesia for trauma-related stimuli and fear generalization to non-trauma-related stimuli in animal models and human patients[12,13] .

Memories relevant to survival or adaptation are resistant to forgetting[14] however, these can be suppressed by another type of learning called active extinction[14]. Paradoxically, these two types of memory consolidation processes compete with each other, perhaps with distinct mechanisms and behavioral outcomes. Experimental and clinical studies have revealed altered memory formation resistant to normal processes of extinction as core features of PTSD[15-18]. Impairments of traumatic memories to be effectively extinguished, resulting in their intrusion into inappropriate contexts and ultimately becoming maladaptive.

A large amount of evidence suggests that fear memory traces can be updated with emotional information after reactivation[19-21] or during extinction[22-24], leading to fear attenuation.

Over the last decades, after the successful application of deep-brain stimulation (DBS) to movement disorders, different clinical trials have been conducted to evaluate the effects of DBS in psychiatric conditions such as obsessive-compulsive disorder, addiction, and depression[25]. However, although promising, these “open-loop” (OL) approaches, based

on the introduction of external stimulation without the feedback of internal oscillatory activity fail in terms of specificity like traditional treatments.

The recent incorporation of on-demand "closed-loop" (CL) electrical stimulation has been used to treat "oscillophaties" to quickly terminate certain types of epileptic seizures[26-29] and is gradually incorporated to treat human psychopathologies as well [30]. Here, I provide the first demonstration of a CL approach to update the emotional content of fear memory traces through intracranial electrical stimulation, offering novel avenues to develop CL neuromodulation technologies for anxiety, trauma- and stressor-related disorders.

3.1. Clinical features and epidemiology of posttraumatic stress disorder

The consensus characteristics of PTSD can be found in the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) from the American Psychiatric Association (APA). The diagnostic criteria include[10]:

- Intrusive manifestations.
- Experience or vicarious learning with a traumatic event.
- Behavior centered on avoidance.
- Negative changes in thought processes and mood.
- Modifications in arousal and reactivity.
- Persistence of symptoms for over a month.
- Impact on functionality.

Data collection from 2018 about prevalence of Posttraumatic-stress disorder (PTSD) worldwide was set in 3.9%. However, the epidemiology of PTSD is geography-dependent and strictly associated with economical, political and local social context. For example, the prevalence in Africa is 5.7% and 2.6% in Eastern Europe[3,31].

As expected, the COVID-19 pandemic was a game changer in the prevalence of PTSD. A meta-analysis of 2021 estimates that the global prevalence of PTSD during the pandemic was 12.9%, 26.9% among health workers, and 23.8% among infected patients[32,33]. In addition, sex differences have been detected with a higher risk of women developing anxiety-related disorders and PTSD compared to men[34,35]. A possible explanation for the higher frequency of PTSD in women might be a combination of greater exposure and greater vulnerability.

The DSM-5 classification is based on symptom reports rather than neurobiological criteria. To overcome this inherent subjective bias, the US National Institute of Mental Health (NIMH) proposed the Research Domain Criteria (RDoC)[36] where 5 constructs based on observable behaviors and neurobiological measures can guide not only diagnostic but clinical and preclinical research. These domains are: (1) negative valence, (2) positive valence, (3) cognitive processes, (4) social processes, and (5) arousal[37]. In the case of PTSD, the majority of these domains (negative valence, positive valence, cognitive processes, and arousal) can be modeled in animal research[10].

3.2. Fear conditioning as an animal model of adaptive and maladaptive fear processing

There is a general agreement that animal models are essential to unravel the neurobiological mechanisms and find novel treatments in psychiatric research. However, there is also a debate about whether the current models meet the requirements of face, construct, and predictive validity. For instance, animal models of psychiatric disorders with a high cognitive impairment domain, such as schizophrenia (e.g., visual or auditory hallucinations), face problems to translate animal knowledge to potential clinical settings, since many symptoms are uniquely human[38]. This problem is less evident in PTSD, since the onset in humans is triggered by a clear stressful event (trauma) and is deeply associated with evolutionary preserved emotions, such as fear and flight responses[39]. In fact, models using chronic stress are used to study comorbidity between depression and PTSD, offering a more realistic scenario for translation to human psychopathology[40].

Although there are different PTSD animal models, Pavlovian fear conditioning has been consistently used over decades to study the cellular basis of fear memory, the genetic and environmental factors contributing to normal and pathological fear and the development of new therapies for anxiety disorders[41].

Pavlovian fear conditioning is a paradigm in which animals learn to associate a neutral stimulus, such as a light or a tone (conditioned stimulus; CS), with an aversive stimulus, such as an electric shock (unconditioned stimulus; US). After repeated pairings of the CS and US, the animal will exhibit a fear response to the CS alone. The etiological validity of fear conditioning is high, as a similar learning mechanism operates during the development of PTSD[42]. For instance, in PTSD, a life-threatening event (US) can elicit responses of hyperarousal, extreme fear, or panic. These responses become associated with perceptual environmental cues, such as olfactory and auditory cues, collectively

referred to as contextual cues. Because of this association, future exposition to the same or similar cues can evoke similar responses without the actual harmful event[43]. Interestingly, subsequent Pavlovian fear learning is impaired in those exposed to traumatic events under experimental settings[44].

Fear conditioning allows researchers to strictly control for the exact parameters of the traumatic event (e.g., the consistent frequency, intensity, duration, and intervals of electrical shocks), to select the CS that elicits the fear response, such as a tone (i.e. cued fear conditioning) or a context (i.e. contextual fear conditioning). This controllable environment allows to implement reproducible fear extinction protocols also (i.e. prolonged exposure to CS leading to fear reduction), a well-known paradigm used in exposure-based therapies[45]. Fear conditioning can be used alone or in conjunction with other protocols to ensure persistent fear memories. It is crucial to note that the conditioned fear response is, in essence, adaptive. Some critics of the fear conditioning model as a PTSD paradigm argue that this model allows the examination of non-pathological responses[11]. However, in recent years, it has been demonstrated that various experimental conditions under which fear conditioning is conducted can indeed give rise to maladaptive fear responses in animals[46].

Different studies have demonstrated that fear extinction can temporarily control or inhibit the expression of conditioned fear[47]. Considering the adaptive response that ensures survival by preserving defensive reactions in the face of threats[9], conditioned fear responses can reemerge after extinction in various ways. For instance, the mere passage of time is associated with the recovery of fear reactions (spontaneous recovery), and fear can also reappear after direct exposure to learning stimuli (CS or US; renewal and reinstatement, respectively)[48]. At present, achieving long-term extinction expression is considered a significant challenge in both basic and clinical research[49] .

Fear conditioning is inescapable, and the expected fear response is “freezing” (the absence of all movements, except those related to breathing, while the animal is alert and awake), an ethologically adaptive reaction in situations in which “fight” and “flight” are ineffective[50]. Similar defensive responses are displayed by humans under certain circumstances[51].

Since defensive responses to threatening events are initially adaptive for the organism, the flexibility of fear conditioning allows to establish enhanced models of fear

conditioning by incorporating additional peri-stress events or increasing the intensity and frequency of CS- US presentations. Ultimately, PTSD models based on fear conditioning are in search of persistent, generalized, inflexible, and resistant fear responses that mimic pathological fear, increasing the validity and translational value of preclinical findings[46].

3.3. Key brain regions and circuits for processing fear memories

Learning and expression of threat responses are dependent on the amygdala. This structure is consisted of multiple interconnected nuclei located deep in the temporal lobe, where the basolateral complex (lateral, basal, and basomedial amygdala) has been consistently implicated in fear expression[52]. However, reward-based behavior can also be modified by local manipulation of amygdala[53,54].

Unilateral, as well as bilateral amygdala lesions result in deficient learning of conditioning responses in humans[55,56]. Studies using fMRI have shown that fear expression increases blood oxygenation level dependent (BOLD) in the amygdala and the intensity is associated with the strength of the conditioned response. However, the activity of the amygdala seems to be critical during early stages of threat learning with a gradual decrease over time. Nevertheless, studies in animals have shown that basolateral (BLA) and central (CEA) amygdala lesions cause deficits in the expression of fear conditioning, independent of when the lesion is performed after learning. [57-59] that can only be overcome by overtraining during fear conditioning[60,61].

One of the main brain regions modulating amygdala activity during fear expression is the prefrontal cortex (PFC). The human prefrontal cortex is divided into dorsolateral (dlPFC), medial (dmPFC), and ventral regions (vmPFC)[62] and the rodent prefrontal cortex shares homology with these human structures. Functionally, the human dmPFC is homologous to the rodent prelimbic cortex (PL) and the anterior cingulate cortex (ACC), and the vmPFC is homologous to the infralimbic cortex (IL). In the case of fear expression, PL and IL have a prominent role in aversive emotional processing. Thus, PL is required for learning and expressing conditioned fear responses[63,64], while IL controls fear suppression after extinction[65] . In humans, the thickness of the vmPFC correlates with their ability to recall extinction memory and express lower fear reactions[66,67]. In agreement with the functional division verified in rodents, the human ACC thickness is positively correlated with skin conductance response during conditioning[67].

Finally, imaging studies have reported smaller hippocampal volume in patients with PTSD[68-70]. However, it remains under debate whether these differences represent a vulnerability for the development of PTSD or a direct consequence of stress during trauma. It is worth noting that the hippocampus has a high concentration of receptors for corticosteroids that participate in glucocorticoid-mediated negative feedback of the HPA axis[71] and consequently make that structure particularly vulnerable to stress. Both endogenous and exogenous prolonged exposure to corticosterone in rodents produces deficits in spatial memory tasks involving the hippocampus[72]. Some of these impairments seem to be associated with modifications of the firing properties of hippocampal place cells supporting spatial navigation and contextual memory[73] .

The firing of place cells is associated to the generation of periodic transients of high-frequency (120–250 Hz) oscillations called sharp-wave ripples (SWRs) during wakefulness and non-REM sleep. SWRs are critical for information integration at both systemic and localized levels, playing a fundamental role in memory consolidation, and are typically affected in certain pathological states [74,75]. A recent study found that after immobilization stress, the coactivity of pyramidal cells was significantly greater than that observed in a wakefulness state before the onset of stress[76,77], suggesting that stress can modify the features of oscillatory markers necessary for memory consolidation. Hippocampal lesions in rodents elicit lower fear expression after contextual fear conditioning, results that have been proposed to reflect amnesia for context-shock pairing[78,79]. Interestingly, this suggestion is in line with the clinical observation of dissociative amnesia, where individuals who have experienced trauma often describe a difficulty in recalling precise details of the primary events or unrelated occurrences closely associated with the traumatic experience[80].

On the other hand, fear extinction, an exposure-based procedure used in animals as well as during cognitive-behavioral therapy in anxiety-related disorders was found to reduce fear in a context-dependent manner, suggesting that the hippocampal representation of the extinction context drives fear attenuation[81]. Indeed, neuronal activity in the basolateral amygdala decreases when (CS+) are presented in the same context used for extinction but increases following non-extinction exposure to the (CS+) [82]. Furthermore, inactivation of the hippocampus was found to enhance extinction to the CS+ and promote low fear expression in environments different from the extinction context[83,84]. Together, these

findings allow us to hypothesize that hippocampal oscillations and in particular SWRs are candidates for neuromodulation aiming to control fear responses.

3.4. Pathophysiology of cortico-amygdala circuits in fear-related disorders

Classically, PTSD has been considered to establish a deficient top-down control from mPFC over amygdala during exposure to trauma-related stimuli[85,86]. The absence of this control leads to amygdala hyperresponsivity in face of negative emotional content, correlating with symptoms such as increased anxiety, hyperarousal and vigilance[86.] The reversal of mPFC hypofunction is desired to gain emotional regulation and fear suppression. For instance, cognitive reappraisal (reinterpretion of emotional events) is associated with increased activation of the left dorsal and ventral lateral prefrontal cortex, dmPFC, left temporal pole, right supramarginal (SMG), and left lateral occipital gyrus. The activity of these structures is negatively correlated with amygdala activity[87].

Patients with PTSD show less activity in the dmPFC[88] and vmPFC[89] during scary faces or image expositions related to the trauma. Thus, the evaluation and control of emotional contents are partially impaired in this condition. This prefrontal lower activity is linked to elevated activity in the amygdala, which supports the idea of a deficient top-down control[90].

Interestingly, greater hippocampal engagement during exposure to negative stimuli and trauma-specific cues correlates with increased amygdala activity in patients with PTSD[91] suggesting that emotional detection encoded by the amygdala can trigger the hippocampus to promote a memory recall with emotional content. However, the hippocampus and amygdala are also coactivated during learning of emotional memories[92] and artificial stimulation of the amygdala is enough to enhance contextual memories in animals and humans[93,94].

The evidence showing PFC hypofunction during PTSD, is supported by studies showing that deep brain stimulation (DBS) of the subgenual cingulum, a fiber tract that connecting the PFC and the amygdala, induces a significant reduction of PTSD symptoms[95] as well as several preclinical studies about the enhancement of fear extinction and anxiety reduction through the stimulation of mPFC[96-98].

3.5. Memory alterations in pathological fear

Experimental and clinical studies reveal altered memory in PTSD that contributes to the persistence of the symptoms[15-18]. Memory alterations include involuntary hypermnesia or

explicit amnesia for trauma-related stimuli and fear generalization to non-trauma related stimuli in animal models and in human patients[12,13,99].

Nevertheless, memory alteration in PTSD seems to be a generalized cognitive impairment rather than specific to trauma cues. For instances, verbal memory (declarative) is impaired in combat-related and child abuse PTSD patients relative to controls[100,101]. Two meta-analyses with 55 studies, showed that PTSD patients had worse verbal and visual memory for neutral information than controls without PTSD[102,103]. These observations have been extended to other cognitive domains, where speed of information processing, attention and working memory are also affected in PTSD[104].

A close relationship has been proposed between declarative memory impairments and the efficacy of psychotherapy, since verbal memory performance was significantly lower in PTSD patients unresponsive to cognitive behavioral therapy[105]. This suggests that assessment and training aimed to reverse this memory deficit and could be crucial prior to the psychotherapeutic treatment[106].

Memory generalization is crucial for cognitive flexibility and extending behavioral repertoires to similar situations. Although generalization can be considered an adaptive response, in the context of emotional processing, overgeneralization of fear memories contributes to pathological states. In fact, overgeneralization is considered a transdiagnostic factor in anxiety-related disorders[107-110]. Fear overgeneralization can be conceptualized as a memory deficit as fear expression is driven by inappropriate predictors such as stimuli that are similar but different from those directly related to trauma[109]. Moreover, fear generalization is a time-dependent process, as animals are able to discriminate some days after learning, but there is a gradual decay in memory quality over time leading to generalization[111]. In fact, fear generalization has been proposed as a behavioral outcome of natural forgetting[112].

As previously mentioned for declarative memories, there is a link between fear generalization and therapeutic outcomes[113,114]. For instance, the chronic administration of the antidepressant fluoxetine can prevent fear generalization that occurs over time[115]. By preserving memory precision, the subsequent fear extinction is more effective compared with the control group that expresses fear generalization. On the other hand, poor context encoding causes maladaptive overgeneralization resistant to extinction. This effect can be

reversed by allowing animals to update the contextual features through context reexposure, restoring the capacity for extinction[116].

Different studies have shown that fear generalization and resistance to fear attenuation are closely related to training intensity. Thus, strong memories produced by high intensity foot-shocks become generalized and resistant to extinction soon after training, while using lower stimulation intensities generates precise fear memories and more susceptible to conventional extinction protocols[117-120]. Overcoming fear generalization before any intervention has been proposed as a critical step to enhance current treatments for fear-related disorders[121] .

3.6. Current treatments for posttraumatic stress disorder and their efficacy

Available treatments for PTSD can be classified into three categories: 1) psychotherapy, 2) pharmacological approaches, and 3) neuromodulation.

Psychotherapy in PTSD

Trauma-focused therapy is considered a first-line intervention for PTSD even before medication. This indication is based on large meta-analyses showing that psychotherapy alone had long-lasting effects in resolving symptoms and reducing the risk of continued use of antidepressants (the first pharmacological choice in PTSD)[122].

Psychotherapy for PTSD is not a unified form of intervention; instead, there are different approaches with high clinical evidence regarding their efficacy, such as cognitive behavioral therapy, narrative exposure therapy, and written exposure[123-125]. The remission rate of PTSD using trauma-focused therapy in adults is about 44% after an average of 40 months. However, the rate varies between 8% and 89%, depending on the type of trauma, diagnosis, and sociocultural factors[126].

Importantly, these types of therapies rely on extinction learning to reduce conditioned fear responses to stimuli that provoke anxiety and trauma reconstruction[48]. Thus, although effective, the chance for fear reemergence in patients treated with psychological intervention is high[127].

Pharmacological Approaches

Selective serotonin reuptake inhibitors (SSRIs) are the first-line pharmacological treatment in PTSD[128], with fluoxetine, venlafaxine, and paroxetine preferred as monotherapy. Selective norepinephrine reuptake inhibitors (SNRIs) such as bupropion

are also well tolerated with reduced side effects compared to SSRIs[129]. However, approximately 60% of patients respond to the treatment, with only 20-30% achieving criteria for full remission[130].

Surprisingly, since the introduction of fluoxetine in 1988, only minor improvements have been made in the pharmacological approaches to PTSD[131]. In addition, SSRIs have well-known drawbacks, such as slow onset of action, incomplete response or complete lack of response, and several side effects, including reduced sexual desire and weight changes, among others.

Recently, the reemergence of psychedelic compounds such as MDMA, ketamine, and psilocybin seem to overcome several limitations of classical psychopharmacology. For instance, the first randomized placebo-controlled trial using MDMA-assisted psychotherapy for PTSD showed that 83% of the patients treated with MDMA achieved full remission[132]. These results are encouraging and may be a potential breakthrough for psychiatry.

Neuromodulation

Neuromodulation therapies revolve modifying cell activity using biophysical interactions or by delivering chemical agents. Here, we focus on electrical stimulation, considering the methodology used in the current thesis. Electrical neuromodulation can be non-invasive and invasive, with transcranial direct current stimulation (tDCS) and deep brain stimulation (DBS) representing the standard approaches, respectively.

Deep brain stimulation consists of placing penetrating electrodes in specific brain regions connected to a pulse generator implanted subcutaneously. The electrical pulses can change the physiological properties of brain cells depending on preset stimulation parameters based on amplitude, pulse width, and pulse frequency. While the development and implementation of electrical stimulation techniques on deep brain structures in clinical contexts for Parkinsonian hyperkinesia dates back to the 1960s and 70s[133], the FDA approval of DBS in 1997 for Parkinson's disease can be considered as a historical milestone for the acceptance of DBS as a therapeutic option[134]. Over the last decades, following the successes of DBS in movement disorders, different clinical trials have been conducted to evaluate the effects of DBS in psychiatric conditions such as obsessive-compulsive disorder, addiction, and depression[25].

In the specific case of PTSD, currently, only two clinical trials are ongoing using DBS for refractory-PTSD[135-137]. In the 2016 study conducted by Langevin et al. [135] showed that DBS stimulation of (BLA) induced substantial clinical improvement after eight months, with a 37.8% reduction, and a 48% reduction after 15 months evaluated with the clinician-administered PTSD scale (CAPS) score in a combat veteran resistant to pharmacological and psychotherapy treatments during 20 years.

In the second clinical trial, DBS stimulation of the medial prefrontal cortex (mPFC) and the uncinate fasciculus was used, which showed a 100% improvement in CAPS, with a substantial decrease in comorbid depressive symptomatology and an increase in quality of life after six months [95]. However, the follow up of these outcomes in a long-term treatment remains to be demonstrated.

These clinical trials have been motivated by substantial evidence from the last decade about the possibility of enhancing fear extinction through DBS stimulation of the mPFC[138-141] and the amygdala[142,143] in animal models. Although DBS has been used to control fear expression in animal models, this approach introduces preset electrical stimulation in an open-loop manner, without being aligned with the internal oscillatory activity. This may lead to excessive stimulation and potentially disrupt normal physiological oscillations[27].

3.7. Changing the memory, changing the symptoms

A cognitive model of PTSD has been relatively recently proposed. In this view, PTSD is a disorder in which the core problem is a threatening memory of a past event. Therefore, a disturbance of autobiographical memory with poor elaboration, as well as excessive reconciliation and sometimes a lack of contextualization, generates a strong associative memory with strong perceptual priming (hypervigilance to environmental triggers)[144].

According to this model, PTSD involves the involuntary recollection of trauma (e.g., unwanted thoughts, flashbacks), generating negative appraisals. Thus, a logical target to control PTSD symptoms is the memory itself. In fact, behavioral interventions in PTSD (in vivo, imaginal, and cognitive reappraisal) involve the opposite of involuntary recollection, encouraging intentional self-referential retrieval of memories associated with the trauma[145].

This cognitive model is supported by basic research showing that individual stages of memory processing can be selectively manipulated. For instance, secondary prevention

of PTSD involves early behavioral and pharmacological interventions after trauma aimed to prevent the consolidation of fear memories[146]. Administration of hydrocortisone, propranolol, or dexamethasone is effective in decreasing symptoms and the development rate of PTSD when administered up to 12 hours after trauma[147]. In animal models, the inhibitor of endogenous adrenal corticosteroid synthesis, methyrapone, and the administration of propranolol before contextual fear conditioning prevents memory generalization induced by high training intensity[117]. Together, these studies suggest that stress during traumatic events influences emotional memory consolidation directly and the future expression of fear reactions. As mentioned before, overcoming fear generalization seems to be a critical step to enhance fear extinction[115,116].

On the other hand, after consolidation, when memory is recalled, it becomes unstable and sensitive to manipulation (destabilization). The memory trace requires a re-stabilization process called "reconsolidation" to persist[148]. Administration of different drugs such as protein synthesis inhibitors, blockers of the mammalian target of rapamycin, specific antagonists of NMDA and adrenergic receptors, and immediate extinction after retrieval have shown promising results in animal models to disrupt or update memory reconsolidation, decreasing fear reaction to reminders[149-151]. However, excluding propranolol, these pharmacological alternatives are not available for human testing[152].

In the case of fear extinction, a similar panorama emerges. The administration of L-DOPA enhances fear in both mice and humans[153]. Yohimbine, an α2-adrenergic receptor antagonist, enhances fear extinction in rodents[154] and, in combination with cognitive-behavioral therapy, reduces social anxiety and claustrophobic fear[155]. In addition, the allosteric modulation of NMDARs via the L-glycine binding site using D-serine or D-cycloserine promotes the generalization of fear extinction in animals[156]. These results are supported by clinical data showing also positive outcomes in PTSD patients[157].

Finally, there is growing evidence suggesting the possibility of selectively forgetting of emotional memories, mainly through the administration of the antagonist of the NMDA glutamate receptors, memantine, via a putative enhancement of hippocampal neurogenesis[158,159]. The therapeutic effect of memantine in humans has been recently demonstrated, where thirteen adult women with PTSD, reported significant improvements of the symptoms after 12 weeks of memantine intake[160].

Taken together, these evidence suggest a memory-based framework to investigate and treat PTSD. Targeting the memory processes (i.e. consolidation, reconsolidation, extinction) and its selective manipulation could alleviate intrusive recollection, decreasing the triggers for the entire symptomatology manifestation. This view differs from the classical clinical approach based on anxiety management during early intervention (e.g., benzodiazepines, without any strong scientific support despite their use in clinical practice[161], as well as during chronic treatments (e.g., antidepressants).

4. AIMS OF THE STUDY

The general aim is to establish a closed-loop neuromodulation approach guided by oscillatory markers of memory consolidation to electrically modulate brain regions involved in reward and anxiety responses. Further on, we sought to evaluate if the resistance to extinction and fear generalization in a cued fear conditioning paradigm could be overcome using this method applied to specific memory processes (consolidation, reconsolidation and extinction).

Our specific aims were:

a) To develop a closed-loop neuromodulation system detecting real time sharp wave ripples (SWRs) and deliver an intracranial anxiolytic signal through IL stimulation and evaluate its effects during the consolidation of fear conditioning.

b) To use the previously developed closed-loop neuromodulation system detecting real time SWRs and deliver basolateral Amygdala stimulation and evaluate its effects during the reconsolidation of fear conditioning.

c) To use the previously developed closed-loop neuromodulation system detecting real time SWRs and deliver an intracranial reward signal through medial forebrain bundle (MFB) stimulation and evaluate its effects during the consolidation of fear extinction.

5. MATERIALS AND METHODS

5.1. Animals

Rats (40 adult male Long-Evans and 60 adult male Wistar, 300-450 g, 3-6 months old) were kept in a 12-hour light/ dark cycle. All experiments were performed in accordance with the European Union guidelines (2003/65/CE) and the National Institutes of Health Guidelines for the Care and Use of Animals for Experimental Procedures. The experimental protocols were approved by the Ethical Committee for Animal Research at the Albert Szent-Györgyi Medical and Pharmaceutical Center of the University of Szeged (XIV/218/2016 and XIV/824/2021).

5.2. Construction of recording and stimulation electrodes

Tripolar tungsten wire electrodes were built as shown in [162]. In short, we inserted three 50 µm diameter polyimide-insulated tungsten wires (Tungsten 99.95%, California Fine Wire, Grover Beach, CA, USA) into a 180-µm inner diameter stainless steel tube. Tips of the tungsten wires were spaced between 0.2- 0.4 mm axial distance depending on the target structure. The impedance of the electrodes ranged between 30–90 kΩ at 1 kHz.

The bipolar stimulus electrode was built using the same tungsten wire as tripolar recording electrodes, but the tips were spaced 0.2 mm axial distance and the insulation was removed 0.1 mm around the tips.

Stainless-steel screws were used as ground and reference electrodes respectively.

5.3. Surgery

The animals were anesthetized with 2% isoflurane and craniotomies performed in the specific stereotaxic coordinates. Intracortical electrode triplets (interwire spacing, 0.2-0.4 mm)[26] targeting the infralimbic cortex (IL) (AP: +3.2, ML: 0.5, DV: 4.5,) anterior cingulate cortex (ACC) (AP: +1.0, ML: 0.5, DV: 1.4,) bilateral BLA (AP:-2.8, ML: 4.6, DV: 7.8 mm from the dura) and the bilateral CA1 subfield of the dorsal hippocampus (DH) (AP: -3.5, -4.5 and -5.5, ML: 2.0, 3.0 and 4.0, DV: 2.9 and 3.0 all mm from Bregma). A custom-built bipolar stimulus electrode was placed in (IL), left medial-forebrain bundle (AP: −2.8, ML: 2.0 mm, DV: 8.1) or ventral hippocampal commissure (VHC) (AP: −1.3, ML: 1.1 mm, DV: 3.8 all mm from Bregma) depending on the experiment conducted. The electrodes and the base of the microdrive were attached to the skull with dental acrylic (Unifast Trad, USA).

To improve DH-SWRs detection, a custom-built microdrive[163] was used in some experiments, allowing the vertical movement within the CA1 subfield after implantation. In those cases, the craniotomies were sealed with sterile wax.

Two stainless-steel screws (serving as reference and ground) were placed in the skull above the cerebellum. Some additional screws were drilled into the skull and covered with dental cement to strengthen the implant. A Faraday cage was built using copper mesh where the ground screw was connected and finally, it was attached to the skull around the implanted electrodes with dental acrylic.

In experiments involving pharmacological infusion, rats were bilaterally implanted with 25-gauge guide cannulas, 11mm length (Bilaney Consultants GmbH, Germany) above the BLA (AP: -2.8, ML: 4.7, DV: 6.9 all mm from Bregma). Cannulae were fixed to the skull with dental acrylic (Unifast Trad, USA). The dummy cannula fits 11mm guide without projection to avoid any accidental occlusion.

Post-surgical analgesics and antibiotics were applied lege artis. After recovery, the electrodes were moved in daily steps of 50 to 150 µm until the desired position was reached. Experimental protocol started after a recovery period of at least 15 days following surgery.

5.4. Electrophysiological recordings and stimulation

Rats were housed individually in Plexiglass home cages (42 × 38 cm, 18 cm tall). LFP recordings were conducted in both the home cage and the fear conditioning box (see below). Recording and stimulation sessions for CL or OL interventions were carried out for one or three hours following the training, reactivation, or extinction session, depending on the specific experiment. Food and water were provided ad libitum.

All recording sessions took place in the same room using 12/12 h light/dark cycle with light onset and offset at 7 am and 7 pm, respectively.

The electrodes were linked to a signal multiplexing headstage (HS3_v1.3, Amplipex, Szeged, Hungary) that was connected to a lightweight cable (36AWG Nylon Kerrigan-Lewis Litz wire, Alpha Wire, Elizabeth, NJ, USA. This cable hung from a trolley system on the ceiling of the room, allowing the freely moving. To prevent any twisting or excessive tension in the recording cables, a bore-through electrical commutator (VSR-TC-15-12; Victory-Way Electronic, Shenzhen,China. was used. The multiplexed signals

were acquired at 500 Hz per channel for closed-loop neuromodulation experiments[26] . The neuronal signals were preamplified (total gain 400X), multiplexed and stored after digitalization at 20-kHz sampling rate per channel (KJE1001, Amplipex, Szeged, Hungary)[164]. During home cage stimulation, preamplified signals were analyzed on-line by a programmable digital signal processor (RX-8, Tucker-Davis Technologies, Alachua, FL, USA) using a custom made SWRs detection algorithm, as follows.

Two LFP signals were used for real-time SWRs detection. For ripple detection, a channel from the tripolar electrodes from CA1 pyramidal layer with the largest ripple amplitude was selected, band- pass filtered (150–250 Hz), and root-mean square (RMS) power was calculated in real time for ripple detection. For noise detection, manual inspection from channels of the ACC was performed to select the signal with no ripple-like activity and lower noise incidence to enhance signal-to-noise ratio during detection. In case of the ACC, the signal was filtered between 80 and 500 Hz. SWRs were defined as events crossing the ripple thresholds in the absence of the noise signal. Amplitude thresholds for ripple detection were adjusted for each animal before fear conditioning training. Threshold crossings triggered a stimulation (10 pulses, 0.2-ms width at 100 μA, 50 Hz) in the IL or BLA single pulse (5-15V in the ventral hippocampal commissure) in VHC or stimulation train (lasting 100 ms and composed of fourteen 1-ms long, 100 μA square-wave pulses at 140 Hz) in the MFB (STG4008; Multi Channel Systems, Reutlingen, Germany) depending on the experiment performed. The electrical stimulation of the IL, BLA and MFB stimulation was performed under current-controlled mode and VHC stimulation in voltage-controlled mode. Individual thresholds were set for each animal.

5.5. Electrophysiological data analysis

The offline ripples were analyzed using custom-made MATLAB (R2017b, Natick, Massachusetts, USA) routines. Raw signals were down sampled from 20 kHz to 500 Hz and bandpass filtered in the ripple band (150-250 Hz) from the hippocampal channels. Then, normalized squared signals were calculated. Putative SWRs events were defined as those where the beginning/end cutoffs exceeded 2 SDs and the peak power 3 SDs. The detection window was set as 150 ms. SWRs duration limits were set between 20 and 200 ms, otherwise the events were excluded to minimize artifacts. All ripple events were inspected manually following off-line detection. The closest stimulation onset from the digital channel was selected for further analysis. The time delay between the successfully detected ripples events and stimulation time were next quantified. For the brain states

classifications (SWS/REM), SleepScoreMaster toolbox from Buzcode (https://github.com/buzsakilab/buzcode) was employed combined with manual corrections. Time frequency spectrum was calculated in MATLAB using Multitaper Spectral Estimation from the Chronux Toolbox (http://chronux.org/). A 2s sliding window with a 50% overlap, a time-bandwidth product of 5 and tapers of 3 were chosen.

5.6. Drugs and infusions

Anisomycin (125 µg/µl; Sigma-Aldrich) was dissolved in equimolar HCl and sterile saline and infused bilaterally into the BLA using needle inserted into the implanted cannula with 1mm projection connected to Hamilton syringes via 20-gauge plastic tubes. A total volume of 0.5 µl per side was infused by a microinfusion pump at a rate of 0.125 µl/min. Injectors were left in place for an additional minute to allow diffusion of the drug away from the cannula tip. The drug was infused immediately after the reactivation session in experiments aimed to confirm that reconsolidation was taking place.

5.7. Auditory fear conditioning

The experiments were performed in a fear conditioning apparatus comprising three contextual Plexiglas boxes (42 × 38 cm, 18 cm tall) placed within a soundproof chamber.4 different contextual configurations were used (Habituation, Reactivation and Test Context (A): square configuration, white walls with black vertical horizontal lines, white smooth floor, washed with 70% ethanol; Training Context (B): square configuration, grey walls, metal grid on black floor, washed 30% ethanol; Extinction Context (C): rectangular configuration, white walls with black dots, white smooth floor; and Renewal and Remote/Reinstatement context (D): hybrid context comprising a square configuration, grey walls from training context, white smooth floor, washed with 70% ethanol. All sessions were controlled using a MATLAB based custom script.

Habituation

On day 1, animals were exposed to the habituation session in context A. After 2 min of contextual habituation, they were exposed to 5 alternating presentations of 2 different tones (2.5 or 7.5 kHz, 85 dB, 30 s). Tone time intervals were randomized (30-40 s) during the session. No behavioral differences were detected under exposition to the 2 frequencies.

Training

On day 2, cue fear conditioning was performed in context B. After 2 min of contextual habituation, animals received 5 trials of one tone (CS+: 7.5 kHz) immediately followed by a 2 s long footshock as unconditioned stimulus (US: 1.0mA, 0.8 mA or 0.7mA depending on the experiment performed). The other tone (CS−: 2.5 kHz) was presented 5 times intermittently but never followed by the US.

Reactivation

Reactivation took place in context A following 2 days after training and entailed four 30 s tone presentations without footshock after an initial 60 s acclimation. Rats remained in the boxes for 60 s after the tone presentation.

Test

The tests were performed on days 3-4 and 5-6 in context A. After 2 min of contextual habituation, rats were exposed to presentations of the CS+ or CS- in 2 different sessions. Each session consisted of a block of 5 tones. The order of the CS+ and the CS− in each session was randomized. Sessions were repeated every 4-6 h.

Extinction

The extinction was performed on context C, 24 h after the last fear conditioning test. In experiments with manipulations during consolidation and reconsolidation, extinction was performed as a single session. In experiments using the extinction criterion, consecutive extinction sessions were performed until reaching the remission criterion (see below). Extinction training consisting of 20 CS+ presentations without the US (unreinforced tones). Tones were repeated with randomized intervals (30-40 s) during the session.

Fear remission from extinction

We stablished an extinction threshold criterion to evaluate the effectiveness of fear reduction following extinction sessions, similar to the approach of Shumake et al.[165]. The block of the first five tones during each extinction session was analyzed to determine the fear reduction percentage. Considering individual differences under fear conditioning[165,166] fear reduction during extinction was expressed as a fraction of the percentage of freezing expressed during the CS+ test, performed on day 3 (% Freezing Reduction = Freezing extinction × 100/Freezing test CS+). Fear remission was achieved

when animals expressed <20% of the initial freezing during the first block of 5 CS+ presentations during the extinction session). Extinction training was performed for a maximum duration of 7 days.

Renewal and Remote Test

Animals were exposed to context D (Hybrid context) as a renewal or remote test, respectively, 24 h or 25 days after achieving the remission. In each test, rats were exposed to a block of 5 CS+ presentations after 2 min of contextual habituation. Time intervals between tones were randomized (30-40 s) during the session.

Immediate Footshock

To promote fear recovery, animals were placed in a neutral environment outside the conditioning box and received an unconditioned foot shock after 30 s contextual exposition, with the same intensity used during fear conditioning. The animals were returned to their home cage 30 s following the footshock.

Reinstatement Test

Animals were submitted to a reinstatement test in context D 24 h after the immediate footshock. Rats were exposed to a block of 5 CS+ presentations without the US after 2 min of contextual habituation. Time intervals between tones were randomized during the session.

Behavioral Assessment

The measure of freezing behavior was used as an indicator of memory in the fear conditioning task. Freezing was analyzed off-line using Solomon software (SOLOMON CODER, (© András Péter, Budapest, Hungary), for behavioral coding by an experienced observer that was blind to the experimental group. Freezing was defined as the absence of all movements, except those related to breathing, while the animal was alert and awake. An index discrimination analysis between tones was used based on previous studies: [Freezing CS+/(Freezing (CS+) + Freezing (CS-))][115].

5.8. Histology

Following the termination of the experiments, animals were deeply anesthetized with 1.5 g/kg urethane (i.p.) and the recording sites of each electrode were lesioned with 100 µA anodal direct current for 10 s. Then, the animals were transcardially perfused with 0.9%

saline solution followed by 4% paraformaldehyde solution and 0.2% picric acid in 0.1 M phosphate buffer saline. After postfixation overnight, 50-µm thick coronal sections were prepared with a microtome (VT1000S, Leica), stained with 1 µg/ml DAPI in distilled water (D8417; Sigma-Aldrich), coverslipped and examined using a Zeiss LSM880 scanning confocal microscope (Carl Zeiss) for histological verification of the recording electrode and cannulae locations.

5.9. Statistical analysis and data presentation

Statistical analyses were performed using GraphPad Prism 8 software. Significance was set at $p < 0.05$. Data were analyzed using two-tailed Mann–Whitney U test, Kruskal–Wallis test or ANOVA Mixed-Effect Analysis followed by Dunn's post hoc or Bonferroni's multiple comparisons test. Data are expressed and visualized as median ± IQR, individual data points are also shown where applicable. *, ** and *** denote significance levels smaller than 0.05, 0.01 and 0.001, respectively.

6. RESULTS

6.1. Sharp-wave ripples (SWRs) guided closed-loop stimulation of the infralimbic cortex during memory consolidation prevents fear generalization.

Based on previous studies from our laboratory[26] we developed a real-time detection system to identify dorsal hippocampal SWRs and deliver closed-loop intracranial stimulation to the IL immediately after cued fear conditioning. The choice of IL stimulation was influenced by the fact that it has been shown to enhance fear extinction using conventional DBS[138,139,141]. Furthermore, previous results have demonstrated that IL stimulation can induce anxiolytic and antidepressant-like responses[96,167]. Consequently, by providing IL stimulation during the precise time of memory replay through SWRs, a positive emotional valence could be introduced during the consolidation of a fearful event. This mechanism resembles a counterconditioning process through memory updating but with a high degree of temporal precision[19,168].

Immediately after fear conditioning, one group of rats received closed-loop stimulation of the Infralimbic Cortex (CL-IL) during (SWRs) (ten 200-µs long, 100µA square-wave pulses at 50 Hz; Fig. 1A-B) to assign an anxiolytic signal to the replayed fear memory after training, another group was exposed to the same CL stimulation 4h after training, a group received jittered stimulation (open-loop) (OL) (Fig. 1C), and a control group received no stimulation. The experimental protocol consisted of stimulation and recording sessions conducted for a duration of three hours immediately following the fear conditioning procedure. To minimize the influence of novelty, the stimulation was carried out within the animals' home cage.

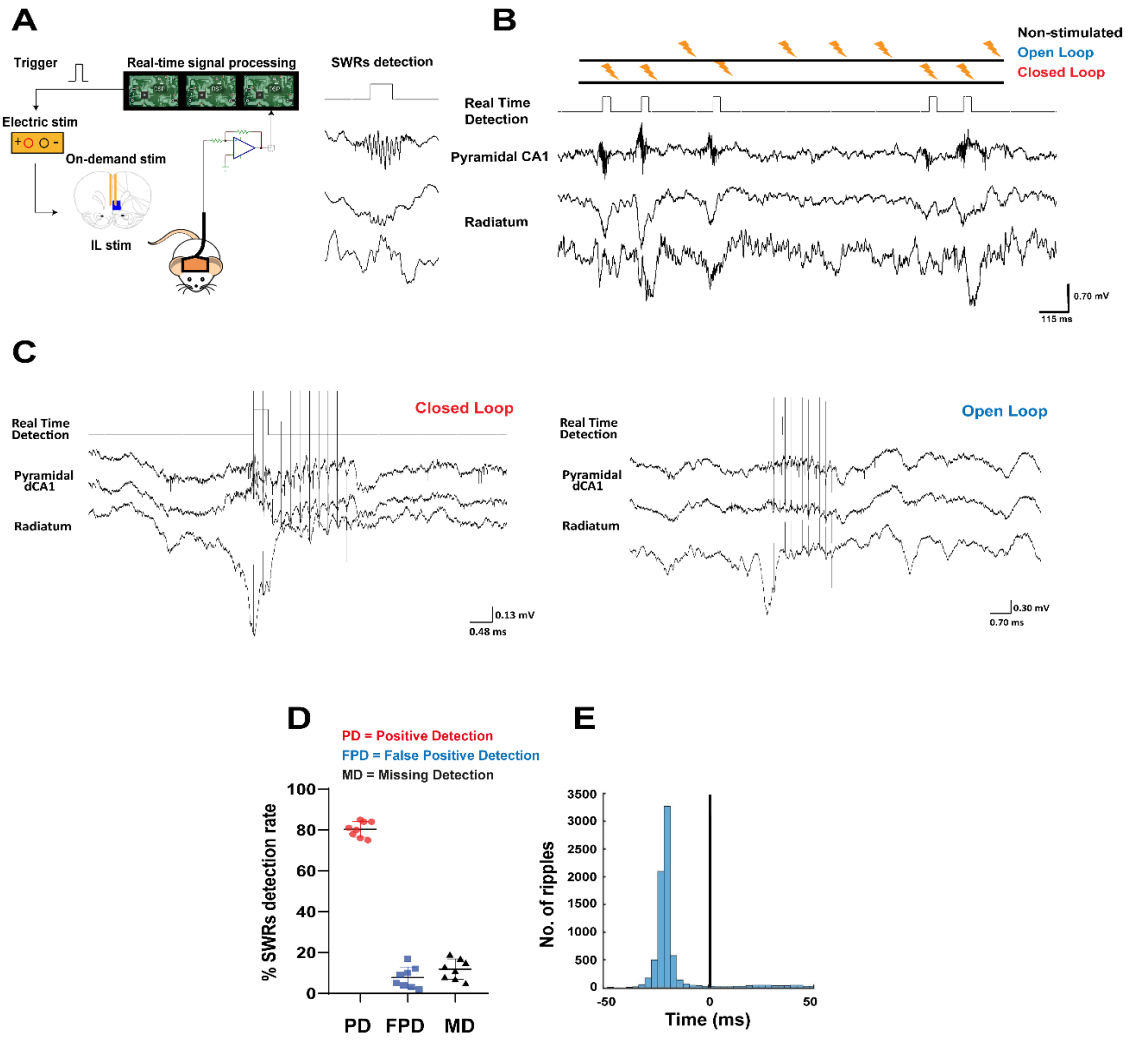


Figure 1. Overview of the closed-loop system and stimulation. ***(A)*** *A custom threshold crossing based detection algorithm was used to trigger the IL stimulation (ten 200-µs long, 100µA square-wave pulses at 50 Hz) following online detections of SWRs.* ***(B)*** *CL stimulation consisted of IL stimulation during the detected SWRs events, OL stimulation was similar to CL, but stimulation was jittered from SWRs (representative LFP signals from dorsal hippocampus showing SWRs events and online detection).* ***(C)*** *Representative LFP signals from dorsal hippocampus showing SWRs detection and stimulation pattern in CL (top) and OL (bottom).* ***(D)*** *Average online detection rate of SWRs events.* ***(E)*** *Delay of SWRs triggered stimulation. The largest number of stimuli (blue peak) were delivered between 18-21ms after the SWRs onset (black line: time of the stimulation).*

We found that the average online detection rate of SWRs was 80.38 ± 1.349% compared to the post hoc detection rate. Our detection method has proven to be highly accurate, as evidenced by a false positive detection rate of 7.750 ± 1.830% and a missed detection rate of 11.88 ± 7.67% (See Figure 1D). The minimum delay for triggering the stimulation after SWRs detection was 15ms, while the maximum was 27ms. Notably, the majority of

SWRs events were detected between 18-21ms before the onset of stimulation (Fig. 1E), highlighting the precise timing of our CL stimulation approach**.**

Fear related behavioral performance was tested using different tests to assess fear generalization (TEST CS+ and TEST CS-), fear extinction (EXTINCTION), exposition exposed to CS+ in a hybrid context mixing new features with the conditioning context following extinction (RENEWAL TEST) and by unpredictable exposure to the US (REINSTATEMENT TEST) (see Material and Methods).

During the test after conditioning, no differences were detected in the fear expression to the CS+, between groups, however, animals exposed to CL immediately after training (CL-10min) expressed less fear responses to the CS- compared with all groups (Mixed-effects ANOVA, interaction tone x groups factor $F(3, 42) = 7.414$, $p = <0.001$) (Fig. 2A). This result was confirmed by the index discrimination analysis (Fig. 2B) showing that CL-10min allow a significant discrimination between CS+ and CS- in these animals (Kruskal-Wallis test, $H = 14.04$, $p = <0.01$).

During the extinction, there is a general decrease of fear expression between early (0-5 trial) and late (15-20 trial) extinction (Mixed-effects ANOVA, time factor $F(1, 20) = 37.35$, $p = <0.001$) (Fig. 2C). However, no differences were detected between groups. During the renewal in the hybrid context, animals exposed immediately to CL (CL-10min) expressed lower fear reaction compared to non-stimulated animals ($p = 0.0461$) and OL ($p = 0.0029$) (after Kruskal-Wallis test, $H = 9.200$, $p = <0.05$) (Fig. 2D). Surprisingly, no differences were detected between animals exposed immediately to CL compared to animals exposed 48h after training (CL-48h) ($p = 0.0711$), suggesting a potential extinction enhancement even when CL is not contingent with fear memory consolidation. Finally, during reinstatement, non-stimulated animals expressed a significant increase of fear reactions compared to OL ($p = 0.0347$), while OL animals expressed significant fear expression compared to CL-10min ($p = 0.0127$) and 4h later (48h; $p = 0.0124$) (after Kruskal-Wallis test, $H = 10.79$, $p = <0.05$) (Fig. 2E).

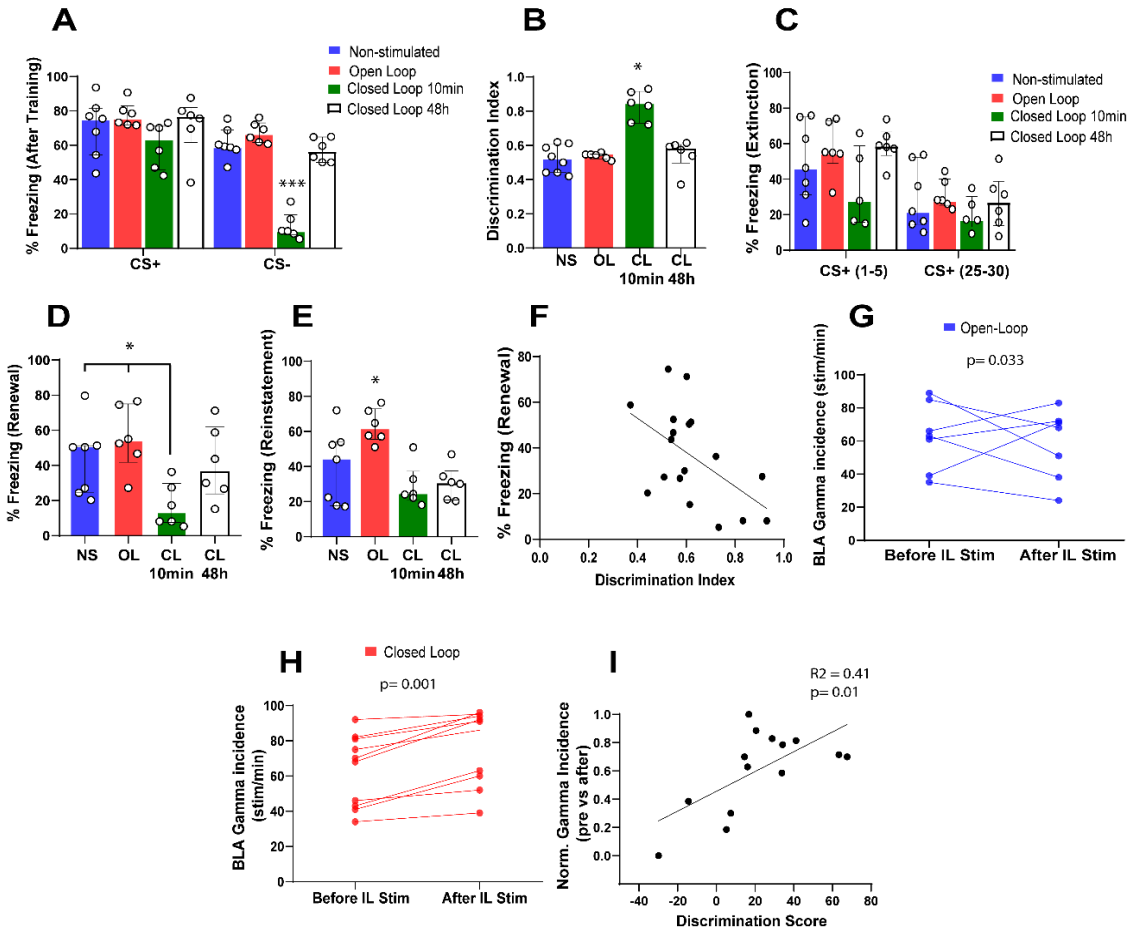


Figure 2. Infralimbic closed-loop stimulation after training prevents fear generalization. *A) Animals exposed to IL closed-loop stimulation (CL-IL) show less fear expression to the CS- compared to all groups. However, no differences were detected in fear expression to CS+. (non-stimulated (NS) n=7; open-loop (OL) n=6; closed-loop 10 min after training (CL-10min) n=7; closed-loop 48 h after training (CL-48h) n=6). (B) These variations were substantiated by the discrimination index, indicating that the animals undergoing closed-loop stimulation displayed the highest discrimination between CS+ and CS-. (C) No differences were noted in the fear responses across all groups. (D) CL-IL stimulation resulted in a reduced fear response during the renewal test compared to all other groups. (E) No significant differences in the fear responses were detected during the reinstatement phase. (F) A significant negative correlation was identified between the discrimination index and fear behavior during the renewal phase. (G) Our findings suggests that OL stimulation does not influence gamma incidence in the BLA pre- and post-stimulation. (H) However, a marked increase was found to be significant in animals treated with CL stimulation. (I) Moreover, a significant positive correlation was identified between gamma incidence and fear discrimination.*

The results during renewal test suggests a potential relationship between memory precision and fear extinction enhancement. This observation was confirmed by the presence of a significant negative correlation between the discrimination index and fear behavior during renewal (slope differed from zero, $p < 0.01$, $r2 = 0.2824$) (Fig. 2F). Thus, animals able to discriminate between CS+ and CS- are prone to successfully mitigate fear.

Exploring the brain dynamics from these experiments, we found that CL stimulation increases gamma incidence in BLA between pre- and after stimulation (Wilcoxon matched-pairs signed rank test, $W = 55.00$, $p = <0.01$) (Fig. 2H), however, this effect was absent in OL animals (Wilcoxon matched-pairs signed rank test, $W = -6.000$, $p = 0.6719$) (Fig. 2G). Interestingly, there is a significant positive correlation between gamma incidence and fear discrimination (slope differed from zero, $p < 0.05$, $r2 = 0.4158$) (Fig. 2I) suggesting that the increase in gamma oscillations in BLA after stimulation are directly associated with memory discrimination and subsequent extinction enhancement.

6.2.SWRs-based closed-loop stimulation of the basolateral amygdala during memory reconsolidation reverts fear generalization.

Considering the prominent projections from IL to BLA[169,170] and IL activity engaging inhibitory circuits in amygdala to reduce fear and anxiety responses[139,171,172], we sought to test if direct BLA stimulation can modify the strength and generalization of fear memory. Preliminary studies in PTSD patients have shown that stimulation of the BLA via the ventral amygdalofugal (VAF) pathway, can elicit positive emotions[136]. It has been suggested that the connections between the BLA and the Nac, through the VAF pathway, play a role in positive reinforcement and appetitive learning[173]. Following the rationale of emotional updating through CL stimulation, we stimulated the BLA after a brief memory reactivation aimed to trigger reconsolidation. Like memory consolidation, the retrieval of a consolidated memory induces a labile state where the memory can be disrupted or updated by novel information. Memory reconsolidation involves a re-stabilization (re-storage) process with identifiable molecular mechanisms boosting the persistence of the memory trace[174]. In this scenario, BLA stimulation is predicted to interact with the labile state of memory after reactivation.

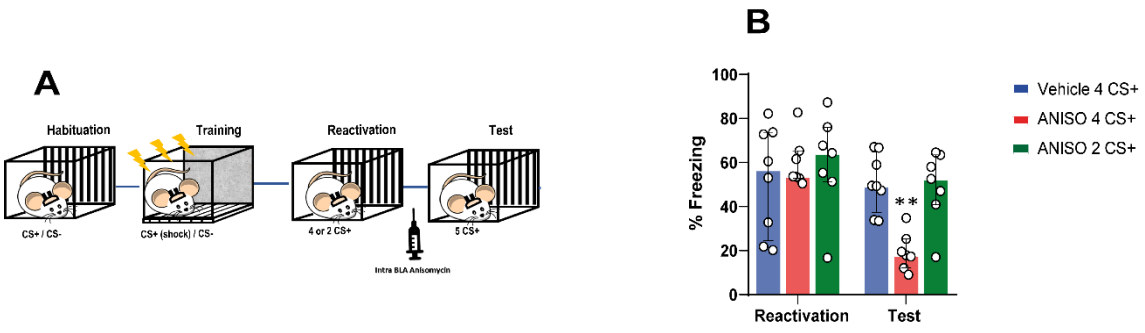


Figure 3. Disruption memory reconsolidation after retrieval. *(A) Schematics of the experimental design. Animals underwent habituation and fear conditioning training. 48 h after conditioning, the animals were subjected to either 2 or 4 presentations of the conditioned stimulus (CS+; reactivation session) to initiate memory reconsolidation. Immediately after reactivation, anisomycin was bilaterally infused into the BLA. On the following day, the animals were tested with the presentation of 5 CS+. B) Animals that underwent reactivation with 4 CS+ and received anisomycin infusion (ANISO 4CS+) showed memory disruption during the test, suggesting an amnesic effect of the treatment. No memory disturbances were detected in the animals exposed to only 2 CS+ presentations and infused with anisomycin (ANISO 2CS+), nor in the vehicle-exposed subjects who experienced 4 CS+ presentations (Vehicle 4CS+).*

To confirm that our reactivation protocol can induce reconsolidation, animals previously implanted with cannulae in the BLA, underwent fear conditioning protocol as previously described. 48h after training one group of animals was exposed to 4 CS+ followed intra-BLA injection of the protein synthesis inhibitor anisomycin, a second group was exposed to 2 CS+ and injected with anisomycin and a third group exposed to 4 CS+ but injected with saline (Fig. 3A).

No differences were detected during reactivation, however, in the test performed 24 h after the administration, animals reactivated with 4 CS+ and anisomycin showed a retrieval impairment in a subsequent test compared with 2 CS+ with anisomycin ($p = 0.0071$) and 4 CS+ with vehicle ($p = 0.0032$) (Mixed-effects ANOVA, time x group interaction $F(2, 19) = 12.82$, $p = <0.001$, followed by Tukey's multiple comparisons test) (Fig. 3C). These results confirm that our reactivation protocol can engage reconsolidation dependent on BLA protein synthesis.

In the next set of experiments, animals were reactivated followed by CL or OL stimulation. After this session, the experimental protocol was the same as previously described. No differences were detected between CL and OL during reactivation (Mann–

Whitney test, U = 20, p = 0.9452) (Fig. 4A). However, after the stimulation, CL animals expressed less freezing compared to the OL group during the CS- test (p = 0.0202). There were no differences during the CS+ test (Mixed-effects ANOVA, time factor F (1, 11) = 11.29, p = <0.01, followed by Sidak's multiple comparisons test) (Fig. 4B). Comparing the discrimination indices of OL and CL animals suggests that CL animals can successfully discriminate between CS+ and CS- (Fig. 4C) (Mann–Whitney test, U = 4, p = 0.0140).

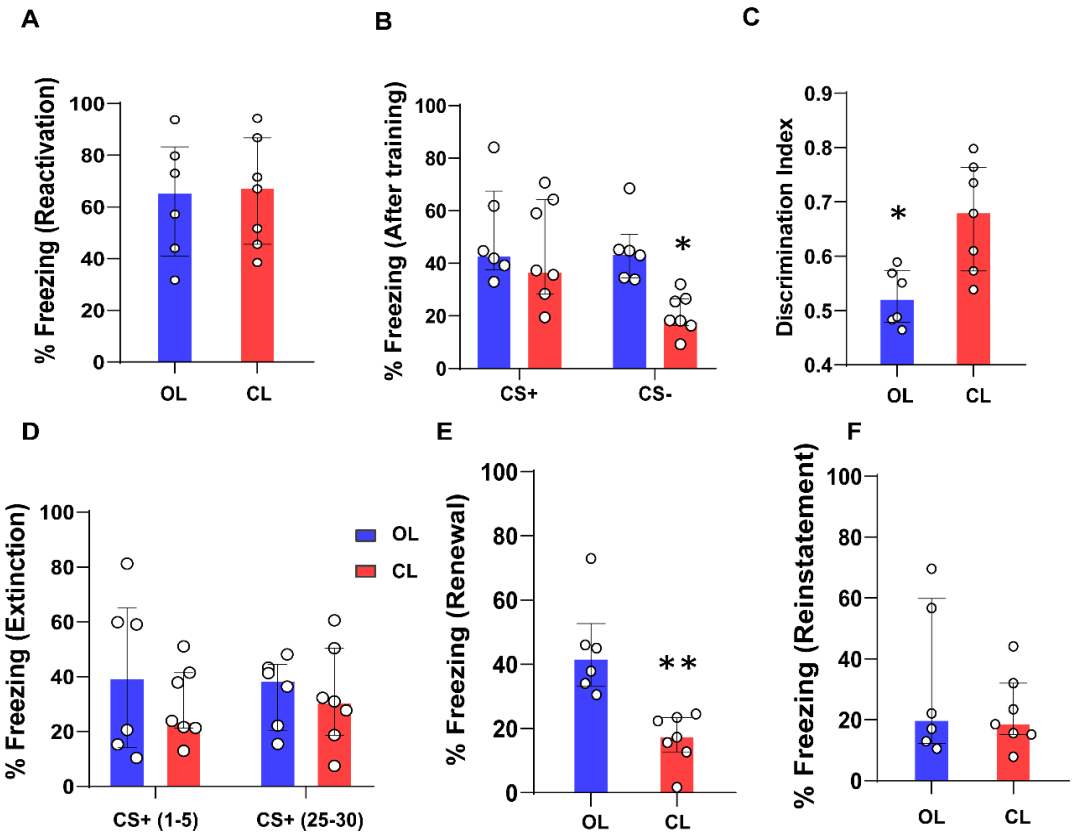


Figure 4. Basolateral amygdala closed-loop stimulation after reactivation revers fear generalization. *(A) No differences were detected in the fear responses to the conditioned stimulus (CS+) following reactivation between the two experimental group (OL n=6; CL n=7). (B) Animals exposed to CL-BLA stimulation demonstrated less fear response to the CS- compared to those exposed to open-loop stimulation. However, no significant variations were observed in the fear responses to CS+. (C) These differences were substantiated by the discrimination index, indicating that the animals subjected to CL stimulation displayed a heightened discrimination between CS+ and CS-. (D) No variations were observed in the fear responses during extinction across all the experimental groups. (E) CL-BLA stimulation resulted in a reduced fear response during the renewal test when compared to animals exposed to OL stimulation. (F) No significant variations were detected in the fear responses during the reinstatement phase.*

No differences were observed during extinction between the first and last 5 trials of CS+ presentations (Mixed-effects ANOVA, time x group interaction F (1, 22) = 0.3629, p = 0.5531) (Fig. 4D). However, during the renewal test, CL animals expressed less freezing compared to the OL group (Mann–Whitney test, U = 0, p = 0.0012) (Fig. 4E) but not during test after reinstatement (Mann–Whitney test, U = 19, p = 0.8357) (Fig. 4F).

6.3.SWRs-based closed-loop stimulation of the MFB enhance fear extinction.

The medial forebrain bundle (MFB) is a group of fibers connecting nodes of mesolimbic and mesocortical dopaminergic pathways involved in reward and emotional processing[175]. The ventral tegmental area (VTA) sends dopaminergic axons to the nucleus accumbens (NAc), amygdala and PFC via the MFB[175]. A cluster of dopaminergic neurons in the anterior VTA/SNc projects to the CA1 region of the hippocampus[176]. A global manipulation of the reward system through MFB-DBS can ameliorate depression-like behaviors in animal models and depression[177]. We hypothesized that SWRs-triggered CL neuromodulation may provide a reward safety signal during memory replay interacting with network activity that encodes fear extinction.

To test this hypothesis, rats were exposed to a single session of fear conditioning (5 pairings of conditions stimulus, CS and unconditioned stimulus, US, i.e. foot shock using 1mA current) followed by fear extinction training over multiple days (20 re-exposures/day in 4 blocks to CS+ in a novel context without US) until a remission criterion (reduction of freezing behavior to ≤ 20 % of the initial freezing) was reached or up to maximum 7 days. Considering the strong intensity of our training, this extinction threshold criterion allows to perform repetitive extinction sessions and stimulation (maximum 7 days). During the extinction protocol, one group of rats received closed-loop stimulation of the MFB (CL-MFB) during hippocampal SWRs events (fourteen 1-ms long, 100µA square-wave pulses at 140 Hz) to assign a reward signal to the replayed extinction memory, another group received jittered stimulation (OL), and a control group received no stimulation. The experimental protocol involved stimulation and recording sessions for a duration of 1 h immediately following the extinction procedure (Fig. 5A).

No significant differences were found in the fear expression between groups in the test after conditioning to CS+ (Kruskal-Wallis test, H = 1.737, p = 0.4195) (Fig. 5B). During the extinction, there is a general decrease of fear expression in all groups comparing early (day 1) and late (extinction criterion) extinction (Mixed-effects ANOVA, time factor F (1, 23) = 164.2, p = <0.001). However, no differences were detected between groups (Fig.

5C). While our findings demonstrate that extinction can lead to the overcoming of fear, animals that were exposed to CL stimulation required fewer extinction sessions to achieve the remission criterion of <20% initial freezing compared to the OL (p = 0.0015) and non-stimulated groups (p = 0.0118) (after Kruskal-Wallis test, H = 13.6, p = <0.**0**1) (Fig. 5D) suggesting that CL-MFB neuromodulation can enhance the effectiveness of fear extinction.

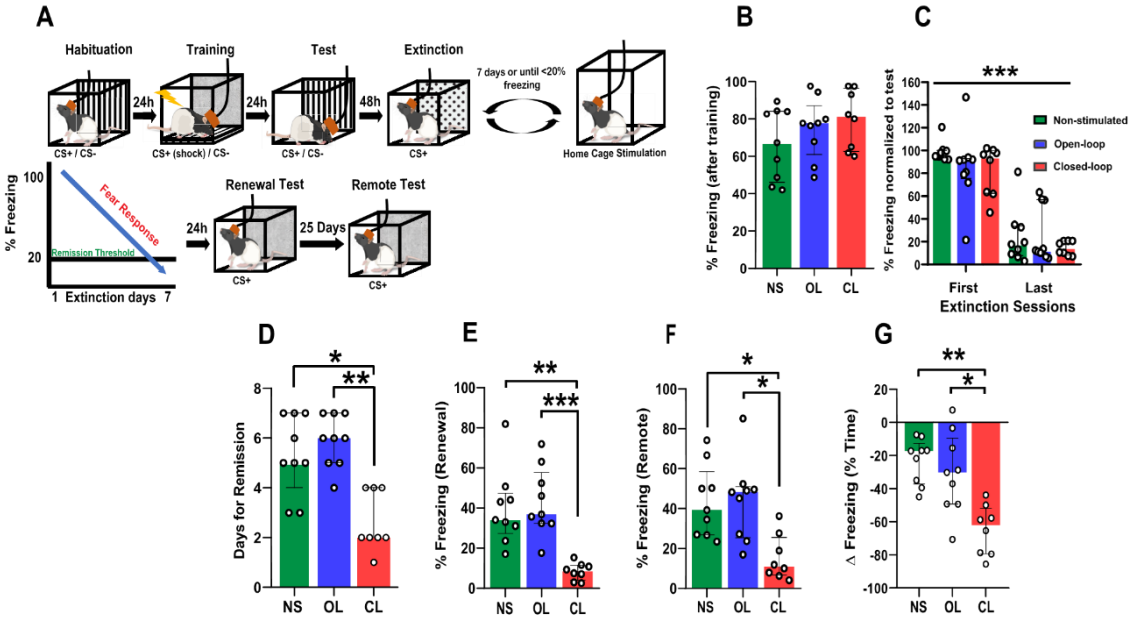


Figure 5. Medial Forebrain Bundle closed-loop stimulation enhance fear extinction. ***(A)*** *Schematics of the experimental design. Animals underwent fear conditioning training followed by a test session to evaluate memory recall, extinction sessions and 1h CL stimulation where the online detected SWRs triggered MFB stimulation until achieving the remission criterion (reduction of freezing behavior to < 20 % of the initial freezing). Renewal and fear recovery were assessed, 1 and 25 days after the last extinction session, respectively.* ***(B)*** *No difference in fear expression in response to the CS+ following training between the three experimental groups (non-stimulated (NS) n=9; (OL) n=9; (CL) n=8).* ***(C)*** *No difference between the fear expression of the three groups during the first 5 CS+ block after first and last extinction days. There was a significant decrease in fear expression over time, suggesting that extinction can attenuate fear. Values are normalized to the freezing expressed immediately after foot shock training (i.e. test after training).* ***(D)*** *Animals exposed to CL stimulation required less extinction sessions to achieve the remission criterion compared to the OL and non-stimulated groups.* ***(E)*** *CL neuromodulation induced lower fear expression during the renewal test in a hybrid context.* ***(F)*** *CL neuromodulation prevented spontaneous fear recovery 25 days after extinction.* ***(G)*** *CL neuromodulation results in the most pronounced reduction in fear responses throughout the experiment.*

Following the exposure to the 'renewal test' in a hybrid context there was a significant decrease in fear expression in the CL treated animals compared to the OL ($p = 0.0006$) and non-stimulated groups ($p = 0.0032$) (after Kruskal-Wallis test, $H = 16.21$, $p = <0.001$) (Fig. 5E). These results indicate that CL-MFB stimulation during SWRs can enhance fear extinction, decrease the time needed to achieve fear attenuation and maintain freezing levels low in challenging situations such as exposure to hybrid contexts resembling the learning contingencies.

To assess the persistence of the effects, animals were exposed to a 'remote test' 25 days following the renewal in the hybrid context. Animals were kept in their home cages between the renewal and remote tests. Freezing in CL stimulated animals remained at low levels compared to the OL ($p = 0.0152$) and non-stimulated group ($p = 0.0138$) (after Kruskal-Wallis test, $H = 10.38$, $p = <0.01$) (Fig. 5F), suggesting fear attenuation induced by CL-MFB stimulation was resistant to spontaneous recovery and persisted over time.

Finally, we quantified Δ freezing as reduced fear reactions between those after fear conditioning and the remote test (Δ freezing = Freezing extinction – Freezing test CS+) to reveal the overall effect of the interventions. CL simulated animals had stronger fear reduction than OL ($p = 0.0156$) and non-stimulated animals ($p = 0.0019$) (after Kruskal-Wallis test, $H = 13.06$, $p = <0.01$) (Fig. 5G). Together, CL neuromodulation of the reward system triggered by memory consolidation related neuronal oscillations accelerates fear extinction and promotes persistent low fear expression.

6.4.SWRs are required to consolidate contextual-related cues of fear memories.

The results obtained during CL modulation of fear memories guided by SWRs in different stages of memory processing (consolidation, reconsolidation and extinction) allow to hypothesize that SWRs should have a causal role during fear memory consolidation. This suggestion is supported by several lines of evidence regarding the disruption or improvement of memory performance after SWRs modulation[74,178]. Considering that, SWRs have been critical for contextual processing[179,180], we decided to continue with fear extinction to probe this potential causal role, since fear extinction is a context-dependent process[81]. Following the experiment with the extinction criterion, after each extinction, online detected SWRs triggered a single-pulse (0.5 ms) VHC stimulation, with the stimulation intensity tailored to each animal's requirements to disrupt the SWRs (range: 5–15 V) (Fig. 6A-B). OL animals were randomly stimulated within the same voltage range. This stimulation induces phasic silencing of hippocampal pyramidal cells and

interneurons[181]. Since animals trained with high-intensity foot-shocks tend to resist extinction, we reduced the training intensity (5 pairings CS + US at 0.7 mA) to ensure that the extinction criterion was achieved within seven sessions in control conditions.

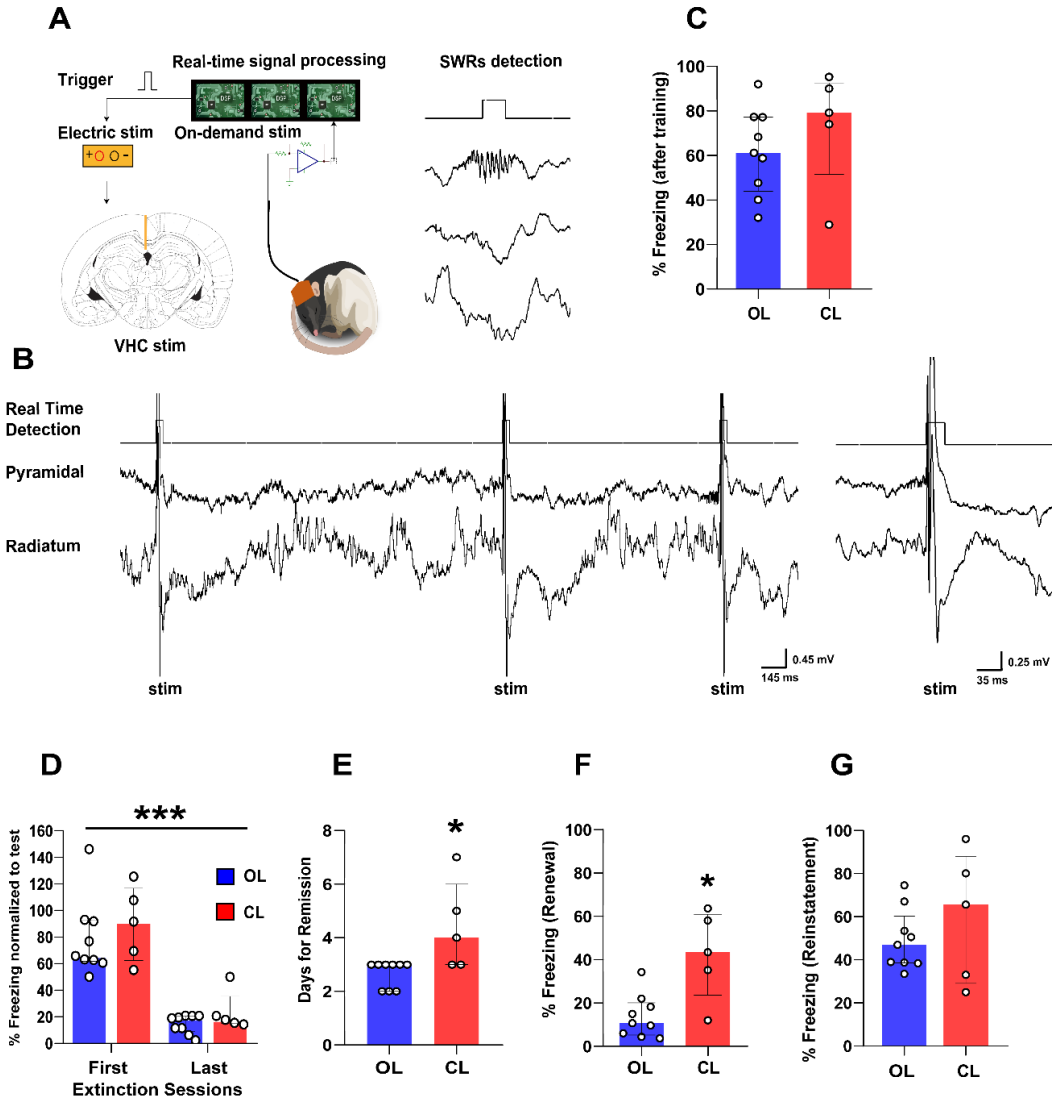


Figure 6. Closed-loop disruption of SWRs delays fear extinction. *(A) Behavioral protocol was performed as before, but SWRs triggered VHC stimulation was performed for 1 h following each extinction session. (B) Representative LFP signals from dorsal hippocampus showing disrupted SWRs events (stim; stimulation time and disruption). (C) No difference in fear expression in response to the CS+ following training between the three experimental groups were detected (open-loop (OL) n=9; closed-loop (CL) n=5). (D) No difference between the fear expression of the three experimental groups during the first 5 CS+ block from first and last extinction day. However, significant decrease in fear expression over time was verified in both groups. (E) SWRs disrupted animals require more days to achieve the extinction criterion. (F) Animals displaying disrupted SWRs exhibit elevated fear response during the renewal test. (G) No difference in fear expression during reinstatement were detected.*

No significant differences were detected between groups in fear expression following conditioning to CS+ (Mann–Whitney test, U = 14, p = 0.2977) (Fig. 6C). During extinction, there were no differences between groups, but a general fear attenuation was induced by extinction during the first and last extinction days (Mixed-effects ANOVA, time factor F (1, 12) = 65.00, p = <0.001) (Fig. 6D). Animals that experienced SWRs disruption required more extinction sessions to achieve an 80% reduction in freezing compared to those in the OL group (Mann–Whitney test, U = 6, p = 0.028) (Fig. 6E). Additionally, these SWRs-disrupted animals expressed elevated levels of fear reaction during the renewal test compared to the non-stimulated and OL groups (Mann–Whitney test, U = 4, p = 0.012) (Fig. 6F). No differences were detected during the reinstatement test (Mann–Whitney test, U = 20, p = 0.797) (Fig. 6H). These results suggests that hippocampal SWRs are essential for consolidating fear extinction. The disruption of SWRs results in slow extinction learning and fear persistence in different environments beyond the extinction context.

7. DISCUSSION

To the best of our knowledge, this series of experiments represents the first experimental demonstration that SWRs, a well-known marker of memory consolidation, can be used as an intrinsic signal to trigger CL brain stimulation, resulting in fear attenuation. Our approach can be applied to various memory processes such as consolidation, reconsolidation, and extinction, exhibiting high translational value. In this study, we provide evidence that: 1) CL-IL stimulation during consolidation can prevent the generalization of fear expression while subsequently enhancing extinction. 2) This window of opportunity can be extended upon memory reactivation and concurrent reconsolidation, where CL-BLA stimulation reverses fear generalization. 3) Extinction can be enhanced through CL-MFB, providing reward to a replayed extinction memory, thus accelerating fear suppression. Finally, 4) we demonstrate that SWRs have a causal role in memory processing, as SWRs disruption can impair extinction, a highly context-dependent process.

7.1.Memory processes as a target for the modification of fear memories

Memory has been conceptualized as a continuous process involving several stages, including periods of instability and stabilization[182]. Various studies have demonstrated that memory is particularly vulnerable to interference shortly after learning (during early consolidation) and after retrieval (when reconsolidation occurs)[183-187]. Consequently, animal models for suppressing fear memories have focused on pharmacological, behavioral, or neuromodulatory techniques that disrupt memory consolidation or impair reconsolidation after retrieval, thereby suppressing the fear memory trace[188]. In contrast, fear attenuation during extinction results from new inhibitory learning that can control the original fear memory. However, this learning is not persistent, and over time, the original fear can be recovered. Enhancing extinction and disrupting reconsolidation represent the primary strategies and challenges for both basic and clinical science in treating fear and anxiety-related disorders[189].

Another approach for disrupting memory involves updating its emotional content. For example, reactivating memory under rewarding stimuli can decrease the aversiveness of fear conditioning[20,21,190]. During this procedure, conditioned stimuli, which originally predicted punishment, are associated with a novel contingency, a rewarding stimulus. Similarly, the initial association between stimuli and reward can be updated by pairing the stimuli with punishment. This process is called counterconditioning and has been

applied in clinical interventions such as aversion therapies[191] and systematic desensitization[192].

This perspective of memory as independent processes that can be individually manipulated, is not limited to studies with animal models. The number of studies in clinical contexts, specifically interfering with the consolidation of a traumatic event or the reconsolidation of anxiety-provoking memories[193], has increased in recent years. This strengthens the possibility that precise neuromodulation, as provided by closed-loop approaches, may be one of the potential tools to be used.

7.2. Emotional updating: enhanced discrimination and fear attenuation as a result of closed-loop stimulation of the infralimbic cortex

Conventional deep brain stimulation of the infralimbic cortex (DBS-IL) has been shown to enhance fear extinction[138,139,141]. Interestingly, it has been reported that IL stimulation can induce anxiolytic and antidepressant-like responses[96,167]. In fact, the firing rate of IL neurons increased before entering the open arms of the elevated plus maze, suggesting that IL activity is required to overcome anxiety and motivate exploration of open spaces[194]. Our results indicate that IL stimulation during SWRs events can update the replayed fear memory trace with an anxiolytic response, reducing the aversiveness of fear conditioning through a precise counterconditioning process, as previously described.

Although our findings are supported by CL-IL stimulation preventing fear generalization (fear to CS-), no differences were detected in the expression of the fear memory (fear to CS+). Considering that SWRs are associated with contextual encoding and our task involves cued fear conditioning primarily associated with amygdala activity, raises the possibility that SWRs play a role in discrete features of less hippocampal-dependent memories, such as generalization. Despite the predominant view of the hippocampus as critical for the encoding of contextual information[195], there is evidence that the hippocampus participates in the consolidation of memories traditionally believed to be hippocampal-independent [196]. Also, SWRs contribute to the consolidation of social memory[197] as well as the representation of events in the absence of place cell activity[198]. While the discussion about the specific role of certain structures in different emotional processes is a debate intrinsic to the history of neuroscience[199], our results suggest that SWRs may participate in the consolidation of memories where the predictive capacity of the context during conditioning is low, for instance, when compared with context fear conditioning[187].

It is worth noting that lesioning or inactivation of the IL before cued and contextual fear conditioning results in memory generalization and resistance to extinction[114,200] as IL activity immediately following acquisition controls memory generalization. This evidence is in line with our results, as CL-IL stimulation affects only fear generalization. Importantly, our findings were limited to CL animals, indicating that the pairing between SWRs and IL stimulation is necessary for the effect. Despite open-loop animals receiving same amount of stimulation (not contingent with SWRs), no differences were detected in fear expression or generalization. Together, these results support our hypothesis that SWRs in cued fear memories specifically mediate fear generalization but not memory consolidation. Thus, the anxiolytic signal from IL stimulation during SWRs only modifies the response to CS-.

Finally, preventing fear generalization was sufficient to enhance fear extinction as showed by the correlations between these processes. This relationship has been previously reported[115,201] and overgeneralization and resistance to extinction are hallmarks in PTSD[4,5,15,17]. Importantly, overcoming fear generalization has been proposed to be a critical step to ensure successful extinction by slowing down systems consolidation[121].

7.3. Gamma activity in the basolateral amygdala as a neurobiological scaffolding for fear discrimination and extinction

The replication of these results using CL-BLA stimulation suggests that IL exerts its inhibitory role through an increase in gamma incidence in the BLA. Indeed, studies have shown that synchronization between the primary auditory cortex and prefrontal regions in gamma oscillations correlates with fear discrimination, and optogenetic inhibition of this pathway induces generalization[202]. This allows us to hypothesize that artificially increasing gamma activity in the amygdala could reduce generalization. Functional brain imaging studies have shown that AMY hyperactivity is present in PTSD[203] and studies in humans have demonstrated an increase in gamma activity in response to fearful stimuli, as compared to neutral ones[204]. Interestingly, brain lesions compromising the amygdala in humans act as a protective factor against the development of PTSD[205]. Classical DBS stimulation reduces PTSD-like behaviors in animal models[142,143] and clinical trials involving humans using OL[206] and CL[207] amygdala stimulation have shown significant reduction in PTSD symptomatology.

Different studies have suggested that high-frequency electrical stimulation generates suppressive effects in the targeted areas of the brain[208,209]. Although speculative, some of our results could be explained by a potential mechanism of DBS to preferentially activate "extinction cells" relative to "fear cells" in the amygdala[206]. Considering that both IL and BLA stimulation share the same neural signature of increased gamma activity in the BLA during CL stimulation, we propose that gamma oscillations play a critical role in decreasing fear responses. We hypothesize that gamma oscillation in the BLA provides the foundation for fear extinction. This hypothesis is supported by predominant oscillations in the BLA specifically associated with fear expression or extinction. For instance, some study demonstrated that following extinction learning, PV interneurons in the BLA enable a competing interaction between 6–12 Hz oscillations associated with safety and 3–6 Hz oscillations associated with fear[210]. A theta-fast gamma coupling has been shown to be strongest when the theta frequency was 6 Hz in BLA, correlating with high fear expression to CS+, while safety periods (e.g., CS- and pre-tone conditions) are characterized by enhanced BLA fast gamma power[211]. Closed-loop gamma modulation of the BLA can enhance or disrupt contextual fear memories derived from emotional experiences[212]. Action potentials of neurons recorded in the BLA are phase-locked to gamma oscillations, particularly when animals express low levels of freezing[211]. This oscillatory substrate in the amygdala, associated with safety, appears to be a shared mechanism between fear generalization and extinction[213]. Accordingly, we propose that future studies should evaluate whether selective gamma suppression in the BLA could induce fear generalization and impair fear extinction.

Despite several behavioral and cellular distinctions between consolidation and reconsolidation, the similarity between the results of CL-IL and CL-BLA suggests that our interpretation of emotional updating applies to both conditions. Similar to consolidation, the reconsolidation of destabilized memories requires sleep to avoid potential interference and promote long-term retrieval[214], potentially involving similar oscillatory coupled activity between ripples and slow-wave/spindles. Induced hippocampal theta-gamma phase amplitude coupling triggers destabilization and subsequent reconsolidation of naturally resistant avoidance memory tasks[215]. However, studies focused on oscillatory patterns during reconsolidation are still limited.

7.4. Enhancing fear extinction through closed-loop stimulation of the reward system

Compared to reconsolidation, neural oscillations have been well characterized in extinction[216], where phase-locked theta oscillations between the infralimbic cortex (IL) and the CA1 region of the hippocampus have been found associated with the gradual process of fear reduction[217]. In the case of the amygdala, increases in gamma oscillations during extinction can predict spontaneous recovery. However, whether the content of oscillatory patterns can be changed or updated with new information remains unknown [218]. To extend this emotional updating framework, we intend to enhance fear extinction based on the same principles of closed-loop stimulation used with IL and BLA. However, potential anxiolysis induced by IL stimulation was changed to a reward signal. For this purpose, SWRs detection delivered electrical stimulation of the MFB, chosen for its rewarding properties upon stimulation (aimed at reinforcing fear extinction) and its translational value is recognized by FDA-approved clinical trials[177,219].

The MFB is a principal pathway providing dopaminergic fibers to various brain regions involved in reward and emotional processing, including the amygdala[175]. The VTA sends dopaminergic axons to the NAc, amygdala, and PFC via the MFB[175], thus manipulation of the MFB has a global effect on the dopaminergic systems. Indeed, MFB deep brain stimulation can alleviate symptoms in patients with major depression[177,219-221].

Dopamine release in the BLA during fear learning controls the saliency of the foot shock and the extinction through prediction error signaling of non-reinforced CS+ presentation[24,176,222], thereby generating a reinforced fear extinction process based on reward derived from the omission of predicted punishment[222]. Notably, dopamine can enhance the excitability, evoked firing, and input resistance of BLA projection neurons via D1 and D2 receptor activation[223].

A recent report demonstrated that the BLA expresses two genetically distinct populations of excitatory neurons responding to positive and negative behaviors. These neurons participate in a mutual inhibition process[224]. Specifically, one of these subpopulations, Ppp1r1b+ neurons, are activated during extinction encoding and are responsive to natural reward[24]. Thus, it has been hypothesized that Ppp1r1b+ neurons in the BLA participate in the reward signaling induced by US omission[24].

We propose that SWRs-triggered CL-MFB stimulation and the reward from precise stimulation coincide with the widespread ongoing brain network activity orchestrating

the consolidation of fear extinction[225,226] during SWRs events. As neuronal activity in the BLA increases during SWRs[225,227], SWRs-triggered CL neuromodulation may provide a reward safety signal to the network activity that encodes fear extinction[218] thereby decreasing the aversiveness in both contextual and emotional memory content. This mechanism could be summarized as follows: 1) SWRs reactivate the memory trace in the BLA, 2) CL- MFB stimulation promotes concurrent dopamine release in the BLA and dHPC, and 3) dopamine release onto "extinction cells" enhances fear attenuation while protecting against increasing activity of "fear cells". This model can also be applied to the anxiolysis induced by CL-IL stimulation.

7.5.Causal role of SWRs in the consolidation of fear memories

Lastly, since our project heavily involved the detection of SWRs, an important question is revealing the role of SWRs during the consolidation of emotional memories. SWRs encode and consolidate spatial memories, playing a critical role in integrating information across neocortical and subcortical structures. Since our model was based on cued-fear conditioning, some aspects of the encoding and retrieval are not strictly associated with the context, thus diminishing the involvement of hippocampal activity. However, fear extinction is a highly context-dependent process[81]. CL disruption of SWRs delayed but did not completely block fear extinction, as animals were able to achieve the remission criterion, consistent with the contextual dependence of fear extinction previously reported[81,83,84]. Although SWRs were crucial for updating the emotional content of cued fear memories and enabling the extinction, we believe that SWRs are not sufficient to explain the context-dependent processes taking place during fear conditioning. It is well-known that slow cortical oscillations (<1 Hz) and spindles (7–15 Hz) are associated with long-term memory, and studies suggest that temporal coupling and phase coupling of ripples, spindles, and slow oscillations mediate hippocampal-to-neocortical information transfer during sleep[228]. These oscillations, although having different spatial and frequency characteristics, form a nested global framework of timing in the brain. Therefore, even in the absence of SWRs, cortical oscillations may have a compensatory role during ripple disruption. Future studies conducting simultaneous SWRs-spindle disruption or modifying (shifting, reversing) the coupling between SWRs and slow cortical oscillation could provide answers in this regard.

7.6. Future perspectives and limitations

The hippocampal-neocortical connection during memory consolidation opens new avenues to develop CL approaches that decrease the invasiveness of the procedure. For instance, conventional EEG could be used to detect slow cortical oscillations to trigger brain stimulation via transcranial direct current stimulation (tDCS) or transcranial magnetic stimulation (TMS).

Some open questions arise from our findings that could be addressed by future studies: 1) Although the role of SWRs is evident in these experiments, it remains unclear how SWRs can mediate the consolidation of less hippocampal dependent memories, such as cued-fear conditioning. 2) Can these findings be extended to a less invasive form of neuromodulation, such as spindle/slow wave stimulation? Considering the shared mechanisms of these oscillations with SWRs during memory consolidation, it is possible that similar results could be obtained using cortical biomarkers. 3) Are there other structures suitable for stimulation? For instance, DBS of the NAc induces striatal dopamine release in humans[229]. Similarly, could cortical structures more accessible to non-invasive stimulation be targeted to produce reward signals or anxiolysis? Studies in animals and humans suggest that the orbitofrontal cortex (OFC) could be a potential target, as it is engaged in rewarding activities with strong connection with the mesolimbic dopaminergic pathway [230,231]. Is there any relationship between the DA release induced in the CL and the gamma activity in the BLA that might explain the enhanced extinction? Lastly, are the changes observed in extinction a result of the intrinsic reinforcement of the SWRs characteristics such as frequency, incidence, or amplitude? Could our results be explained by the intrinsic conditioning of the cells involved in the generation of the SWRs?

I strongly believe that a systematic, carefully designed set of studies, performed either by me throughout my future career path or by others, may give reveal the answers to these questions in the near future.

7.7. Conclusions

In summary, our framework for studying and mitigating fear-related memories relies on CL stimulation guided by classical biomarkers of memory consolidation. If fear memories can be attenuated through oscillatory activity related to memory consolidation, we propose that disorders such as PTSD could be conceptualized as memory-based rather than anxiety-based disease. Even though anxiety is a transdiagnostic factor in populations

suffering from neuropsychiatric disorders, it may be an epiphenomenon resulting from the memory reconstruction of traumatic events. Therefore, the modification of memory engrams related to these memories could lead to an immediate decrease in anxiety-related symptomatology. This suggestion, although highly speculative, is partially supported by the results showed here. While decades of emotional memory research have aimed to disrupt, enhance, update, or modify memory traces to overcome fear reactions[182], there are few formal proposals from a translational standpoint to target memory in clinical populations[160]. This is particularly promising given current attempts to enhance reconsolidation protocols in humans, which have the capability of reactivating specific memories. The coupling between SWRs, cortical slow-waves, and spindles may provide a potential pathway to translate our approach towards a non-invasive therapy in the future, guided by the hypothesis of memory as a clinical target.

8. SUMMARY

In my current thesis, I present and interpret our experiments providing the first demonstration of a CL intervention to update the emotional information of fear memory traces through intracranial electrical stimulation. Our results suggest new directions for developing CL neuromodulation technologies for anxiety, trauma- and stressor-related disorders.

We developed a real-time detection system to identify dorsal hippocampal SWRs and deliver intracranial stimulation to the IL immediately after cued fear conditioning. IL stimulation has been shown to enhance fear extinction using conventional DBS as well as anxiolytic and antidepressant-like responses. We hypothesized that by providing IL stimulation during the precise timing of memory replay via SWRs, an artificial anxiolytic response could be introduced during the consolidation of a fearful event.

Immediately after fear conditioning, one group of rats received CL stimulation, another group was exposed to the same CL stimulation 48 h after training, a group received jittered stimulation (OL), and a control group received no stimulation.

We found that only animals exposed to CL immediately after training expressed less fear responses to the CS- compared with all groups during test. This procedure was successful in enhancing extinction, resulting in an attenuated expression of fear in a subsequent renewal test. This outcome demonstrated long-lasting effects as animals treated with the closed-loop stimulation showed resistance to fear recovery. Interestingly, a relationship

between memory precision and fear extinction enhancement was confirmed by significant negative correlation between the discrimination index and fear behavior during renewal.

These results are mediated by an enhanced gamma-band activity in the BLA in CL animals, as a significant increase in gamma incidence was observed following the stimulation period. Moreover, this increase was positively correlated with memory discrimination.

Beyond its interference with memory consolidation, reconsolidation—a plasticity-dependent process that occurs after reactivation—presents a potential target for persistently modifying memory traces. Anatomically connection between IL and BLA provides inhibitory circuits in amygdala implicated in the reduction of fear and anxiety responses. We evaluated whether direct CL-BLA stimulation could modify fear memory intensity or generalization when applied after reactivation, following the rationale of emotional updating. We showed that after the stimulation, CL animals expressed less fear behavior compared OL during the CS- test. This facilitation was confirmed in the discrimination index. Analogous to our results observed during consolidation, this precise stimulation approach enhances subsequent fear extinction. In summary, direct CL amygdala stimulation can replicate the results obtained through (IL) stimulation.

Considering that extinction-based treatments are the gold standard psychological approach for fear and anxiety disorders, we decided to translate CL stimulation to an extinction procedure. The MFB is a group of fibers connecting nodes of dopaminergic pathways involved in reward. We hypothesized that SWRs-triggering CL-MFB neuromodulation may provide a reward signal during memory replay. For this purpose, rats underwent fear conditioning followed by fear extinction training over multiple days until a remission criterion or up to maximum seven days. During the extinction protocol, one group of rats received CL-MFB during hippocampal SWRs events, another group OL stimulation, and a control group received no stimulation.

Animals exposed to CL stimulation required fewer extinction sessions to achieve the remission criterion compared to all groups suggesting that CL-MFB neuromodulation can enhance the effectiveness of fear extinction. In the renewal test there was a significant decrease in fear expression in the CL treated animals compared to all groups. This result was maintained even 25 days after the last extinction session.

A Δ freezing (fear response) showed that the most significant decrease in fear occurred between the post-training period and the remote testing following the extinction in the CL simulated animals, supporting the hypothesis that neuromodulation of the reward system accelerates fear extinction.

Finally, to evaluate the role of SWRs during fear processing, ripples were disrupted after extinction sessions in a CL fashion. Following the experiment with extinction criterion, after each extinction session, online SWRs triggered a single-pulse (0.5 ms stimulation of the ventral hippocampal commissure. Animals that experienced SWRs disruption required more extinction sessions to achieve an 80% reduction in freezing compared the OL group. Additionally, CL animals expressed elevated levels of fear reaction during the renewal test. These results suggest that hippocampal SWRs are essential for consolidating fear extinction.

Our study demonstrates the potential of SWRs, a memory consolidation marker, for triggering CL brain stimulation to attenuate fear. We found the feasibility of this method in various memory processes with high translational value. We found: 1) CL stimulation during consolidation prevents fear generalization and enhances extinction. 2) CL-BLA after memory reactivation reverses fear generalization. 3) CL-MFB accelerates fear suppression and 4) disruption of SWRs impairs extinction, proving its causal role in fear processing. The feasibility of our method and positive results raises questions about strategies to reduce the invasiveness of the stimulation and detection procedures for future human testing.

9. ACKNOWLEDMENTS

I would like to thank to Dr. Antal Berényi for the opportunity to do my PhD and learning a lot in his lab.

I thank to Dr Magor L. Lőrincz for his support and scientific discussions.

I thank all the people who in one way or another have contributed to my being here at this moment.

I am also very thankful to Professor Ferenc Bari for accepting me in the PhD Program of the University and to Professor Gyula Sáry for allowing me to work in the department and to everyone in the Department of Physiology.

I thank to Dr. József Maléth and Professor Péter Hegyi, for providing access to the confocal microscope.

I thank my labmates Livia, Qun, Andi, Yuichi, Levente and Gábor for their support in many of the stages of this work, providing insightful discussions and professional skills.

My special gratitude to Lívia Barcsai and Péter Ráfi for their kindness over the years in Hungary.

I thank to Csenge and Tamás for helping me with the bureaucratic issues in Hungary.

I thank to Éva Csajbók and Krisztina Pál for being the doctors who cared for me so carefully during these challenging years of my health.

I thank my family (Los Magníficos), for their unconditional love.

Finally, I thank Rodrigo for our love, the adventures we have lived and all the memories and learnings we take with us from here.

10. REFERENCES

1. McLaughlin, K.A., *et al.* Trauma exposure and posttraumatic stress disorder in a national sample of adolescents. *J Am Acad Child Adolesc Psychiatry* **52**, 815-830 e814 (2013).
2. Perrin, S. Prolonged exposure therapy for PTSD in sexually abused adolescents. *JAMA* **310**, 2619-2620 (2013).
3. Morina, N., Stam, K., Pollet, T.V. & Priebe, S. Prevalence of depression and posttraumatic stress disorder in adult civilian survivors of war who stay in war-afflicted regions. A systematic review and meta-analysis of epidemiological studies. *J Affect Disord* **239**, 328-338 (2018).
4. Bryant, R.A., *et al.* Acute and Chronic Posttraumatic Stress Symptoms in the Emergence of Posttraumatic Stress Disorder: A Network Analysis. *JAMA Psychiatry* **74**, 135-142 (2017).
5. Ross, D.A., *et al.* An Integrated Neuroscience Perspective on Formulation and Treatment Planning for Posttraumatic Stress Disorder: An Educational Review. *JAMA Psychiatry* **74**, 407-415 (2017).
6. Bradley, R., Greene, J., Russ, E., Dutra, L. & Westen, D. A multidimensional meta-analysis of psychotherapy for PTSD. *Am J Psychiatry* **162**, 214-227 (2005).
7. Simon, N.M., *et al.* Paroxetine CR augmentation for posttraumatic stress disorder refractory to prolonged exposure therapy. *J Clin Psychiatry* **69**, 400-405 (2008).
8. Berger, W., *et al.* Pharmacologic alternatives to antidepressants in posttraumatic stress disorder: a systematic review. *Prog Neuropsychopharmacol Biol Psychiatry* **33**, 169-180 (2009).
9. Izquierdo, I., Furini, C.R. & Myskiw, J.C. Fear Memory. *Physiol Rev* **96**, 695-750 (2016).
10. Richter-Levin, G., Stork, O. & Schmidt, M.V. Animal models of PTSD: a challenge to be met. *Mol Psychiatry* **24**, 1135-1156 (2019).
11. Bienvenu, T.C.M., *et al.* The advent of fear conditioning as an animal model of post-traumatic stress disorder: Learning from the past to shape the future of PTSD research. *Neuron* **109**, 2380-2397 (2021).
12. Al Abed, A.S., *et al.* Preventing and treating PTSD-like memory by trauma contextualization. *Nat Commun* **11**, 4220 (2020).
13. Cottencin, O., *et al.* Directed forgetting in PTSD: a comparative study versus normal controls. *J Psychiatr Res* **40**, 70-80 (2006).
14. Hardt, O., Nader, K. & Nadel, L. Decay happens: the role of active forgetting in memory. *Trends Cogn Sci* **17**, 111-120 (2013).
15. McNally, R.J. Memory and anxiety disorders. *Philos Trans R Soc Lond B Biol Sci* **352**, 1755-1759 (1997).
16. Golier, J.A., Harvey, P.D., Legge, J. & Yehuda, R. Memory performance in older trauma survivors: implications for the longitudinal course of PTSD. *Ann N Y Acad Sci* **1071**, 54-66 (2006).
17. Desmedt, A., Marighetto, A. & Piazza, P.V. Abnormal Fear Memory as a Model for Posttraumatic Stress Disorder. *Biol Psychiatry* **78**, 290-297 (2015).
18. Brewin, C.R. Memory and Forgetting. *Curr Psychiatry Rep* **20**, 87 (2018).
19. Redondo, R.L., *et al.* Bidirectional switch of the valence associated with a hippocampal contextual memory engram. *Nature* **513**, 426-430 (2014).
20. Haubrich, J., *et al.* Reconsolidation allows fear memory to be updated to a less aversive level through the incorporation of appetitive information. *Neuropsychopharmacology* **40**, 315-326 (2015).

21. Arellano Perez, A.D., Popik, B. & de Oliveira Alvares, L. Rewarding information presented during reactivation attenuates fear memory: Methylphenidate and fear memory updating. *Neuropharmacology* **171**, 108107 (2020).
22. Correia, S.S., McGrath, A.G., Lee, A., Graybiel, A.M. & Goosens, K.A. Amygdala-ventral striatum circuit activation decreases long-term fear. *Elife* **5**(2016).
23. Keller, N.E., Hennings, A.C., Leiker, E.K., Lewis-Peacock, J.A. & Dunsmoor, J.E. Rewarded Extinction Increases Amygdalar Connectivity and Stabilizes Long-Term Memory Traces in the vmPFC. *J Neurosci* **42**, 5717-5729 (2022).
24. Zhang, X., Kim, J. & Tonegawa, S. Amygdala Reward Neurons Form and Store Fear Extinction Memory. *Neuron* **105**, 1077-1093 e1077 (2020).
25. Holtzheimer, P.E. & Mayberg, H.S. Deep brain stimulation for psychiatric disorders. *Annu Rev Neurosci* **34**, 289-307 (2011).
26. Kozak, G. & Berenyi, A. Sustained efficacy of closed loop electrical stimulation for long-term treatment of absence epilepsy in rats. *Sci Rep* **7**, 6300 (2017).
27. Takeuchi, Y. & Berenyi, A. Oscillotherapeutics - Time-targeted interventions in epilepsy and beyond. *Neurosci Res* **152**, 87-107 (2020).
28. Takeuchi, Y., *et al.* Closed-loop stimulation of the medial septum terminates epileptic seizures. *Brain* **144**, 885-908 (2021).
29. Berenyi, A., Belluscio, M., Mao, D. & Buzsaki, G. Closed-loop control of epilepsy by transcranial electrical stimulation. *Science* **337**, 735-737 (2012).
30. Scangos, K.W., *et al.* Closed-loop neuromodulation in an individual with treatment-resistant depression. *Nat Med* **27**, 1696-1700 (2021).
31. Koenen, K.C., *et al.* Posttraumatic stress disorder in the World Mental Health Surveys. *Psychol Med* **47**, 2260-2274 (2017).
32. Yuan, K., *et al.* Prevalence of posttraumatic stress disorder after infectious disease pandemics in the twenty-first century, including COVID-19: a meta-analysis and systematic review. *Mol Psychiatry* **26**, 4982-4998 (2021).
33. Zhang, L., Pan, R., Cai, Y. & Pan, J. The Prevalence of Post-Traumatic Stress Disorder in the General Population during the COVID-19 Pandemic: A Systematic Review and Single-Arm Meta-Analysis. *Psychiatry Investig* **18**, 426-433 (2021).
34. McLean, C.P., Asnaani, A., Litz, B.T. & Hofmann, S.G. Gender differences in anxiety disorders: prevalence, course of illness, comorbidity and burden of illness. *J Psychiatr Res* **45**, 1027-1035 (2011).
35. Maeng, L.Y. & Milad, M.R. Sex differences in anxiety disorders: Interactions between fear, stress, and gonadal hormones. *Horm Behav* **76**, 106-117 (2015).
36. Insel, T., *et al.* Research domain criteria (RDoC): toward a new classification framework for research on mental disorders. *Am J Psychiatry* **167**, 748-751 (2010).
37. Morris, S.E. & Cuthbert, B.N. Research Domain Criteria: cognitive systems, neural circuits, and dimensions of behavior. *Dialogues Clin Neurosci* **14**, 29-37 (2012).
38. Canetta, S. & Kellendonk, C. Can we use mice to study schizophrenia? *Philos Trans R Soc Lond B Biol Sci* **373**(2018).
39. Schoner, J., Heinz, A., Endres, M., Gertz, K. & Kronenberg, G. Post-traumatic stress disorder and beyond: an overview of rodent stress models. *J Cell Mol Med* **21**, 2248-2256 (2017).
40. Radell, M.L., Hamza, E.A. & Moustafa, A.A. Depression in post-traumatic stress disorder. *Rev Neurosci* **31**, 703-722 (2020).

41. Bowers, M.E. & Ressler, K.J. An Overview of Translationally Informed Treatments for Posttraumatic Stress Disorder: Animal Models of Pavlovian Fear Conditioning to Human Clinical Trials. *Biol Psychiatry* **78**, E15-27 (2015).
42. Johnson, L.R., McGuire, J., Lazarus, R. & Palmer, A.A. Pavlovian fear memory circuits and phenotype models of PTSD. *Neuropharmacology* **62**, 638-646 (2012).
43. Blechert, J., Michael, T., Vriends, N., Margraf, J. & Wilhelm, F.H. Fear conditioning in posttraumatic stress disorder: evidence for delayed extinction of autonomic, experiential, and behavioural responses. *Behav Res Ther* **45**, 2019-2033 (2007).
44. Harnett, N.G., *et al.* Trauma exposure acutely alters neural function during Pavlovian fear conditioning. *Cortex* **109**, 1-13 (2018).
45. Wenzel, A. Basic Strategies of Cognitive Behavioral Therapy. *Psychiatr Clin North Am* **40**, 597-609 (2017).
46. Gazarini, L., Stern, C.A.J. & Bertoglio, L.J. On making (and turning adaptive to) maladaptive aversive memories in laboratory rodents. *Neurosci Biobehav Rev* **147**, 105101 (2023).
47. Craske, M.G., Sandman, C.F. & Stein, M.B. How can neurobiology of fear extinction inform treatment? *Neurosci Biobehav Rev* **143**, 104923 (2022).
48. Maren, S. & Holmes, A. Stress and Fear Extinction. *Neuropsychopharmacology* **41**, 58-79 (2016).
49. Goode, T.D. & Maren, S. Animal models of fear relapse. *ILAR J* **55**, 246-258 (2014).
50. Matar, M.A., Zohar, J. & Cohen, H. Translationally relevant modeling of PTSD in rodents. *Cell Tissue Res* **354**, 127-139 (2013).
51. Azevedo, T.M., *et al.* A freezing-like posture to pictures of mutilation. *Psychophysiology* **42**, 255-260 (2005).
52. Janak, P.H. & Tye, K.M. From circuits to behaviour in the amygdala. *Nature* **517**, 284-292 (2015).
53. Murray, E.A. The amygdala, reward and emotion. *Trends Cogn Sci* **11**, 489-497 (2007).
54. Lichtenberg, N.T., *et al.* The Medial Orbitofrontal Cortex-Basolateral Amygdala Circuit Regulates the Influence of Reward Cues on Adaptive Behavior and Choice. *J Neurosci* **41**, 7267-7277 (2021).
55. Bechara, A., *et al.* Double dissociation of conditioning and declarative knowledge relative to the amygdala and hippocampus in humans. *Science* **269**, 1115-1118 (1995).
56. LaBar, K.S., LeDoux, J.E., Spencer, D.D. & Phelps, E.A. Impaired fear conditioning following unilateral temporal lobectomy in humans. *J Neurosci* **15**, 6846-6855 (1995).
57. LeDoux, J.E., Cicchetti, P., Xagoraris, A. & Romanski, L.M. The lateral amygdaloid nucleus: sensory interface of the amygdala in fear conditioning. *J Neurosci* **10**, 1062-1069 (1990).
58. Maren, S., Aharonov, G. & Fanselow, M.S. Retrograde abolition of conditional fear after excitotoxic lesions in the basolateral amygdala of rats: absence of a temporal gradient. *Behav Neurosci* **110**, 718-726 (1996).
59. Goosens, K.A. & Maren, S. Contextual and auditory fear conditioning are mediated by the lateral, basal, and central amygdaloid nuclei in rats. *Learn Mem* **8**, 148-155 (2001).

60. Goosens, K.A. & Maren, S. Pretraining NMDA receptor blockade in the basolateral complex, but not the central nucleus, of the amygdala prevents savings of conditional fear. *Behav Neurosci* **117**, 738-750 (2003).
61. Maren, S. Neurotoxic basolateral amygdala lesions impair learning and memory but not the performance of conditional fear in rats. *J Neurosci* **19**, 8696-8703 (1999).
62. Bicks, L.K., Koike, H., Akbarian, S. & Morishita, H. Prefrontal Cortex and Social Cognition in Mouse and Man. *Front Psychol* **6**, 1805 (2015).
63. Sotres-Bayon, F., Sierra-Mercado, D., Pardilla-Delgado, E. & Quirk, G.J. Gating of fear in prelimbic cortex by hippocampal and amygdala inputs. *Neuron* **76**, 804-812 (2012).
64. Mendoza, J., Sanio, C. & Chaudhri, N. Inactivating the infralimbic but not prelimbic medial prefrontal cortex facilitates the extinction of appetitive Pavlovian conditioning in Long-Evans rats. *Neurobiol Learn Mem* **118**, 198-208 (2015).
65. Milad, M.R. & Quirk, G.J. Neurons in medial prefrontal cortex signal memory for fear extinction. *Nature* **420**, 70-74 (2002).
66. Milad, M.R., *et al.* Thickness of ventromedial prefrontal cortex in humans is correlated with extinction memory. *Proc Natl Acad Sci U S A* **102**, 10706-10711 (2005).
67. Milad, M.R., *et al.* Recall of fear extinction in humans activates the ventromedial prefrontal cortex and hippocampus in concert. *Biol Psychiatry* **62**, 446-454 (2007).
68. Gurvits, T.V., *et al.* Magnetic resonance imaging study of hippocampal volume in chronic, combat-related posttraumatic stress disorder. *Biol Psychiatry* **40**, 1091-1099 (1996).
69. Gilbertson, M.W., *et al.* Smaller hippocampal volume predicts pathologic vulnerability to psychological trauma. *Nat Neurosci* **5**, 1242-1247 (2002).
70. Villarreal, G., *et al.* Reduced hippocampal volume and total white matter volume in posttraumatic stress disorder. *Biol Psychiatry* **52**, 119-125 (2002).
71. McEwen, B.S. & Sapolsky, R.M. Stress and cognitive function. *Curr Opin Neurobiol* **5**, 205-216 (1995).
72. de Quervain, D.J., Roozendaal, B. & McGaugh, J.L. Stress and glucocorticoids impair retrieval of long-term spatial memory. *Nature* **394**, 787-790 (1998).
73. Kim, J.J., *et al.* Stress-induced alterations in hippocampal plasticity, place cells, and spatial memory. *Proc Natl Acad Sci U S A* **104**, 18297-18302 (2007).
74. Fernandez-Ruiz, A., *et al.* Long-duration hippocampal sharp wave ripples improve memory. *Science* **364**, 1082-1086 (2019).
75. Buzsaki, G. Hippocampal sharp wave-ripple: A cognitive biomarker for episodic memory and planning. *Hippocampus* **25**, 1073-1188 (2015).
76. Tomar, A., Polygalov, D., Chattarji, S. & McHugh, T.J. Stress enhances hippocampal neuronal synchrony and alters ripple-spike interaction. *Neurobiol Stress* **14**, 100327 (2021).
77. Tomar, A. & McHugh, T.J. The impact of stress on the hippocampal spatial code. *Trends Neurosci* **45**, 120-132 (2022).
78. Phillips, R.G. & LeDoux, J.E. Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behav Neurosci* **106**, 274-285 (1992).

79. Anagnostaras, S.G., Maren, S. & Fanselow, M.S. Temporally graded retrograde amnesia of contextual fear after hippocampal damage in rats: within-subjects examination. *J Neurosci* **19**, 1106-1114 (1999).
80. Brewin, C.R. The nature and significance of memory disturbance in posttraumatic stress disorder. *Annu Rev Clin Psychol* **7**, 203-227 (2011).
81. Maren, S., Phan, K.L. & Liberzon, I. The contextual brain: implications for fear conditioning, extinction and psychopathology. *Nat Rev Neurosci* **14**, 417-428 (2013).
82. Herry, C., *et al.* Switching on and off fear by distinct neuronal circuits. *Nature* **454**, 600-606 (2008).
83. Zelikowsky, M., Pham, D.L. & Fanselow, M.S. Temporal factors control hippocampal contributions to fear renewal after extinction. *Hippocampus* **22**, 1096-1106 (2012).
84. Corcoran, K.A. & Maren, S. Hippocampal inactivation disrupts contextual retrieval of fear memory after extinction. *J Neurosci* **21**, 1720-1726 (2001).
85. Badura-Brack, A., *et al.* Veterans with PTSD demonstrate amygdala hyperactivity while viewing threatening faces: A MEG study. *Biol Psychol* **132**, 228-232 (2018).
86. Giustino, T.F. & Maren, S. The Role of the Medial Prefrontal Cortex in the Conditioning and Extinction of Fear. *Front Behav Neurosci* **9**, 298 (2015).
87. Ochsner, K.N., Bunge, S.A., Gross, J.J. & Gabrieli, J.D. Rethinking feelings: an FMRI study of the cognitive regulation of emotion. *J Cogn Neurosci* **14**, 1215-1229 (2002).
88. Williams, L.M., *et al.* Trauma modulates amygdala and medial prefrontal responses to consciously attended fear. *Neuroimage* **29**, 347-357 (2006).
89. Lanius, R.A., *et al.* Neural correlates of trauma script-imagery in posttraumatic stress disorder with and without comorbid major depression: a functional MRI investigation. *Psychiatry Res* **155**, 45-56 (2007).
90. Shin, L.M., *et al.* A functional magnetic resonance imaging study of amygdala and medial prefrontal cortex responses to overtly presented fearful faces in posttraumatic stress disorder. *Arch Gen Psychiatry* **62**, 273-281 (2005).
91. Werner, N.S., *et al.* Hippocampal function during associative learning in patients with posttraumatic stress disorder. *J Psychiatr Res* **43**, 309-318 (2009).
92. Brohawn, K.H., Offringa, R., Pfaff, D.L., Hughes, K.C. & Shin, L.M. The neural correlates of emotional memory in posttraumatic stress disorder. *Biol Psychiatry* **68**, 1023-1030 (2010).
93. Bass, D.I., Partain, K.N. & Manns, J.R. Event-specific enhancement of memory via brief electrical stimulation to the basolateral complex of the amygdala in rats. *Behav Neurosci* **126**, 204-208 (2012).
94. Inman, C.S., *et al.* Direct electrical stimulation of the amygdala enhances declarative memory in humans. *Proc Natl Acad Sci U S A* **115**, 98-103 (2018).
95. Hamani, C., *et al.* Patient With Posttraumatic Stress Disorder Successfully Treated With Deep Brain Stimulation of the Medial Prefrontal Cortex and Uncinate Fasciculus. *Biol Psychiatry* **88**, e57-e59 (2020).
96. Hamani, C., *et al.* Antidepressant-like effects of medial prefrontal cortex deep brain stimulation in rats. *Biol Psychiatry* **67**, 117-124 (2010).
97. Bambico, F.R., *et al.* Neuroplasticity-dependent and -independent mechanisms of chronic deep brain stimulation in stressed rats. *Transl Psychiatry* **5**, e674 (2015).

98. Reznikov, R., Binko, M., Nobrega, J.N. & Hamani, C. Deep Brain Stimulation in Animal Models of Fear, Anxiety, and Posttraumatic Stress Disorder. *Neuropsychopharmacology* **41**, 2810-2817 (2016).
99. Hurlemann, R. Noradrenergic-glucocorticoid mechanisms in emotion-induced amnesia: from adaptation to disease. *Psychopharmacology (Berl)* **197**, 13-23 (2008).
100. Bremner, J.D., *et al.* Deficits in short-term memory in posttraumatic stress disorder. *Am J Psychiatry* **150**, 1015-1019 (1993).
101. Bremner, J.D., *et al.* Deficits in short-term memory in adult survivors of childhood abuse. *Psychiatry Res* **59**, 97-107 (1995).
102. Brewin, C.R., Kleiner, J.S., Vasterling, J.J. & Field, A.P. Memory for emotionally neutral information in posttraumatic stress disorder: A meta-analytic investigation. *J Abnorm Psychol* **116**, 448-463 (2007).
103. Johnsen, G.E. & Asbjornsen, A.E. Consistent impaired verbal memory in PTSD: a meta-analysis. *J Affect Disord* **111**, 74-82 (2008).
104. Scott, J.C., *et al.* A quantitative meta-analysis of neurocognitive functioning in posttraumatic stress disorder. *Psychol Bull* **141**, 105-140 (2015).
105. Wild, J. & Gur, R.C. Verbal memory and treatment response in post-traumatic stress disorder. *Br J Psychiatry* **193**, 254-255 (2008).
106. Petzold, M. & Bunzeck, N. Impaired episodic memory in PTSD patients - A meta-analysis of 47 studies. *Front Psychiatry* **13**, 909442 (2022).
107. Lissek, S., *et al.* Classical fear conditioning in the anxiety disorders: a meta-analysis. *Behav Res Ther* **43**, 1391-1424 (2005).
108. Lissek, S., *et al.* Overgeneralization of conditioned fear as a pathogenic marker of panic disorder. *Am J Psychiatry* **167**, 47-55 (2010).
109. Kaczkurkin, A.N., *et al.* Neural Substrates of Overgeneralized Conditioned Fear in PTSD. *Am J Psychiatry* **174**, 125-134 (2017).
110. McTeague, L.M., *et al.* Fearful imagery in social phobia: generalization, comorbidity, and physiological reactivity. *Biol Psychiatry* **65**, 374-382 (2009).
111. Wiltgen, B.J. & Silva, A.J. Memory for context becomes less specific with time. *Learn Mem* **14**, 313-317 (2007).
112. Migues, P.V., *et al.* Blocking Synaptic Removal of GluA2-Containing AMPA Receptors Prevents the Natural Forgetting of Long-Term Memories. *J Neurosci* **36**, 3481-3494 (2016).
113. Gazarini, L., Stern, C.A., Piornedo, R.R., Takahashi, R.N. & Bertoglio, L.J. PTSD-like memory generated through enhanced noradrenergic activity is mitigated by a dual step pharmacological intervention targeting its reconsolidation. *Int J Neuropsychopharmacol* **18**(2014).
114. Bayer, H. & Bertoglio, L.J. Infralimbic cortex controls fear memory generalization and susceptibility to extinction during consolidation. *Sci Rep* **10**, 15827 (2020).
115. Pedraza, L.K., *et al.* Chronic fluoxetine prevents fear memory generalization and enhances subsequent extinction by remodeling hippocampal dendritic spines and slowing down systems consolidation. *Transl Psychiatry* **9**, 53 (2019).
116. Zinn, R., *et al.* Maladaptive Properties of Context-Impoverished Memories. *Curr Biol* **30**, 2300-2311 e2306 (2020).
117. Pedraza, L.K., *et al.* The dynamic nature of systems consolidation: Stress during learning as a switch guiding the rate of the hippocampal dependency and memory quality. *Hippocampus* **26**, 362-371 (2016).

118. Pedraza, L.K., Sierra, R.O., Crestani, A.P., Quillfeldt, J.A. & de Oliveira Alvares, L. Sequential learning during contextual fear conditioning guides the rate of systems consolidation: Implications for consolidation of multiple memory traces. *Hippocampus* **27**, 518-528 (2017).
119. Dos Santos Correa, M., *et al.* Relationship between footshock intensity, post-training corticosterone release and contextual fear memory specificity over time. *Psychoneuroendocrinology* **110**, 104447 (2019).
120. Dos Santos Correa, M., *et al.* Corticosterone differentially modulates time-dependent fear generalization following mild or moderate fear conditioning training in rats. *Neurobiol Learn Mem* **184**, 107487 (2021).
121. Pedraza, L.K., Sierra, R.O. & de Oliveira Alvares, L. Systems consolidation and fear memory generalisation as a potential target for trauma-related disorders. *World J Biol Psychiatry* **23**, 653-665 (2022).
122. Lee, D.J., *et al.* Psychotherapy Versus Pharmacotherapy for Posttraumatic Stress Disorder: Systemic Review and Meta-Analyses to Determine First-Line Treatments. *Depress Anxiety* **33**, 792-806 (2016).
123. Blanchard, E.B., *et al.* A controlled evaluation of cognitive behavioural therapy for posttraumatic stress in motor vehicle accident survivors. *Behav Res Ther* **41**, 79-96 (2003).
124. Sloan, D.M., Marx, B.P., Bovin, M.J., Feinstein, B.A. & Gallagher, M.W. Written exposure as an intervention for PTSD: a randomized clinical trial with motor vehicle accident survivors. *Behav Res Ther* **50**, 627-635 (2012).
125. Stenmark, H., Catani, C., Neuner, F., Elbert, T. & Holen, A. Treating PTSD in refugees and asylum seekers within the general health care system. A randomized controlled multicenter study. *Behav Res Ther* **51**, 641-647 (2013).
126. Morina, N., Wicherts, J.M., Lobbrecht, J. & Priebe, S. Remission from post-traumatic stress disorder in adults: a systematic review and meta-analysis of long term outcome studies. *Clin Psychol Rev* **34**, 249-255 (2014).
127. Vervliet, B., Craske, M.G. & Hermans, D. Fear extinction and relapse: state of the art. *Annu Rev Clin Psychol* **9**, 215-248 (2013).
128. Steckler, T. & Risbrough, V. Pharmacological treatment of PTSD - established and new approaches. *Neuropharmacology* **62**, 617-627 (2012).
129. Schrader, C. & Ross, A. A Review of PTSD and Current Treatment Strategies. *Mo Med* **118**, 546-551 (2021).
130. Stein, M.B., Kline, N.A. & Matloff, J.L. Adjunctive olanzapine for SSRI-resistant combat-related PTSD: a double-blind, placebo-controlled study. *Am J Psychiatry* **159**, 1777-1779 (2002).
131. Hillhouse, T.M. & Porter, J.H. A brief history of the development of antidepressant drugs: from monoamines to glutamate. *Exp Clin Psychopharmacol* **23**, 1-21 (2015).
132. Mithoefer, M.C., Wagner, M.T., Mithoefer, A.T., Jerome, L. & Doblin, R. The safety and efficacy of +/-3,4-methylenedioxymethamphetamine-assisted psychotherapy in subjects with chronic, treatment-resistant posttraumatic stress disorder: the first randomized controlled pilot study. *J Psychopharmacol* **25**, 439-452 (2011).
133. Bechtereva, N.P., Bondartchuk, A.N., Smirnov, V.M., Meliutcheva, L.A. & Shandurina, A.N. Method of electrostimulation of the deep brain structures in treatment of some chronic diseases. *Confin Neurol* **37**, 136-140 (1975).

134. Wagle Shukla, A. & Okun, M.S. State of the Art for Deep Brain Stimulation Therapy in Movement Disorders: A Clinical and Technological Perspective. *IEEE Rev Biomed Eng* **9**, 219-233 (2016).
135. Langevin, J.P., *et al*. Deep Brain Stimulation of the Basolateral Amygdala: Targeting Technique and Electrodiagnostic Findings. *Brain Sci* **6**(2016).
136. Avecillas-Chasin, J.M., *et al*. Structural correlates of emotional response to electrical stimulation of the amygdala in subjects with PTSD. *Brain Stimul* **13**, 424-426 (2020).
137. Langevin, J.P., *et al.* Neuromodulation for Treatment-Refractory PTSD. *Fed Pract* **34**, 20S-33S (2017).
138. Milad, M.R., Vidal-Gonzalez, I. & Quirk, G.J. Electrical stimulation of medial prefrontal cortex reduces conditioned fear in a temporally specific manner. *Behav Neurosci* **118**, 389-394 (2004).
139. Vidal-Gonzalez, I., Vidal-Gonzalez, B., Rauch, S.L. & Quirk, G.J. Microstimulation reveals opposing influences of prelimbic and infralimbic cortex on the expression of conditioned fear. *Learn Mem* **13**, 728-733 (2006).
140. Maroun, M., Kavushansky, A., Holmes, A., Wellman, C. & Motanis, H. Enhanced extinction of aversive memories by high-frequency stimulation of the rat infralimbic cortex. *PLoS One* **7**, e35853 (2012).
141. Reznikov, R., *et al.* Prefrontal Cortex Deep Brain Stimulation Improves Fear and Anxiety-Like Behavior and Reduces Basolateral Amygdala Activity in a Preclinical Model of Posttraumatic Stress Disorder. *Neuropsychopharmacology* **43**, 1099-1106 (2018).
142. Langevin, J.P., De Salles, A.A., Kosoyan, H.P. & Krahl, S.E. Deep brain stimulation of the amygdala alleviates post-traumatic stress disorder symptoms in a rat model. *J Psychiatr Res* **44**, 1241-1245 (2010).
143. Stidd, D.A., Vogelsang, K., Krahl, S.E., Langevin, J.P. & Fellous, J.M. Amygdala deep brain stimulation is superior to paroxetine treatment in a rat model of posttraumatic stress disorder. *Brain Stimul* **6**, 837-844 (2013).
144. Ehlers, A. & Clark, D.M. A cognitive model of posttraumatic stress disorder. *Behav Res Ther* **38**, 319-345 (2000).
145. Oulton, J.M., Strange, D., Nixon, R.D.V. & Takarangi, M.K.T. Imagining trauma: Memory amplification and the role of elaborative cognitions. *J Behav Ther Exp Psychiatry* **60**, 78-86 (2018).
146. Maccani, M.A., Delahanty, D.L., Nugent, N.R. & Berkowitz, S.J. Pharmacological secondary prevention of PTSD in youth: challenges and opportunities for advancement. *J Trauma Stress* **25**, 543-550 (2012).
147. Roque, A.P. Pharmacotherapy as prophylactic treatment of post-traumatic stress disorder: a review of the literature. *Issues Ment Health Nurs* **36**, 740-751 (2015).
148. Nader, K., Schafe, G.E. & Le Doux, J.E. Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. *Nature* **406**, 722-726 (2000).
149. Debiec, J. & LeDoux, J.E. Noradrenergic signaling in the amygdala contributes to the reconsolidation of fear memory: treatment implications for PTSD. *Ann N Y Acad Sci* **1071**, 521-524 (2006).
150. Blundell, J., Kouser, M. & Powell, C.M. Systemic inhibition of mammalian target of rapamycin inhibits fear memory reconsolidation. *Neurobiol Learn Mem* **90**, 28-35 (2008).
151. Monfils, M.H., Cowansage, K.K., Klann, E. & LeDoux, J.E. Extinction-reconsolidation boundaries: key to persistent attenuation of fear memories. *Science* **324**, 951-955 (2009).

152. Brunet, A., *et al.* Reduction of PTSD Symptoms With Pre-Reactivation Propranolol Therapy: A Randomized Controlled Trial. *Am J Psychiatry* **175**, 427-433 (2018).
153. Haaker, J., *et al.* Single dose of L-dopa makes extinction memories context-independent and prevents the return of fear. *Proc Natl Acad Sci U S A* **110**, E2428-2436 (2013).
154. Cain, C.K., Blouin, A.M. & Barad, M. Adrenergic transmission facilitates extinction of conditional fear in mice. *Learn Mem* **11**, 179-187 (2004).
155. Smits, J.A., *et al.* Yohimbine enhancement of exposure therapy for social anxiety disorder: a randomized controlled trial. *Biol Psychiatry* **75**, 840-846 (2014).
156. Ledgerwood, L., Richardson, R. & Cranney, J. D-cycloserine facilitates extinction of learned fear: effects on reacquisition and generalized extinction. *Biol Psychiatry* **57**, 841-847 (2005).
157. Difede, J., *et al*. D-cycloserine augmentation of exposure therapy for post-traumatic stress disorder: a pilot randomized clinical trial. *Neuropsychopharmacology* **39**, 1052-1058 (2014).
158. Ishikawa, R., *et al.* Time-dependent enhancement of hippocampus-dependent memory after treatment with memantine: Implications for enhanced hippocampal adult neurogenesis. *Hippocampus* **24**, 784-793 (2014).
159. Ishikawa, R., Fukushima, H., Frankland, P.W. & Kida, S. Hippocampal neurogenesis enhancers promote forgetting of remote fear memory after hippocampal reactivation by retrieval. *Elife* **5**(2016).
160. Hori, H., *et al.* The efficacy of memantine in the treatment of civilian posttraumatic stress disorder: an open-label trial. *Eur J Psychotraumatol* **12**, 1859821 (2021).
161. Cloos, J.M. & Ferreira, V. Current use of benzodiazepines in anxiety disorders. *Curr Opin Psychiatry* **22**, 90-95 (2009).
162. Kozak, G., Foldi, T. & Berenyi, A. Chronic Transcranial Electrical Stimulation and Intracortical Recording in Rats. *J Vis Exp* (2018).
163. Vandecasteele, M., *et al.* Large-scale recording of neurons by movable silicon probes in behaving rodents. *J Vis Exp*, e3568 (2012).
164. Berenyi, A., *et al*. Large-scale, high-density (up to 512 channels) recording of local circuits in behaving animals. *J Neurophysiol* **111**, 1132-1149 (2014).
165. Shumake, J., Jones, C., Auchter, A. & Monfils, M.H. Data-driven criteria to assess fear remission and phenotypic variability of extinction in rats. *Philos Trans R Soc Lond B Biol Sci* **373**(2018).
166. Monfils, M.H., *et al*. Predicting extinction phenotype to optimize fear reduction. *Psychopharmacology (Berl)* **236**, 99-110 (2019).
167. Hare, B.D., *et al.* Optogenetic stimulation of medial prefrontal cortex Drd1 neurons produces rapid and long-lasting antidepressant effects. *Nat Commun* **10**, 223 (2019).
168. Keller, N.E., Hennings, A.C. & Dunsmoor, J.E. Behavioral and neural processes in counterconditioning: Past and future directions. *Behav Res Ther* **125**, 103532 (2020).
169. Pinard, C.R., Mascagni, F. & McDonald, A.J. Medial prefrontal cortical innervation of the intercalated nuclear region of the amygdala. *Neuroscience* **205**, 112-124 (2012).
170. Cheriyan, J., Kaushik, M.K., Ferreira, A.N. & Sheets, P.L. Specific Targeting of the Basolateral Amygdala to Projectionally Defined Pyramidal Neurons in Prelimbic and Infralimbic Cortex. *eNeuro* **3**(2016).

171. Berretta, S., Pantazopoulos, H., Caldera, M., Pantazopoulos, P. & Pare, D. Infralimbic cortex activation increases c-Fos expression in intercalated neurons of the amygdala. *Neuroscience* **132**, 943-953 (2005).
172. Bloodgood, D.W., Sugam, J.A., Holmes, A. & Kash, T.L. Fear extinction requires infralimbic cortex projections to the basolateral amygdala. *Transl Psychiatry* **8**, 60 (2018).
173. Beyeler, A., *et al.* Divergent Routing of Positive and Negative Information from the Amygdala during Memory Retrieval. *Neuron* **90**, 348-361 (2016).
174. Lee, J.L.C., Nader, K. & Schiller, D. An Update on Memory Reconsolidation Updating. *Trends Cogn Sci* **21**, 531-545 (2017).
175. Russo, S.J. & Nestler, E.J. The brain reward circuitry in mood disorders. *Nat Rev Neurosci* **14**, 609-625 (2013).
176. Tang, W., Kochubey, O., Kintscher, M. & Schneggenburger, R. A VTA to Basal Amygdala Dopamine Projection Contributes to Signal Salient Somatosensory Events during Fear Learning. *J Neurosci* **40**, 3969-3980 (2020).
177. Fenoy, A.J., *et al.* A longitudinal study on deep brain stimulation of the medial forebrain bundle for treatment-resistant depression. *Transl Psychiatry* **8**, 111 (2018).
178. Girardeau, G., Benchenane, K., Wiener, S.I., Buzsaki, G. & Zugaro, M.B. Selective suppression of hippocampal ripples impairs spatial memory. *Nat Neurosci* **12**, 1222-1223 (2009).
179. Girardeau, G. & Zugaro, M. Hippocampal ripples and memory consolidation. *Curr Opin Neurobiol* **21**, 452-459 (2011).
180. Dahal, P., Rauhala, O.J., Khodagholy, D. & Gelinas, J.N. Hippocampal-cortical coupling differentiates long-term memory processes. *Proc Natl Acad Sci U S A* **120**, e2207909120 (2023).
181. Zugaro, M.B., Monconduit, L. & Buzsaki, G. Spike phase precession persists after transient intrahippocampal perturbation. *Nat Neurosci* **8**, 67-71 (2005).
182. Kida, S. Reconsolidation/destabilization, extinction and forgetting of fear memory as therapeutic targets for PTSD. *Psychopharmacology (Berl)* **236**, 49-57 (2019).
183. Lee, J.L., Everitt, B.J. & Thomas, K.L. Independent cellular processes for hippocampal memory consolidation and reconsolidation. *Science* **304**, 839-843 (2004).
184. Silva, A.J., Kogan, J.H., Frankland, P.W. & Kida, S. CREB and memory. *Annu Rev Neurosci* **21**, 127-148 (1998).
185. Nader, K., Schafe, G.E. & LeDoux, J.E. The labile nature of consolidation theory. *Nat Rev Neurosci* **1**, 216-219 (2000).
186. Nader, K. Reconsolidation and the Dynamic Nature of Memory. *Cold Spring Harb Perspect Biol* **7**, a021782 (2015).
187. Frankland, P.W. & Bontempi, B. The organization of recent and remote memories. *Nat Rev Neurosci* **6**, 119-130 (2005).
188. Nader, K., Hardt, O. & Lanius, R. Memory as a new therapeutic target. *Dialogues Clin Neurosci* **15**, 475-486 (2013).
189. Post, R.M. & Kegan, R. Prevention of recurrent affective episodes using extinction training in the reconsolidation window: A testable psychotherapeutic strategy. *Psychiatry Res* **249**, 327-336 (2017).
190. Pedraza, L.K., Sierra, R.O., Lotz, F.N. & de Oliveira Alvares, L. Periodical reactivation under the effect of caffeine attenuates fear memory expression in rats. *Sci Rep* **8**, 7260 (2018).

191. Frawley, P.J. & Smith, J.W. Chemical aversion therapy in the treatment of cocaine dependence as part of a multimodal treatment program: treatment outcome. *J Subst Abuse Treat* **7**, 21-29 (1990).
192. Davison, G.C. Systematic desensitization as a counter-conditioning process. *J Abnorm Psychol* **73**, 91-99 (1968).
193. Astill Wright, L., Horstmann, L., Holmes, E.A. & Bisson, J.I. Consolidation/reconsolidation therapies for the prevention and treatment of PTSD and re-experiencing: a systematic review and meta-analysis. *Transl Psychiatry* **11**, 453 (2021).
194. Shimizu, T., Minami, C. & Mitani, A. Effect of electrical stimulation of the infralimbic and prelimbic cortices on anxiolytic-like behavior of rats during the elevated plus-maze test, with particular reference to multiunit recording of the behavior-associated neural activity. *Behav Brain Res* **353**, 168-175 (2018).
195. Eichenbaum, H. The role of the hippocampus in navigation is memory. *J Neurophysiol* **117**, 1785-1796 (2017).
196. Sawangjit, A., *et al.* The hippocampus is crucial for forming non-hippocampal long-term memory during sleep. *Nature* **564**, 109-113 (2018).
197. Oliva, A., Fernandez-Ruiz, A., Leroy, F. & Siegelbaum, S.A. Hippocampal CA2 sharp-wave ripples reactivate and promote social memory. *Nature* **587**, 264-269 (2020).
198. Tanaka, K.Z., *et al.* The hippocampal engram maps experience but not place. *Science* **361**, 392-397 (2018).
199. Kragel, P.A. & LaBar, K.S. Decoding the Nature of Emotion in the Brain. *Trends Cogn Sci* **20**, 444-455 (2016).
200. Zelikowsky, M., *et al.* Prefrontal microcircuit underlies contextual learning after hippocampal loss. *Proc Natl Acad Sci U S A* **110**, 9938-9943 (2013).
201. Vanvossen, A.C., *et al.* Newly acquired and reactivated contextual fear memories are more intense and prone to generalize after activation of prelimbic cortex NMDA receptors. *Neurobiol Learn Mem* **137**, 154-162 (2017).
202. Concina, G., Cambiaghi, M., Renna, A. & Sacchetti, B. Coherent Activity between the Prelimbic and Auditory Cortex in the Slow-Gamma Band Underlies Fear Discrimination. *J Neurosci* **38**, 8313-8328 (2018).
203. Protopopescu, X., *et al.* Differential time courses and specificity of amygdala activity in posttraumatic stress disorder subjects and normal control subjects. *Biol Psychiatry* **57**, 464-473 (2005).
204. Sato, W., *et al.* Rapid amygdala gamma oscillations in response to fearful facial expressions. *Neuropsychologia* **49**, 612-617 (2011).
205. Koenigs, M., *et al.* Focal brain damage protects against post-traumatic stress disorder in combat veterans. *Nat Neurosci* **11**, 232-237 (2008).
206. Langevin, J.P., *et al.* Deep Brain Stimulation of the Basolateral Amygdala for Treatment-Refractory Posttraumatic Stress Disorder. *Biol Psychiatry* **79**, e82-e84 (2016).
207. Gill, J.L., *et al.* A pilot study of closed-loop neuromodulation for treatment-resistant post-traumatic stress disorder. *Nat Commun* **14**, 2997 (2023).
208. Breit, S., Schulz, J.B. & Benabid, A.L. Deep brain stimulation. *Cell Tissue Res* **318**, 275-288 (2004).
209. Grill, W.M., Snyder, A.N. & Miocinovic, S. Deep brain stimulation creates an informational lesion of the stimulated nucleus. *Neuroreport* **15**, 1137-1140 (2004).

210. Davis, P., Zaki, Y., Maguire, J. & Reijmers, L.G. Cellular and oscillatory substrates of fear extinction learning. *Nat Neurosci* **20**, 1624-1633 (2017).
211. Stujenske, J.M., Likhtik, E., Topiwala, M.A. & Gordon, J.A. Fear and safety engage competing patterns of theta-gamma coupling in the basolateral amygdala. *Neuron* **83**, 919-933 (2014).
212. Kanta, V., Pare, D. & Headley, D.B. Closed-loop control of gamma oscillations in the amygdala demonstrates their role in spatial memory consolidation. *Nat Commun* **10**, 3970 (2019).
213. Lopresto, D., Schipper, P. & Homberg, J.R. Neural circuits and mechanisms involved in fear generalization: Implications for the pathophysiology and treatment of posttraumatic stress disorder. *Neurosci Biobehav Rev* **60**, 31-42 (2016).
214. Brawn, T.P., Nusbaum, H.C. & Margoliash, D. Sleep-dependent reconsolidation after memory destabilization in starlings. *Nat Commun* **9**, 3093 (2018).
215. Radiske, A., *et al.* Cross-Frequency Phase-Amplitude Coupling between Hippocampal Theta and Gamma Oscillations during Recall Destabilizes Memory and Renders It Susceptible to Reconsolidation Disruption. *J Neurosci* **40**, 6398-6408 (2020).
216. Fenton, G.E., Halliday, D.M., Mason, R. & Stevenson, C.W. Medial prefrontal cortex circuit function during retrieval and extinction of associative learning under anesthesia. *Neuroscience* **265**, 204-216 (2014).
217. Lesting, J., *et al.* Patterns of coupled theta activity in amygdala-hippocampal-prefrontal cortical circuits during fear extinction. *PLoS One* **6**, e21714 (2011).
218. Courtin, J., Karalis, N., Gonzalez-Campo, C., Wurtz, H. & Herry, C. Persistence of amygdala gamma oscillations during extinction learning predicts spontaneous fear recovery. *Neurobiol Learn Mem* **113**, 82-89 (2014).
219. Fenoy, A.J., *et al.* Deep brain stimulation of the medial forebrain bundle: Distinctive responses in resistant depression. *J Affect Disord* **203**, 143-151 (2016).
220. Fenoy, A.J., *et al.* Deep brain stimulation of the "medial forebrain bundle": sustained efficacy of antidepressant effect over years. *Mol Psychiatry* **27**, 2546-2553 (2022).
221. Dobrossy, M.D., Furlanetti, L.L. & Coenen, V.A. Electrical stimulation of the medial forebrain bundle in pre-clinical studies of psychiatric disorders. *Neurosci Biobehav Rev* **49**, 32-42 (2015).
222. Salinas-Hernandez, X.I., *et al.* Dopamine neurons drive fear extinction learning by signaling the omission of expected aversive outcomes. *Elife* **7**(2018).
223. Kroner, S., Rosenkranz, J.A., Grace, A.A. & Barrionuevo, G. Dopamine modulates excitability of basolateral amygdala neurons in vitro. *J Neurophysiol* **93**, 1598-1610 (2005).
224. Kim, J., Pignatelli, M., Xu, S., Itohara, S. & Tonegawa, S. Antagonistic negative and positive neurons of the basolateral amygdala. *Nat Neurosci* **19**, 1636-1646 (2016).
225. Logothetis, N.K., *et al.* Hippocampal-cortical interaction during periods of subcortical silence. *Nature* **491**, 547-553 (2012).
226. Kaplan, R., *et al.* Hippocampal Sharp-Wave Ripples Influence Selective Activation of the Default Mode Network. *Curr Biol* **26**, 686-691 (2016).
227. Girardeau, G., Inema, I. & Buzsaki, G. Reactivations of emotional memory in the hippocampus-amygdala system during sleep. *Nat Neurosci* **20**, 1634-1642 (2017).

228. Latchoumane, C.V., Ngo, H.V., Born, J. & Shin, H.S. Thalamic Spindles Promote Memory Formation during Sleep through Triple Phase-Locking of Cortical, Thalamic, and Hippocampal Rhythms. *Neuron* **95**, 424-435 e426 (2017).
229. Figee, M., *et al.* Deep brain stimulation induces striatal dopamine release in obsessive-compulsive disorder. *Biol Psychiatry* **75**, 647-652 (2014).
230. Gottfried, J.A., O'Doherty, J. & Dolan, R.J. Encoding predictive reward value in human amygdala and orbitofrontal cortex. *Science* **301**, 1104-1107 (2003).
231. Kringelbach, M.L. The human orbitofrontal cortex: linking reward to hedonic experience. *Nat Rev Neurosci* **6**, 691-702 (2005).

I.

nature communications

Article

https://doi.org/10.1038/s41467-023-39546-7

Closed-loop brain stimulation augments fear extinction in male rats

Received: 26 March 2023

Accepted: 16 June 2023

Published online: 05 July 2023

Check for updates

Rodrigo Ordoñez Sierra[1,10], Lizeth Katherine Pedraza[1,10], Lívia Barcsai[1,2,3], Andrea Pejin[1,2,3], Qun Li[1], Gábor Kozák[1], Yuichi Takeuchi[1,4], Anett J. Nagy[1,2,3], Magor L. Lőrincz[1,5,6], Orrin Devinsky[7], György Buzsáki[8,9] & Antal Berényi[1,2,3,8]

Dysregulated fear reactions can result from maladaptive processing of trauma-related memories. In post-traumatic stress disorder (PTSD) and other psychiatric disorders, dysfunctional extinction learning prevents discretization of trauma-related memory engrams and generalizes fear responses. Although PTSD may be viewed as a memory-based disorder, no approved treatments target pathological fear memory processing. Hippocampal sharp wave-ripples (SWRs) and concurrent neocortical oscillations are scaffolds to consolidate contextual memory, but their role during fear processing remains poorly understood. Here, we show that closed-loop, SWR triggered neuromodulation of the medial forebrain bundle (MFB) can enhance fear extinction consolidation in male rats. The modified fear memories became resistant to induced recall (i.e., 'renewal' and 'reinstatement') and did not reemerge spontaneously. These effects were mediated by D2 receptor signaling-induced synaptic remodeling in the basolateral amygdala. Our results demonstrate that SWR-triggered closed-loop stimulation of the MFB reward system enhances extinction of fearful memories and reducing fear expression across different contexts and preventing excessive and persistent fear responses. These findings highlight the potential of neuromodulation to augment extinction learning and provide a new avenue to develop treatments for anxiety disorders.

Learning unpleasant things and remembering them is advantageous for the organism for avoiding future reoccurrences. Memories that are irrelevant to survival or adaptation tend to fade away either by graceful degradation[1,2] or by another type of learning called active extinction[3,4]. Extinction learning, the process of reducing the expression of learned fear responses, is essential for adaptive behavior in response to traumatic experiences.

However, in some pathological scenarios, extinction learning is often impaired, leading to persistent and maladaptive fear responses[5]. For example, post-traumatic stress disorder (PTSD) is a debilitating psychiatric disorder resulting from direct or indirect exposure to stressful events, threats, or life-threatening events perceived to compromise personal physical or mental safety[6–8]. Symptoms include intense feelings of unprovoked fear, panic attacks, anxiety; intrusive

[1]MTA-SZTE 'Momentum' Oscillatory Neuronal Networks Research Group, Department of Physiology, University of Szeged, Szeged 6720, Hungary. [2]HCEMM-SZTE Magnetotherapeutics Research Group, University of Szeged, Szeged 6720, Hungary. [3]Neunos Inc, Boston, MA 02108, USA. [4]Department of Biopharmaceutical Sciences and Pharmacy, Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo, Japan. [5]Department of Physiology, Anatomy and Neuroscience, Faculty of Sciences University of Szeged, Szeged 6726, Hungary. [6]Neuroscience Division, Cardiff University, Museum Avenue, Cardiff CF10 3AX, UK. [7]Department of Neurology, NYU Langone Comprehensive Epilepsy Center, NYU Grossman School of Medicine, New York, NY 10016, USA. [8]Neuroscience Institute, New York University, New York, NY 10016, USA. [9]Center for Neural Science, New York University, New York, NY 10016, USA. [10]These authors contributed equally: Rodrigo Ordoñez Sierra, Lizeth Katherine Pedraza. e-mail: drberenyi@gmail.com

fear memories during wakefulness or in nightmares, fear generalization, and avoiding similar but neutral stimuli[9,10]. PTSD is highly resistant to psycho- and pharmacotherapy[11–13].

Previous studies have demonstrated that hippocampal sharp-wave ripples (SWRs) play a critical role in the consolidation of fear memories[14], and that closed-loop stimulation of the reward system can enhance memory consolidation[15]. Exposure-based extinction procedures have been found to reduce fear in a context-dependent manner, suggesting that the hippocampal representation of the extinction context drives fear attenuation[16]. The activity in the basolateral amygdala decreases when conditioning stimuli (CS+) are presented in the same context used for extinction but increases following non-extinction exposure to the CS+[17]. Furthermore, inactivation of the hippocampus has been found to enhance extinction to the CS+ and promote low fear expression in environments different from the extinction context[18,19].

Excitatory neurons in the basolateral amygdala have been shown to respond to both reward and punishment and have been proposed to be involved in mediating reward signaling induced by the omission of an unconditioned stimulus during extinction[20]. Additionally, these neurons participate in a mutual inhibition process[21]. Based on these findings, we hypothesize that manipulating internal reward signals during extinction learning could facilitate the extinction of memories, thereby reducing excessive fear reactions in inappropriate contexts.

Here, we explore whether SWR-triggered stimulation of the reward system through medial-forebrain bundle (MFB) can augment extinction learning. Our findings suggest that SWRs are crucial for mediating fear extinction, and that closed-loop neuromodulation targeting oscillatory activity related to memory processing could be a promising intervention for reducing excessive fear reactions in inappropriate contexts. Specifically, our experiments demonstrate that selective suppression of SWRs after extinction delayed fear attenuation, indicating that intact SWRs are necessary for extinction learning. Furthermore, our results show that SWR-triggered closed-loop stimulation of the reward system through MFB enhances the extinction of fearful memories, resulting in reduced fear expression across different contexts and preventing excessive and persistent fear responses. Overall, our study suggests that rewarding brain stimulation may be a promising approach to augment extinction learning, potentially beneficial to alleviate PTSD symptoms.

Results

SWR-driven closed-loop electrical stimulation of the medial-forebrain bundle accelerates extinction and prevents fear recovery

Rats were subjected to a single session of fear conditioning (5 pairings of conditions stimulus, CS+ and unconditioned stimulus, US, i.e., footshock using 1 mA current) to develop PTSD-like phenotypes (Supplementary Fig. 3a–e) followed by fear extinction training over multiple days (twenty re-exposures/day in four blocks to CS+ in a novel context without US) until a remission criterion (reduction of freezing behavior to <20% of the initial freezing) was reached or up to maximum seven days (Fig. 1a). During the extinction protocol, one group of rats received closed-loop stimulation of the MFB during hippocampal sharp-wave ripples (SWRs) (fourteen 1-ms long, 100 μA square-wave pulses at 140 Hz; Fig. 1b) to assign a reward signal to the replayed extinction memory, another group received jittered stimulation (open-loop), and a control group received no stimulation (Fig. 1c). The experimental protocol involved conducting stimulation and recording sessions for a duration of one hour immediately following the extinction procedure. To minimize the influence of novelty, the stimulation was carried out within the animals' home cage. Fear-related behavioral performance was tested using different tests to assess the persistence of the extinction memory as follows. Animals were exposed to CS+ in a hybrid context mixing new features with the conditioning context following extinction ('RENEWAL TEST') and by unpredictable exposure to the US ('REINSTATEMENT TEST'). The persistence of the extinction was assessed by exposing the animals to CS+25 days following extinction ('REMOTE TEST').

We found that the average online detection rate of SWRs was 80.38 ± 1.349% compared to the post hoc detection rate. False positive detection rate was 7.750 ± 1.830%, while the rate of missed detections was 11.88 ± 7.67% (Fig. 1d), which further confirms the high accuracy of our detection method. The minimum delay for triggering the stimulation after SWR detection was 15 ms, while the maximum was 27 ms. Notably, the majority of SWR events were detected between 18 and 21 ms before the onset of stimulation (Fig. 1e; Supplementary Fig. 1), highlighting the precise timing of our closed-loop stimulation approach.

Our results demonstrated that the global architecture of sleep and the distinct sleep stages were not affected by the closed-loop neuromodulation of MFB (Fig. 1f; Supplementary Fig. 2), suggesting the observed effects on fear memories were specific to the closed-loop stimulation and not a result of changes in sleep patterns. The rewarding properties of the MFB stimulation were verified using a conditioned place preference task (Supplementary Fig. 3f). No significant differences were found in the fear expression between groups in the test after conditioning to CS+ (Fig. 1g), contextual fear conditioning (Supplementary Fig. 5) or after the first or the last extinction days (Fig. 1h). Supplementary Data 1 shows the results of descriptive and comparative statistics.

While our findings demonstrated that extinction can lead to the overcoming of fear (as evidenced by individual extinction rates shown in Supplementary Fig. 6), animals that were exposed to closed-loop stimulation required fewer extinction sessions to achieve the remission criterion of <20% initial freezing compared to the open-loop and non-stimulated groups (Fig. 1i) suggesting that closed-loop neuromodulation of MFB can enhance the effectiveness of fear extinction.

Following the exposure to the 'renewal test' in a hybrid context there was a significant decrease in fear expression in the closed-loop treated animals compared to the open-loop and non-stimulated groups (Fig. 1j). These results indicate that closed-loop MFB stimulation during SWRs can enhance fear extinction, decrease the time needed to achieve fear attenuation and maintain freezing levels low in challenging situations such as exposure to hybrid contexts resembling the learning contingencies.

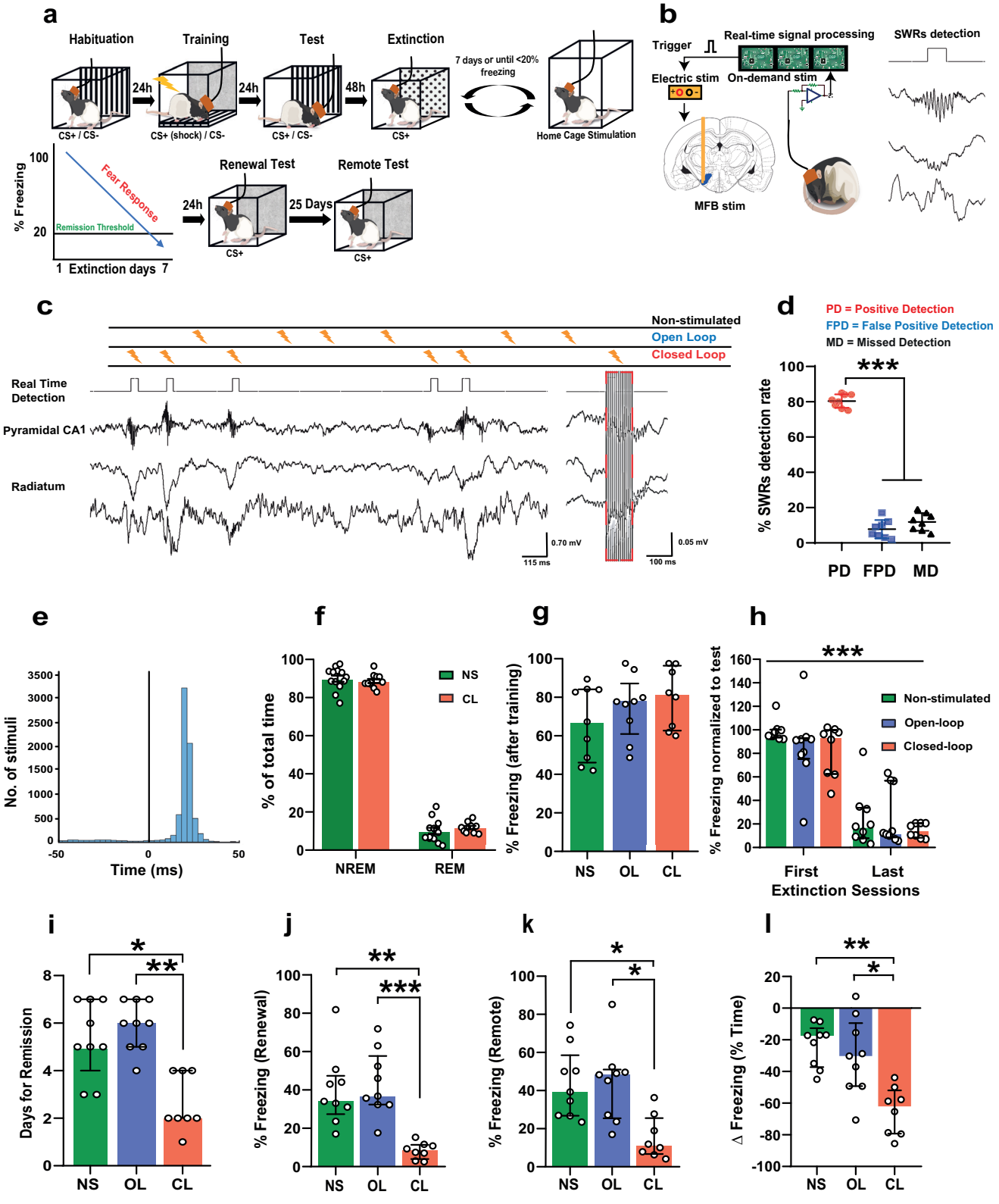
To assess the persistence of the effects, animals were exposed to a 'remote test' 25 days following the renewal in the hybrid context. Animals were kept in their home cages between the renewal and remote tests. Freezing in closed-loop stimulated animals remained at low levels compared to the open-loop and non-stimulated group (Fig. 1k), suggesting fear attenuation induced by closed-loop MFB stimulation was resistant to spontaneous recovery and persisted over time.

Finally, we quantified Δ freezing as reduced fear reactions between those after fear condition and the remote test (Δ freezing = Freezing extinction − Freezing test CS+) to reveal the overall effect of the interventions (Supplementary Fig. 4 shows the performance of individual animals in each group). Closed-loop simulated animals had stronger fear reduction than open-loop and non-stimulated animals (Fig. 1l). Together, closed-loop neuromodulation of the reward system triggered by memory consolidation-related neuronal oscillations accelerates fear extinction and promotes persistent low fear expression.

Exploring the contribution of extinction learning and potential side effects during closed-loop MFB stimulation

We investigated whether closed-loop MFB stimulation without any extinction training could reduce fear, as MFB stimulation is known to be rewarding. To test this, after fear conditioning with 5 pairings of CS

https://doi.org/10.1038/s41467-023-39546-7



+US (1 mA), animals received SWR-triggered closed-loop stimulation during sleep for three consecutive days but were not exposed to the extinction paradigm (Fig. 2a).

To match the mean number of extinction sessions required for closed-loop animals to achieve the remission criterion (Fig. 1i), the number of stimulation sessions was set to 3 days, and the stimulation durations were kept the same as in the previous experiment with extinction. The non-stimulated (NS) control group underwent identical fear conditioning and spent three days in their home cage without any intervention. No significant differences were observed between the two groups immediately after CS+ conditioning (Fig. 2b) or after three days of stimulation sessions (Fig. 2c). Thus, the closed-loop SWR-triggered stimulation alone, without extinction, did not lead to a decrease in fear expression.

Fig. 1 | Closed-loop SWR-timed medial-forebrain bundle electrical stimulation attenuates fear memories. a Schematics of the experimental design. **b** A custom threshold crossing algorithm was used to trigger the MFB stimulation following online detections of SWRs. **c** Closed- loop stimulation consisted of MFB stimulation during the detected SWR events, open-loop stimulation was similar to closed-loop but stimulation was jittered from SWRs (top). Representative LFP signals from dorsal hippocampus showing SWR events and stimulation pattern (right). **d** Average online detection rate of SWR events (PD Positive detection; FPD False positive detection, MD Missed detection; $n = 8$) (one-way ANOVA: $F(2,21) = 602.1$, $P < 0.0001$). **e** Delay of stimulation triggering from the beginning of the SWRs. The largest number of stimuli (blue peak) were delivered between 18 and 21 ms after the SWR onset (black line: time zero). **f** No difference in sleep architecture between closed-loop (CL) and non-stimulated (NS) animals during non-REM (NREM) sleep (Unpaired t test: $t(22) = 0.5977$, $P = 0.5561$, two-tailed) and REM sleep (Unpaired t test: $t(22) = 0.9459$, $P = 0.3545$, two-tailed). Data represent mean ± SEM (Number of sessions: (NS) $n = 13$; (CL) $n = 11$). **g** No difference in fear expression in response to the CS+ following training between the three experimental groups (Kruskal–Wallis test: $H = 1.737$, $P = 0.4195$) (non-stimulated (NS) $n = 9$; open-loop (OL) $n = 9$; closed-loop (CL) $n = 8$). **h** No difference between the fear expression of the three groups during the first 5 CS+ block after first and last extinction days. There was a significant decrease in fear expression over time (mixed ANOVA: $F(1,23) = 164.2$, $P < 0.0001$, time factor). Values are normalized to the freezing expressed immediately after footshock training (i.e., "Test"). **i** Animals exposed to closed-loop stimulation required less extinction sessions to achieve the remission criterion compared to the open-loop and non-stimulated groups (Kruskal–Wallis test: $H = 13.60$, $P = 0.0011$). **j** Closed-loop neuromodulation-induced lower fear expression during the renewal test in a hybrid context (Kruskal–Wallis test: $H = 16.21$, $P = 0.0003$). **k** Closed-loop neuromodulation prevented spontaneous fear recovery 25 days after extinction (Kruskal–Wallis test: $H = 10.38$, $P = 0.0056$). **l** Closed-loop neuromodulation produces the greatest reduction in fear between post-training and remote testing following extinction (Kruskal–Wallis test: $H = 13.06$, $P = 0.0015$). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Bar plots and error bars represent medians and interquartile ranges, individual data points are also displayed. Detailed statistics are shown in Supplementary Data 1. Source data provided as a Source Data file. Silhouettes on **a**, **b** are obtained from https://github.com/eackermann/ratpack under MIT License.

We next tested if the SWR-triggered closed-loop stimulation interferes with already consolidated non-fear-related memories as a non-specific detrimental effect. For this purpose, the animals were trained in a spatial memory task, in which a randomly alternated visual cue indicated the correct choice in a T-maze to receive a reward (frootloops pellet). They underwent a total of 20 trials per day until achieving 80% of correct choice. After completing the spatial memory task, the animals underwent fear conditioning, extinction, and stimulation sessions in the same way as in the previous experiment until achieving remission (Fig. 2d). During the extinction procedure, the animals were also retested in the same spatial memory task each day, with a five-hour gap between the extinction + stimulation sessions and T-maze task. The order of the behavioral tasks was randomized across the experiment. Both OL and CL stimulated animals maintained performance in the T-maze task (Fig. 2e). The individual performance of animals during the fear conditioning and extinction procedure is shown in Fig. 2f, g. Moreover, the extinction enhancement induced by CL neuromodulation was preserved (Supplementary Fig. 8). These results suggest that closed-loop stimulation alone is not sufficient to reduce fear expression and must be coupled with extinction learning. Moreover, already consolidated spatial memories are not affected by the stimulation.

SWRs are required to consolidate fear extinction

We postulated that fear extinction, being context-dependent[16], required SWRs as they play a crucial role in contextual memory consolidation through cortico-hippocampal circuits[22,23]. To test this, we suppressed SWRs by ventral hippocampal commissural electrical stimulation that induces phasic silencing of hippocampal pyramidal cells and interneurons[24–26]. Since animals trained with high-intensity footshocks tend to resist extinction, we reduced the training intensity (5 pairings CS+US at 0.7 mA) to ensure that the extinction criterion was achieved within seven sessions in control conditions. During stimulation following each extinction, online detected SWRs triggered a single-pulse (0.5 ms) ventral hippocampal commissural stimulation (Fig. 3a, b), with the stimulation intensity tailored to each animal's requirements to disrupt the SWRs (range: 5–15 V). Open-loop animals were randomly stimulated within the same voltage range.

The test results showed no significant differences in fear expression between groups after conditioning to CS+ (Fig. 3c) or during the first and last extinction day's initial 5 CS+ blocks, after conditioning to CS+ (Fig. 3d). However, animals that experienced SWR disruption required more extinction sessions to achieve an 80% reduction in freezing compared to those in the open-loop group (Fig. 3e). Additionally, these SWR-disrupted animals expressed elevated levels of freezing in the hybrid context during the renewal test compared to the non-stimulated and open-loop groups (Fig. 3f). No differences were detected during the reinstatement test (Fig. 3g). These results suggest that hippocampal SWRs are essential for consolidating fear extinction. The disruption of SWRs results in slow extinction learning and fear persistence in different environments beyond the extinction context.

The enhancement of extinction induced by closed-loop stimulation is mediated by D2 receptor and G protein Rac1 in BLA

We next explored the plasticity-dependent mechanisms that contribute to enhanced fear extinction induced by closed-loop MFB stimulation. We tested the potential involvement of BLA dopamine receptors and the small G protein Rac1, a Rho family member involved in learning-induced synapse formation[27–30]. After fear conditioning (5 pairings CS+US(1 mA)), animals received bilateral microinfusions of the Rac1 inhibitor NSC2376, D1R antagonist SCH23390, or D2R antagonist sulpiride immediately after each extinction session and before the closed-loop stimulation (Fig. 4a, b). The test results showed no significant differences after conditioning to CS+ (Fig. 4c), or in fear expression during the first 5 CS+ blocks from first and last extinction day (Fig. 4d).

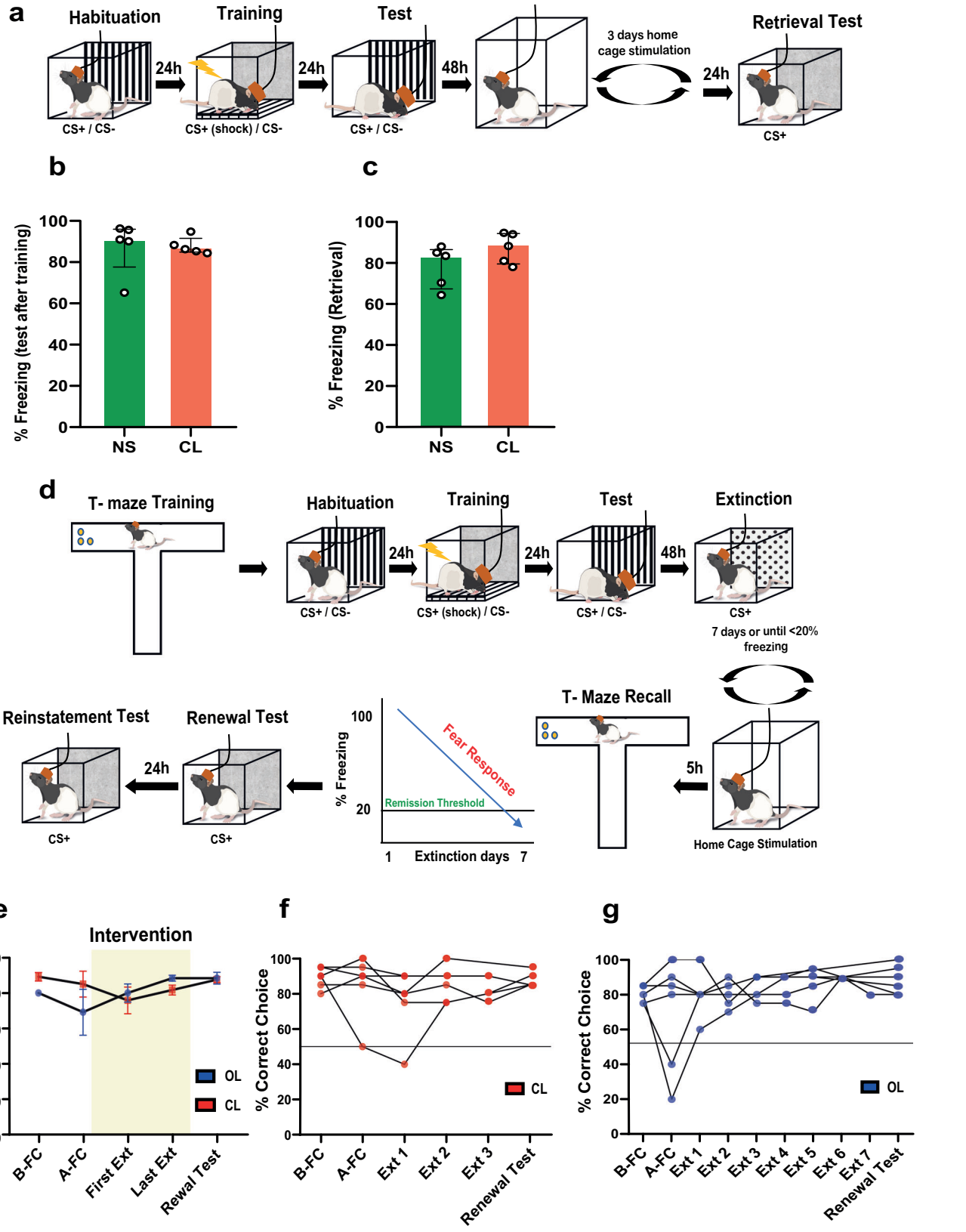
Animals co-infused with NSC2376 and sulpiride required more days to achieve extinction than controls, closed-loop stimulated animals and closed-loop stimulated animals infused with SCH23390 (Fig. 4e). During the renewal test in the hybrid context, only sulpiride suppressed the effect of closed-loop stimulation (Fig. 4f).

Similar to the renewal test, animals infused with sulpiride exhibited a significant fear recovery after exposure to an immediate footshock protocol (Fig. 4g). The pharmacological treatments did not alter the extinction criterion without electrical stimulation. However, NSC2376 appeared to disrupt fear attenuation during renewal, suggesting that RAC1 itself participates in extinction consolidation (Supplementary Fig. 9). Thus, NSC2376 and sulpiride prevented the enhancement of extinction induced by the closed-loop neuromodulation. These findings suggest that closed-loop neuromodulation-induced fear extinction involves dendritic spine plasticity mediated by RAC1 signaling and D2Rs in the BLA.

Discussion

Our study found that closed-loop stimulation of the MFB during SWRs was effective in enhancing the extinction of cued fear conditioning (Supplementary Fig. 10). We observed that stimulation without extinction learning or SWR-independent stimulation was ineffective. Our intervention resulted in a shortened time to reduce fear expression, and the effect persisted even 25 days after treatment, as animals were resistant to induced renewal, reinstatement, and spontaneous reemergence of fear expression.

https://doi.org/10.1038/s41467-023-39546-7



Our findings suggest that SWRs are essential for extinction learning, as disruption of SWRs increases the number of extinction sessions required for remission and predisposes animals to recurrent expression of fear (Supplementary Fig. 10). Closed-loop stimulation effects were mediated by D2 receptors and RAC1 signaling in the BLA, suggesting that closed-loop modulation of the reward pathways promotes a plasticity-dependent mechanism leading to extinction. These results offer novel avenues to develop closed-loop neuromodulation technologies for PTSD and anxiety disorders.

Conventional deep brain stimulation (DBS) introduces preset electrical stimulation in an open-loop manner, without being aligned to the internal oscillatory activity. Although DBS has been used to

Fig. 2 | Contribution of fear extinction and side effects on co-storaged memories during closed-loop MFB stimulation. a Schematics of the experimental design. Fear conditioning and test was performed as before. Closed-loop animals were exposed to 3 consecutive SWR-triggered stimulation sessions without extinction. No difference was found in fear expression in response to the CS+ following training (Mann–Whitney test: $U = 7$, $P = 0.3095$, two-tailed) **b** and renewal (Mann–Whitney test: $U = 6$, $P = 0.2222$, two-tailed) **c** between the groups (non-stimulated (NS) $n = 5$; closed-loop (CL) $n = 5$). **d** Before fear conditioning, animals were trained in a visual cue forced alternation T-maze task until achieving 80% of correct choice. Next, animals were exposed to fear conditioning, extinction and stimulation following Fig. 1. **e** T-maze performance was unaltered during the experiments regardless of the stimulation type (Unpaired t test: $P > 0.05$ on all instances, two-tailed) (open-loop (OL) $n = 6$; closed-loop (CL) $n = 6$). Individual performance of the animals is shown for open-loop **f** and closed-loop **g**. Bar plots and error bars represent medians and interquartile ranges, individual data points are also displayed. Detailed statistics are shown in Supplementary Data 1. Source data provided as a Source Data file. Silhouettes on **a** and **d** are obtained from https://github.com/eackermann/ratpack under MIT License.

control fear expression in animal models[31,32] and humans[33], open-loop approaches may be excessive and disrupt normal physiological oscillations[34]. Closed-loop stimulation may reduce frequent side effects such as strabismus during MFB-DBS stimulation reported by patients with major depression[35].

SWRs encode and consolidate spatial memory and are involved in fear memory processing. Selective pre or post-training inactivation of CA3 disrupts the acquisition and consolidation of contextual fear memory by reducing the number and dominant frequency of CA1 ripples and shifting underlying CA1 ensemble activity[36]. SWRs rely on synchronous CA1 principal neuron activation mainly controlled by PV+ interneurons[37]. Boosting the activity of hippocampal PV+ interneurons results in selective extinction of contextual fear memory and increased SWR incidence[38]. However, suppression of hippocampal PV+ interneurons alters principal neuronal phase coupling to SWRs, decreasing ripple-spindle coupling and consolidation of contextual fear memory[39,40]. Our findings indicate that SWRs are necessary for the extinction of cued fear conditioning and can update the memory trace with rewarding information. Closed-loop disruption of SWRs delayed but did not block extinction since 80% of animals still achieved the remission criterion, consistent with the contextual dependence of fear extinction[16,18,19], although cued fear conditioning is amygdala-dependent[41–43]. Our initial hypothesis that SWRs encode contextual features of 'safety' during the extinction is supported by the decreased time to achieve remission but does not explain the fear reduction to CS+.

SWRs play a critical role in establishing temporally precise 'windows' for integrating information across neocortical and subcortical structures. A widespread increase in neocortical activity precedes SWRs[44] indicating that during SWRs, replay, and information integration involve the contextual features of an engram and the corresponding emotional memory traces. The multiple roles of SWRs and hippocampal place cells in processing contingencies beyond spatial localization support this idea[45,46]. Thus, the SWR-triggered closed-loop MFB stimulation and the resulting reward signal coincide with the widespread ongoing brain network activity orchestrating the consolidation of fear extinction[47] during SWR events. Neuronal activity in the BLA increases during SWRs[48,49] and coordinated reactivation between the dorsal hippocampus and BLA during offline aversive memory processing peaks around the SWRs[50]. Therefore, the SWR-triggered closed-loop neuromodulation may provide a reward safety signal to a consolidating aversive memory[51] and/or enhance the network activity that encodes fear extinction[52]. Since SWRs are also important in encoding context, it cannot be excluded that the enhancement shown in this study might also influence spatial or contextual learning. While we demonstrated that the closed-loop SWR-triggered MFB stimulation does not interfere with already consolidated spatial memories, revealing any effects on their acquisition or extinction may require further studies.

The potential mechanism underlying the closed-loop neuromodulation of SWRs and reward signaling resembles a counter-conditioning process by memory updating with contrasting emotional valence[53–56] characterized by high temporal and neurochemical precision. This hypothesis is supported by the absence of closed-loop effect when animals are not exposed to the extinction learning. In such cases, the reward signal triggered by MFB stimulation does not coincide with extinction-contingent SWRs, which prevent the enhancement of fear attenuation.

Interestingly, when the US is unexpectedly omitted during extinction, there is an increase in the activity of dopaminergic neurons in the VTA[57]. This increase in activity has a positive correlation with extinction learning. In addition, optogenetic excitation of VTA dopaminergic neurons at the time of the US omission accelerates fear extinction[58]. These results suggest that dopaminergic activity during extinction encodes prediction error or mismatch between expectancies[59]. This system is more active during the initial phase (unexpected omission) compared to late phase (expected omission) of extinction learning[58]. Together our results from MFB closed-loop neuromodulation, combined with the assumption that US omission may be rewarding itself[60], suggest that dopaminergic signaling plays a crucial role in the consolidation of extinction during offline states, particularly during SWRs.

The idea that dopaminergic signaling is essential for extinction consolidation is supported by the fact that MFB fibers, which connect nodes involved in reward and emotional processing, play a critical role in this process. The VTA sends dopaminergic axons to the NAc, amygdala and PFC via the MFB[61]. A cluster of dopaminergic neurons in the anterior VTA/SNc directly connect with CA1[62]. A global manipulation of the reward system through MFB deep brain stimulation can ameliorate depression-like behaviors in animal models and depression symptoms in human patients[63]. We found that temporally precise electrical stimulation in these circuits during SWRs may scaffold the extinction enhancement. We argue for a dopamine-dependent mechanism, since previous studies have shown that MFB stimulation leads to an increase in dopamine release in BLA[64–66] and the effects of closed-loop neuromodulation were prevented by a selective local antagonism of D2 but not D1 receptors. Moreover, our stimulation protocol was able to induce conditioned place preference.

Multiple lines of evidence supports that fear conditioning induces long-term potentiation of amygdala principal neurons[67] and fear extinction can revert the enhanced activity of these neurons and decrease AMPAR expression induced by fear conditioning[68]. Dopamine enhances the excitability of BLA projection neurons, and D1 and D2 receptor activation increase excitability and input resistance, respectively[69].

Indeed, dopamine release in the BLA during fear learning is controlling the saliency of the footshock and the extinction through prediction error signaling of non-reinforced CS+ presentation[70]. Fear memories and extinction are encoded by different BLA neuronal populations. Rather than overwriting the original fear learning engrams, extinction engrams can suppress the activity of neurons that were initially engaged in fear learning. Furthermore, since neurons that mediate extinction learning also overlap with those involved in reward processing, the activation of these neurons could also signal reward[20].

Our experimental design cannot differentiate whether post-extinction SWRs are related to the reactivation of the original fear memory or represent the consolidation of the extinction. However, increased dopamine release during SWRs could change the emotional valence of an engram replay or directly suppress neurons engaged in

https://doi.org/10.1038/s41467-023-39546-7

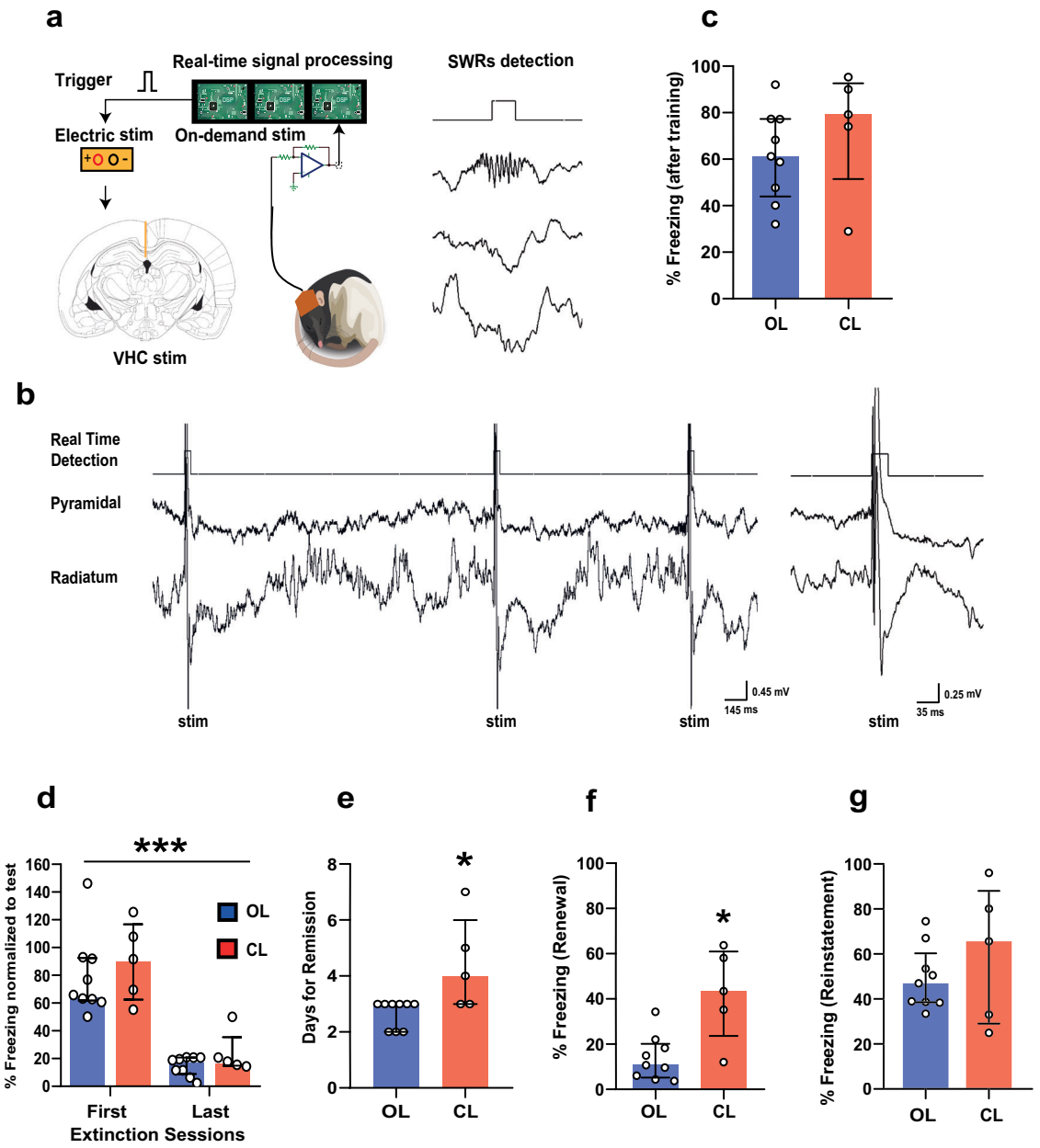
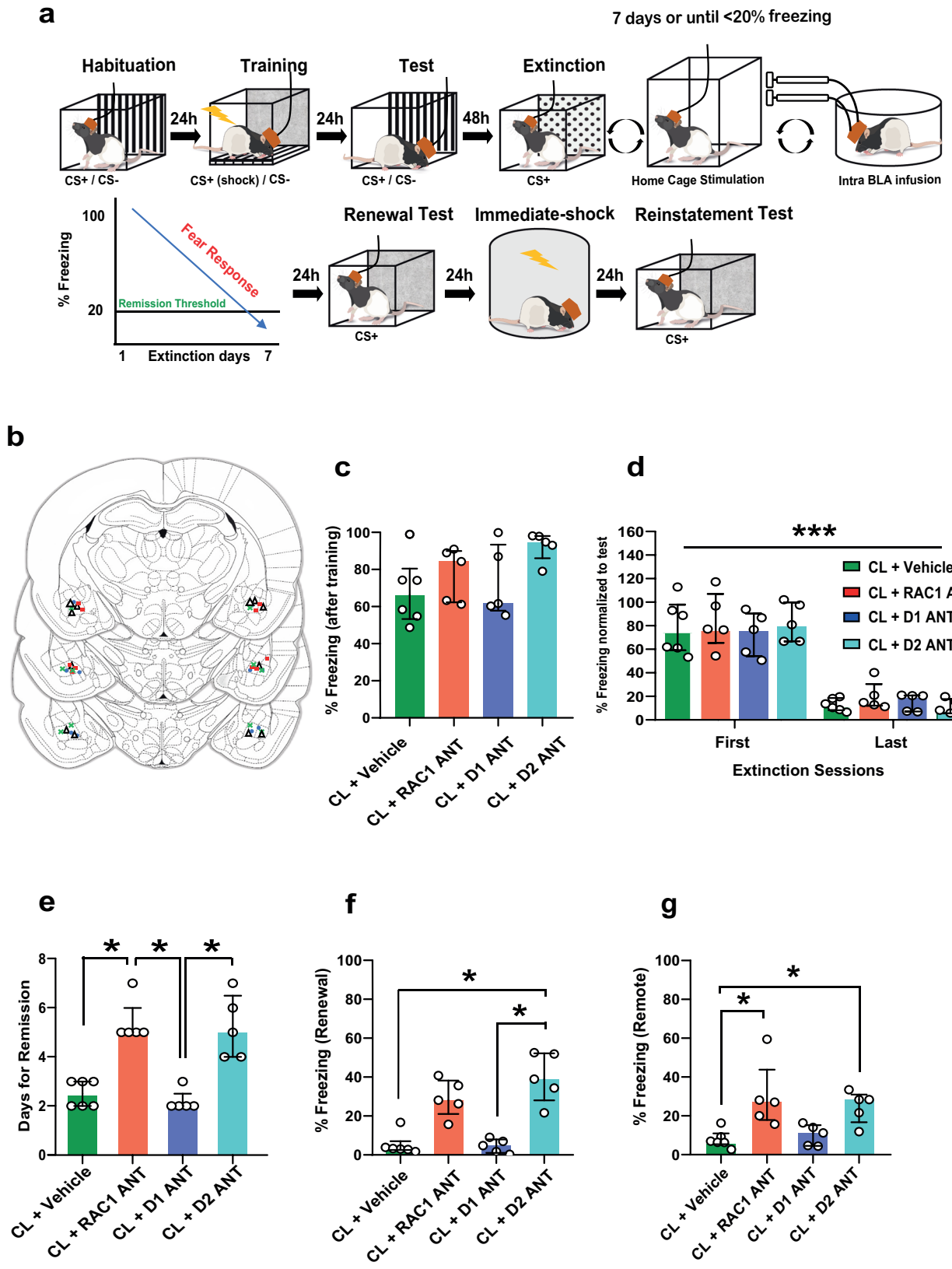


Fig. 3 | SWRs are required for the extinction of fear memories. a The behavioral protocol was performed as before, but SWR-triggered VHC stimulation was performed for 1 h following each extinction session. **b** Representative LFP signals from dorsal hippocampus showing intact and disrupted SWR events. **c** No difference in fear expression in response to the CS+ following training between the two experimental groups (Mann–Whitney test: $U = 14$, $P = 0.2977$, two-tailed; open-loop (OL) $n = 9$; closed-loop (CL) $n = 5$). **d** No difference between the fear expression of the two experimental groups during the first 5 CS+ block from first and last extinction day. However, there was a significant decrease in fear expression over time (mixed ANOVA: F (1,12) = 65.00, $P < 0.0001$, time factor) **e** SWR-disrupted animals require more days to achieve the extinction criterion (Mann–Whitney test: $U = 6$, $P = 0.0280$, two-tailed). **f** SWR-disrupted animals show high fear expression during renewal (Mann–Whitney test: $U = 4$, $P = 0.0120$, two-tailed). **g** No difference in fear expression during reinstatement (Mann–Whitney test: $U = 20$, $P = 0.7972$, two-tailed). $^{*}P < 0.05$, $^{***}P < 0.001$. The bar plots and error bars represent medians and interquartile ranges, and individual data points are also displayed. Detailed statistics are shown in Supplementary Data 1. Source data provided as a Source Data file. Silhouettes on **a** are obtained from https://github.com/eackermann/ratpack under MIT License.

fear learning. Reward-responsive VTA neuronal activity is coupled to SWRs during quiet wakefulness[71], supporting the idea that dopamine release is modulated by SWRs. Dopaminergic projections from VTA innervate PV+ interneurons expressing D2 receptors, contributing to the suppression of BLA principal neurons[72]. The suppression of feed-forward inhibition can induce LTP at excitatory afferent synapses in the BLA, an effect also mediated by D2 receptors[73]. Although the initial fear generalization phenotype was not evaluated after our closed-loop intervention, there is evidence that cue fear generalization is promoted by high-intensity training[74] and is a limiting factor for extinction[75]. Generalization may be mediated by the temporal proximity between CS+ and CS-, linking memory traces by neuronal co-allocation to overlapping engrams[76]. Given this scenario, it is expected that closed-loop MFB stimulation would impact not only the CS+ trace but also the overlapping CS-trace. This hypothesis should be addressed in future studies.

Dopamine stimulation of engram cells may enhance forgetting by activating Rac1/Cofilin, which modulates actin cytoskeleton and

https://doi.org/10.1038/s41467-023-39546-7



cellular morphology[2]. Inhibition of Rac1 activity in the dHPC impairs extinction of contextual fear memories[77] and photoactivation of Rac1 in the motor cortex suppresses motor learning[29].

Our findings suggest three sequential mechanisms underpinning closed-loop extinction enhancement: (1) SWRs reactivate the memory trace in BLA. (2) Closed-loop MFB stimulation promotes concurrent dopamine release in BLA. (3) BLA dopamine release can induce D2 receptor-mediated plasticity processes culminating in Rac1 activation. Blocking Rac1 signaling prevents spontaneous or closed-loop neuromodulation-induced fear reduction during renewal. However, Rac1 inhibition without closed-loop neuromodulation did not extend the number of sessions required for successful fear extinction using the

Fig. 4 | The closed-loop neuromodulation-induced enhancement of extinction is mediated by Rac1 and D2Rs in the BLA. a The behavioral protocol and closed-loop neuromodulation were performed as before and immediately after each extinction session, the BLA was bilaterally microinfused with the Rac1 inhibitor NSC2376, D1R antagonist SCH23390, or D2R antagonist sulpiride. **b** The locations of the cannula tips in each animal are shown, with colors representing the different experimental groups. **c** No significant difference in fear expression was observed in response to the CS+ following training between the four experimental groups (Kruskal–Wallis test: $H = 5.430$, $P = 0.1429$) (closed loop (CL + Vehicle) $n = 6$; closed-loop + NSC2376 (CL + RAC1 ANT) $n = 5$; closed-loop + SCH23390 (CL + D1 ANT) $n = 5$; closed-loop + sulpiride (CL + D2 ANT) $n = 5$). **d** No difference between the fear expression of the four experimental groups during the first 5 CS+ block from first and last extinction day. However, there was a significant decrease in fear expression over time (mixed ANOVA: F (1,34) = 175.1, $P < 0.0001$, time factor). **e** NSC2376 and sulpiride injected animals required more extinction sessions to achieve the extinction criterion (Kruskal–Wallis test: $H = 16.16$, $P = 0.0011$). **f** Sulpiride suppress the extinction enhancement induced by closed-loop neuromodulation during renewal (Kruskal–Wallis test: $H = 14.84$, $P = 0.0020$). **g** Animals treated with NSC2376 and sulpiride exhibited fear recovery compared to animals injected with vehicle (Kruskal–Wallis test: $H = 12.55$, $P = 0.0057$). Bar plots and error bars represent medians and interquartile ranges, individual data points are also displayed. Detailed statistics are shown in Supplementary Data 1. Source data provided as a Source Data file. Silhouettes on **a** and **b** are obtained from https://github.com/eackermann/ratpack under MIT License.

remission criterion. This partial disruption without electrical stimulation is expected since previous studies have shown that RAC1 inhibition impairs the extinction of contextual fear memories[77] and RAC1 activation is required for plasticity-related mechanism during fear extinction[78]. Since the disruption in fear attenuation was more pronounced under closed-loop stimulation, the synaptic plasticity may differ between normal and enhanced extinction. Additional work is required to determine the mechanisms of interaction between dopamine receptors and Rac1 modulation during fear extinction.

Our results suggest a novel translational treatment of fear-related disorders. The US Food and Drug Administration (FDA) approved MFB stimulation for treatment-resistant depression in clinical trials, with promising efficacy[63,79]. It should be noted that in addition to the MFB, other components of the reward system, as the nucleus accumbens (NAc), may also be viable targets for closed-loop neuromodulation. This is supported by evidence demonstrating that NAc-DBS can elicit striatal dopamine release in humans[80]. Although in our experiments the detection of SWRs was invasive, alternatively, cortical slow-waves and spindles concurring with SWRs in animals[81,82] may be detected non-invasively to align stimulation. Thus, closed-loop stimulation triggered by cortical EEG activity could replace SWRs detection. Further, non-invasive techniques (e.g., tDCS, TMS) could stimulate reward-associated cortical areas instead of penetrating electrodes. Importantly, our experiments were performed in male animals only. Considering sex differences in the renewal and the context-dependence of extinction in rodents[83] as well as the higher risk of women to develop anxiety-related disorder compared to men[84,85], future experiments are needed to assess if the closed-loop neuromodulation approach can be extended to females.

Our framework to study and attenuate fear-related memories relies on closed-loop stimulation guided by classical biomarkers of memory consolidation. Closed-loop stimulation can reduce the side effects from chronic and excessive stimulation of DBS approaches. Temporally precise manipulation of the reward system during SWRs overcomes the resistance to extinction in an animal model with key features of PTSD. Moreover, SWRs are critical for extinction learning. Although dopaminergic agonists can enhance fear extinction[86,87], our intervention avoids the side effects of systemic treatments (e.g., psychosis, pathological gambling). Coupling between SWRs, cortical slow-waves, and spindles may offer a potential way to translate our approach towards a non-invasive therapy in the future.

Methods

Animals

Rats (120 adult male Long-Evans, 300–450 g, 3–6 months old) were kept in a 12-hour light/ dark cycle. All experiments were performed in accordance with the European Union guidelines (2003/65/CE) and the National Institutes of Health Guidelines for the Care and Use of Animals for Experimental Procedures. The experimental protocols were approved by the Ethical Committee for Animal Research at the Albert Szent-Györgyi Medical and Pharmaceutical Center of the University of Szeged (XIV/218/2016 and XIV/824/2021).

Surgery

The animals were anesthetized with 2% isoflurane and craniotomies were performed according to stereotaxic coordinates. Intracortical electrode triplets (interwire spacing, 0.2–0.4 mm)[88] targeting the anterior cingulate cortex (ACC) (AP: +1.0, ML: 0.5, DV: 1.4), bilateral BLA (AP:−2.8, ML: 4.6, DV: 8.1 mm from the dura) and the bilateral CA1 subfield of the dorsal hippocampus (AP: −3.5, −4.5, and −5.5, ML: 2.0, 3.0 and 4.0, DV: 2.9 and 3.0 all mm from Bregma). To improve DH-SWRs detection, a custom-built microdrive[89] was used in some experiments, allowing the vertical adjustment over the CA1 subfield. A custom-built bipolar electrode consisting of two insulated (except 200 μm at the tip) Tungsten wires (interwire spacing, 0.4 mm) was implanted in the left medial-forebrain bundle (AP: −2.8, ML: 2.0 mm, DV: 8.1 all mm from Bregma). LFP electrodes and the base of the microdrive were secured to the skull with dental acrylic (Unifast Trad, USA). Two stainless-steel screws above the cerebellum served as ground and reference for the recordings, respectively. A Faraday cage was built using copper mesh and dental acrylic on the skull around the implanted electrodes.

In experiments involving concomitant electrophysiological recording and local pharmacological infusion, in addition to electrodes, rats were bilaterally implanted with 25-gauge guide cannulas above the BLA (AP: −2.8, ML: 4.7, DV: 6.9 all mm from Bregma). Cannulae were fixed to the skull with dental acrylic (Unifast Trad). Caps were used to cover cannulae to avoid any accidental occlusion.

Electrophysiological recordings and stimulation

Rats were housed individually in Plexiglass home cages (42 × 38 cm, 18 cm tall). LFP recordings were performed in the home cage and the fear conditioning box (see below). Recording and stimulation sessions for closed-loop or open-loop interventions were performed during the first hour following the extinction protocol. To avoid any twisting and over-tension of the recording cables, a bore-through electrical commutator (VSR-TC-15-12; Victory-Way Electronic) was used. Food and water were available *ad libitum*. All recording sessions took place in the same room using 12/12 h light/dark cycle with light onset/offset at 7 h/ 19 h The multiplexed signals were acquired at 500 Hz per channel for closed-loop neuromodulation experiments[88]. The neuronal signals were preamplified (total gain 400×), multiplexed on head, and stored after digitalization at 20 kHz sampling rate per channel (KJE1001, Amplipex, Szeged, Hungary). During home cage stimulation, pre-amplified signals were analyzed online by a programmable digital signal processor (RX-8, Tucker-Davis Technologies, Alachua, FL, USA) using a custom-made sharp-wave ripple detection algorithm, as follows.

Two LFP signals were used for real-time SWRs detection. For ripple detection, a channel from the tripolar electrodes from CA1 pyramidal layer with the largest ripple amplitude was selected and band-pass filtered (150–250 Hz), and root-mean-square (RMS) power was calculated in real-time for ripple detection. For noise detection, manual inspection from channels of the ACC, VHC, or AMY was performed to select the signal with no ripple-like activity and lower noise

incidence to enhance signal-to-noise ratio during detection. In case of the ACC, signal was filtered between 80 and 500 Hz. SWRs were defined as events crossing the ripple thresholds in the absence of the noise signal. Amplitude threshold for ripple was adjusted for each animal before fear conditioning training. SWRs were defined as events crossing ripple thresholds in the absence of the noise signal in the neocortical site. Threshold crossings triggered a stimulation train lasting 100 ms and composed of fourteen 1-ms long, 100 μA square-wave pulses at 140 Hz) in the MFB or single pulse (5–15 V in the ventral hippocampal commissure (VHC) (STG4008; Multi Channel Systems, Reutlingen, Germany) depending on the experiment performed. MFB stimulation was performed under current mode and VHC stimulation in voltage-controlled mode. The threshold of the detection algorithm was set for each rat separately. Behavioral (i.e., rewarding) effect of MFB stimulation was confirmed with a place preference task (see below).

Electrophysiological data analysis

The offline ripples were analyzed using custom-made MATLAB (R2017b, Natick, Massachusetts, USA) routines. Raw signals were down sampled from 20 kHz to 500 Hz and bandpass filtered in the ripple band (150–250 Hz) of hippocampal channels. Normalized squared signal was calculated. Putative SWRs events were defined as those where the beginning/end cutoffs exceeded 2 SDs and the peak power 3 SDs. The detection window was set in 150 ms. SWR duration limits were set to be between 20 and 200 ms, otherwise the events were excluded to minimize artifacts. All ripple events were drawn out for manually speculations after offline detection. The closest stimulation onset from the digital channel was selected for further analysis. Then calculated the time delay between the successfully detected ripples events and the stimulation time. For the brain states classifications (SWS/REM), SleepScoreMaster toolbox from Buzcode (https://github.com/buzsakilab/buzcode) was employed combined with post manually corrections. Time-frequency spectrum was calculated in MATLAB using Multitaper Spectral Estimation from the Chronux Toolbox (http://chronux.org/). A 2 s sliding window with a 50% overlap, a time-bandwidth product of 5 and tapers of 3 were chosen.

Drugs and infusions

The Rac1 inhibitor NSC2376 (10 μg/μl), D1 dopamine receptor antagonist SCH23390 (0.50 μg/μl), and D2 dopamine receptor antagonist sulpiride (1 μg/μl) were dissolved in sterile physiological saline (0.9% NaCl). NSC2376, SCH23390, and sulpiride were infused bilaterally into the BLA using a 33 G gauge injectors connected to Hamilton syringes via 20-gauge plastic tubes. The infusion injectors tip protruding 2.0 mm below the tip of the cannula and aimed the BLA center. A total volume of 0.5 μl per side was infused by a microinfusion pump at a rate of 0.125 μl/min. Injectors were left in place for an additional minute to ensure proper drug diffusion. All drugs were infused after the extinction sessions.

Auditory fear conditioning

The experiments were carried out in a fear conditioning apparatus comprising three contextual Plexiglas boxes (42 × 38 cm, 18 cm tall) placed within a soundproof chamber. Four different contextual configurations were used (Habituation and Test Context (A): square configuration, white walls with black vertical horizontal lines, white smooth floor, washed with 70% ethanol; Training Context (B): square configuration, gray walls, metal grid on black floor, washed 30% ethanol; Extinction Context (C): rectangular configuration, white walls with black dots, white smooth floor; and Renewal and Remote/Reinstatement context (D): hybrid context comprising a square configuration, gray walls from training context, white smooth floor, washed with 70% ethanol. All sessions were controlled using a MATLAB custom script.

Habituation. On day 1, animals were exposed to the habituation session in context A. After 2 min of contextual habituation, they were exposed to 5 alternating presentations of two different tones (2.5 or 7.5 kHz, 85 dB, 30 s). Tone time intervals were randomized (30–40 s) during the session. No behavioral differences were detected under exposition to the two frequencies.

Training. On day 2, cue fear conditioning was performed in context B. After 2 min of contextual habituation, animals received 5 trials of one tone (CS+: 7.5 kHz) immediately followed by a 2 s long footshock as unconditioned stimulus (US: 1.0 mA, 0.7 mA or 0.5 mA, depending on the experiment performed). The other tone (CS−: 2.5 kHz) was presented 5 times intermittently but never followed by the US.

Test. On day 3, animals underwent fear retrieval in context A. After 2 min of contextual habituation, rats were exposed to presentations of the CS+ or CS- in two different sessions. Each session consisted of a block of five tones. The order of the CS+ and the CS− in each session was randomized. Sessions were repeated every 4–6 h.

Extinction. In context C, from day 5 until reaching the remission criterion (see below), rats received extinction training consisting of twenty CS+ presentations without the US (unreinforced tones). Tones were repeated with randomized intervals (30–40 s) during the session.

Fear remission from extinction. We used an extinction threshold criterion to assess the efficacy of fear reduction after extinction sessions similar to[90]. The block of the first five tones during each extinction session was assessed to determine fear reduction level of the given day. Considering individual differences under fear conditioning[90–92] fear reduction during extinction was expressed as a fraction of the percentage of freezing expressed during the CS+ test (Day 3) (% Freezing Reduction = Freezing extinction × 100/Freezing test CS+). Fear remission was considered achieved when animals expressed <20% of the initial freezing during the first block of the day (i.e., first 5 CS+ presentations during the extinction session). Extinction training was repeated for maximum 7 days.

Renewal and remote test. Twenty-four hours or 25 days after achieving the remission, animals were exposed to context D (Hybrid context) as a renewal or remote test, respectively. In each test, rats were exposed to a block of five CS+ presentations after 2 min of contextual habituation. Time intervals between tones were randomized (30–40 s) during the session.

Immediate footshock. To promote fear recovery, animals were placed in a neutral environment outside the conditioning box and received an unconditioned footshock after 30 s contextual exposition, with the same intensity used during fear conditioning. The animals were returned to their home cage 30 s following the footshock.

Reinstatement test. Animals were submitted to a reinstatement test in context D 24 hours after the immediate footshock. Rats were exposed to a block of 5 CS+ presentations after 2 min of contextual habituation. Time intervals between tones were randomized during the session.

Behavioral assessment. Freezing behavior was used as a memory index in the fear conditioning task. Freezing was analyzed offline using Solomon software (SOLOMON CODER, © András Péter, Budapest, Hungary), for behavioral coding by an experienced observer that was blinded to the experimental group. Freezing was defined as the absence of all movements, except those related to breathing, while the animal was alert and awake.

Conditioned place preference

The conditioning box consisted of three chambers, two for the conditioning session having the same dimensions (24 × 40 × 50 cm), and the other serving as a central/start chamber (10 × 40 × 50 cm). Each chamber was employed with contextual cues and floor texture to distinguish them.

Conditioned place preference test consisted of three phases: preconditioning (day 1), conditioning (days 2–6), and test (day 7). The preconditioning session (15-min) was intended to reduce novelty and determine initial preferences for any of the two chambers by assessing the time spent in each compartment. Conditioning always took place in the initially less preferred chamber. Conditioning sessions were performed during the following five days. Animals underwent two conditioning sessions each day with 6–8 h intervals between sessions. In one session, animals were placed in the initially less preferred compartment and received MFB stimulation (duration: 20 min, same intensity as used during fear conditioning experiments). During the other session, the animals were placed in the opposite compartment without stimulation. The order of the sessions was randomized between animals and days. A 15 min place preference test was conducted in the absence of stimulation 24 h after the last conditioning day. The video of the animal behavior was recorded and analyzed offline using the ANY-Maze (Stoelting, Wood Dale, IL, USA, Version 7.20) video tracking software.

T-maze task

Animals on food restriction (no less than 85% of their baseline weight) were habituated to the T-maze during 5 days before the training. The T-maze was constructed from black acrylic, with 80 cm long and 30 cm wide alleys and 40 cm high walls. Two removable doors closed the side alleys. During training, a light cue indicated the correct arm to receive a reward (froot-loops pellet). A total of 20 trials per day were performed until achieving 80% of correct choice. A removable door in the central arm was used to confine the animal at the starting point during cue presentation. After 3 min, the alley was removed, and the animal allowed to run in the maze. After arm selection, the alley was closed and the animal remains additional 3 min in the maze before next trial. Afterwards, fear conditioning, extinction, and stimulation sessions started. Animals were tested in the T-maze after the extinction sessions to verify any disruption of the consolidated spatial memory. Extinction and stimulation sessions and T-maze tests were separated by five hours and the order of the behavioral tasks were randomized each day.

Histology

Following the termination of the experiments, animals were deeply anesthetized with 1.5 g/kg urethane (i.p.), and the recording sites of each electrode were lesioned with 100 μA anodal direct current for 10 s (Supplemental Fig. 1C). Then, the animals were transcardially perfused with 0.9% saline solution followed by 4% paraformaldehyde solution and 0.2% picric acid in 0.1 M phosphate buffer saline. After postfixation overnight, 50 μm thick coronal sections were prepared with a microtome (VT1000S, Leica), stained with 1 μg/ml DAPI in distilled water (D8417; Sigma-Aldrich), coverslipped, and examined using a Zeiss LSM880 scanning confocal microscope (Carl Zeiss) and the software ZEN Digital Imaging for Light Microscopy (RRID: SCR_013672) for histological verification of the recording electrode and cannulae locations (Fig. 4b and Supplementary Fig. 7).

Statistical analysis

Statistical analyses were performed using GraphPad Prism 8 software. Significance was set at $p < 0.05$. Data were analyzed using two-tailed Mann–Whitney *U* test, Kruskal–Wallis test, or Mixed ANOVA followed by Dunn's post hoc or Bonferroni's multiple comparisons test. Data are expressed and visualized as median ± IQR, individual data points are also shown where applicable. Detailed statistics are shown in Supplementary Data 1.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The data generated in this study (in the main manuscript and in the Supplementary Information) are provided in the Source Data file and Supplementary Data 1, or from the corresponding author upon request. Source data are provided with this paper.

Code availability

All custom code is freely available from the corresponding author on request.

References

1. Hardt, O., Nader, K. & Nadel, L. Decay happens: the role of active forgetting in memory. *Trends Cogn. Sci.* **17**, 111–120 (2013).
2. Davis, R. L. & Zhong, Y. The biology of forgetting-A perspective. *Neuron* **95**, 490–503 (2017).
3. LeDoux, J. Fear and the brain: where have we been, and where are we going? *Biol. Psychiatry* **44**, 1229–1238 (1998).
4. Quirk, G. J. & Mueller, D. Neural mechanisms of extinction learning and retrieval. *Neuropsychopharmacology* **33**, 56–72 (2008).
5. Wen, Z., Seo, J., Pace-Schott, E. F. & Milad, M. R. Abnormal dynamic functional connectivity during fear extinction learning in PTSD and anxiety disorders. *Mol. Psychiatry* **27**, 2216–2224 (2022).
6. McLaughlin, K. A. et al. Trauma exposure and posttraumatic stress disorder in a national sample of adolescents. *J. Am. Acad. Child Adolesc. Psychiatry* **52**, 815–830 e814 (2013).
7. Perrin, S. Prolonged exposure therapy for PTSD in sexually abused adolescents. *JAMA* **310**, 2619–2620 (2013).
8. Morina, N., Stam, K., Pollet, T. V. & Priebe, S. Prevalence of depression and posttraumatic stress disorder in adult civilian survivors of war who stay in war-afflicted regions. A systematic review and meta-analysis of epidemiological studies. *J. Affect. Disord.* **239**, 328–338 (2018).
9. Bryant, R. A. et al. Acute and chronic posttraumatic stress symptoms in the emergence of posttraumatic stress disorder: a network analysis. *JAMA Psychiatry* **74**, 135–142 (2017).
10. Ross, D. A. et al. An integrated neuroscience perspective on formulation and treatment planning for posttraumatic stress disorder: an educational review. *JAMA Psychiatry* **74**, 407–415 (2017).
11. Bradley, R., Greene, J., Russ, E., Dutra, L. & Westen, D. A multidimensional meta-analysis of psychotherapy for PTSD. *Am. J. Psychiatry* **162**, 214–227 (2005).
12. Berger, W. et al. Pharmacologic alternatives to antidepressants in posttraumatic stress disorder: a systematic review. *Prog Neuropsychopharmacol. Biol. Psychiatry* **33**, 169–180 (2009).
13. Simon, N. M. et al. Paroxetine CR augmentation for posttraumatic stress disorder refractory to prolonged exposure therapy. *J. Clin. Psychiatry* **69**, 400–405 (2008).
14. Steadman, P. E. et al. Disruption of oligodendrogenesis impairs memory consolidation in adult mice. *Neuron* **105**, 150–164 e156 (2020).
15. de Lavilleon, G., Lacroix, M. M., Rondi-Reig, L. & Benchenane, K. Explicit memory creation during sleep demonstrates a causal role of place cells in navigation. *Nat. Neurosci.* **18**, 493–495 (2015).
16. Maren, S., Phan, K. L. & Liberzon, I. The contextual brain: implications for fear conditioning, extinction and psychopathology. *Nat. Rev. Neurosci.* **14**, 417–428 (2013).
17. Herry, C. et al. Switching on and off fear by distinct neuronal circuits. *Nature* **454**, 600–606 (2008).

18. Corcoran, K. A. & Maren, S. Hippocampal inactivation disrupts contextual retrieval of fear memory after extinction. *J. Neurosci.* **21**, 1720–1726 (2001).
19. Zelikowsky, M., Pham, D. L. & Fanselow, M. S. Temporal factors control hippocampal contributions to fear renewal after extinction. *Hippocampus* **22**, 1096–1106 (2012).
20. Zhang, X., Kim, J. & Tonegawa, S. Amygdala reward neurons form and store fear extinction memory. *Neuron* **105**, 1077–1093 e1077 (2020).
21. Kim, J., Pignatelli, M., Xu, S., Itohara, S. & Tonegawa, S. Antagonistic negative and positive neurons of the basolateral amygdala. *Nat. Neurosci.* **19**, 1636–1646 (2016).
22. Maingret, N., Girardeau, G., Todorova, R., Goutierre, M. & Zugaro, M. Hippocampo-cortical coupling mediates memory consolidation during sleep. *Nat. Neurosci.* **19**, 959–964 (2016).
23. Skelin, I. et al. Coupling between slow waves and sharp-wave ripples engages distributed neural activity during sleep in humans. *Proc. Natl. Acad. Sci. USA* **118**, e2012075118 (2021).
24. Girardeau, G., Benchenane, K., Wiener, S. I., Buzsaki, G. & Zugaro, M. B. Selective suppression of hippocampal ripples impairs spatial memory. *Nat. Neurosci.* **12**, 1222–1223 (2009).
25. Girardeau, G., Cei, A. & Zugaro, M. Learning-induced plasticity regulates hippocampal sharp wave-ripple drive. *J. Neurosci.* **34**, 5176–5183 (2014).
26. Zugaro, M. B., Monconduit, L. & Buzsaki, G. Spike phase precession persists after transient intrahippocampal perturbation. *Nat. Neurosci.* **8**, 67–71 (2005).
27. Martinez, L. A. & Tejada-Simon, M. V. Pharmacological inactivation of the small GTPase Rac1 impairs long-term plasticity in the mouse hippocampus. *Neuropharmacology* **61**, 305–312 (2011).
28. Haditsch, U. et al. Neuronal Rac1 is required for learning-evoked neurogenesis. *J. Neurosci.* **33**, 12229–12241 (2013).
29. Hayashi-Takagi, A. et al. Labelling and optical erasure of synaptic memory traces in the motor cortex. *Nature* **525**, 333–338 (2015).
30. Hedrick, N. G. et al. Rho GTPase complementation underlies BDNF-dependent homo- and heterosynaptic plasticity. *Nature* **538**, 104–108 (2016).
31. Rodriguez-Romaguera, J., Do Monte, F. H. & Quirk, G. J. Deep brain stimulation of the ventral striatum enhances extinction of conditioned fear. *Proc. Natl. Acad. Sci. USA* **109**, 8764–8769 (2012).
32. Reznikov, R. et al. Prefrontal cortex deep brain stimulation improves fear and anxiety-like behavior and reduces basolateral amygdala activity in a preclinical model of posttraumatic stress disorder. *Neuropsychopharmacology* **43**, 1099–1106 (2018).
33. Hamani, C. et al. Patient with posttraumatic stress disorder successfully treated with deep brain stimulation of the medial prefrontal cortex and uncinate fasciculus. *Biol. Psychiatry* **88**, e57–e59 (2020).
34. Takeuchi, Y. & Berenyi, A. Oscillotherapeutics—Time-targeted interventions in epilepsy and beyond. *Neurosci. Res.* **152**, 87–107 (2020).
35. Coenen, V. A. et al. Superolateral medial forebrain bundle deep brain stimulation in major depression: a gateway trial. *Neuropsychopharmacology* **44**, 1224–1232 (2019).
36. Nakashiba, T., Buhl, D. L., McHugh, T. J. & Tonegawa, S. Hippocampal CA3 output is crucial for ripple-associated reactivation and consolidation of memory. *Neuron* **62**, 781–787 (2009).
37. Buzsaki, G. Hippocampal sharp wave-ripple: a cognitive biomarker for episodic memory and planning. *Hippocampus* **25**, 1073–1188 (2015).
38. Caliskan, G. et al. Identification of parvalbumin interneurons as cellular substrate of fear memory persistence. *Cereb. Cortex* **26**, 2325–2340 (2016).
39. Ognjanovski, N. et al. Parvalbumin-expressing interneurons coordinate hippocampal network dynamics required for memory consolidation. *Nat. Commun.* **8**, 15039 (2017).
40. Xia, F. et al. Parvalbumin-positive interneurons mediate neocortical-hippocampal interactions that are necessary for memory consolidation. *Elife* **6**, e27868 (2017).
41. Fanselow, M. S. & LeDoux, J. E. Why we think plasticity underlying Pavlovian fear conditioning occurs in the basolateral amygdala. *Neuron* **23**, 229–232 (1999).
42. Gale, G. D. et al. Role of the basolateral amygdala in the storage of fear memories across the adult lifetime of rats. *J. Neurosci.* **24**, 3810–3815 (2004).
43. Sigurdsson, T., Doyere, V., Cain, C. K. & LeDoux, J. E. Long-term potentiation in the amygdala: a cellular mechanism of fear learning and memory. *Neuropharmacology* **52**, 215–227 (2007).
44. Buzsáki, G. Rhythms of the brain. *Oxford University Press* (2006).
45. Tanaka, K. Z. et al. The hippocampal engram maps experience but not place. *Science* **361**, 392–397 (2018).
46. Oliva, A., Fernandez-Ruiz, A., Leroy, F. & Siegelbaum, S. A. Hippocampal CA2 sharp-wave ripples reactivate and promote social memory. *Nature* **587**, 264–269 (2020).
47. Tovote, P., Fadok, J. P. & Luthi, A. Neuronal circuits for fear and anxiety. *Nat. Rev. Neurosci.* **16**, 317–331 (2015).
48. Logothetis, N. K. et al. Hippocampal-cortical interaction during periods of subcortical silence. *Nature* **491**, 547–553 (2012).
49. Pare, D., Collins, D. R. & Pelletier, J. G. Amygdala oscillations and the consolidation of emotional memories. *Trends Cogn. Sci.* **6**, 306–314 (2002).
50. Girardeau, G., Inema, I. & Buzsaki, G. Reactivations of emotional memory in the hippocampus-amygdala system during sleep. *Nat. Neurosci.* **20**, 1634–1642 (2017).
51. Kitamura, T. et al. Engrams and circuits crucial for systems consolidation of a memory. *Science* **356**, 73–78 (2017).
52. Totty, M. S. & Maren, S. Neural oscillations in aversively motivated behavior. *Front. Behav. Neurosci.* **16**, 936036 (2022).
53. Haubrich, J. et al. Reconsolidation allows fear memory to be updated to a less aversive level through the incorporation of appetitive information. *Neuropsychopharmacology* **40**, 315–326 (2015).
54. Goltseker, K., Bolotin, L. & Barak, S. Counterconditioning during reconsolidation prevents relapse of cocaine memories. *Neuropsychopharmacology* **42**, 716–726 (2017).
55. Keller, N. E., Hennings, A. C. & Dunsmoor, J. E. Behavioral and neural processes in counterconditioning: past and future directions. *Behav. Res. Ther.* **125**, 103532 (2020).
56. Redondo, R. L. et al. Bidirectional switch of the valence associated with a hippocampal contextual memory engram. *Nature* **513**, 426–430 (2014).
57. Luo, R. et al. A dopaminergic switch for fear to safety transitions. *Nat. Commun.* **9**, 2483 (2018).
58. Salinas-Hernandez, X. I. et al. Dopamine neurons drive fear extinction learning by signaling the omission of expected aversive outcomes. *Elife* **7**, e38818 (2018).
59. Kalisch, R., Gerlicher, A. M. V. & Duvarci, S. A dopaminergic basis for fear extinction. *Trends Cogn. Sci.* **23**, 274–277 (2019).
60. Josselyn, S. A. & Frankland, P. W. Fear extinction requires reward. *Cell* **175**, 639–640 (2018).
61. Russo, S. J. & Nestler, E. J. The brain reward circuitry in mood disorders. *Nat. Rev. Neurosci.* **14**, 609–625 (2013).
62. Tang, W., Kochubey, O., Kintscher, M. & Schneggenburger, R. A VTA to basal amygdala dopamine projection contributes to signal salient somatosensory events during fear learning. *J. Neurosci.* **40**, 3969–3980 (2020).

63. Fenoy, A. J. et al. Deep brain stimulation of the medial forebrain bundle: distinctive responses in resistant depression. *J. Affect. Disord.* **203**, 143–151 (2016).
64. Garris, P. A. & Wightman, R. M. Different kinetics govern dopaminergic transmission in the amygdala, prefrontal cortex, and striatum: an in vivo voltammetric study. *J. Neurosci.* **14**, 442–450 (1994).
65. Jones, S. R., Garris, P. A., Kilts, C. D. & Wightman, R. M. Comparison of dopamine uptake in the basolateral amygdaloid nucleus, caudate-putamen, and nucleus accumbens of the rat. *J. Neurochem.* **64**, 2581–2589 (1995).
66. Holloway, Z. R. et al. Comparing phasic dopamine dynamics in the striatum, nucleus accumbens, amygdala, and medial prefrontal cortex. *Synapse*, **73**, e22074 (2019).
67. McKernan, M. G. & Shinnick-Gallagher, P. Fear conditioning induces a lasting potentiation of synaptic currents in vitro. *Nature* **390**, 607–611 (1997).
68. Kim, J. et al. Amygdala depotentiation and fear extinction. *Proc. Natl. Acad. Sci. USA* **104**, 20955–20960 (2007).
69. Kroner, S., Rosenkranz, J. A., Grace, A. A. & Barrionuevo, G. Dopamine modulates excitability of basolateral amygdala neurons in vitro. *J. Neurophysiol.* **93**, 1598–1610 (2005).
70. Cahill, E. N., Wood, M. A., Everitt, B. J. & Milton, A. L. The role of prediction error and memory destabilization in extinction of cued-fear within the reconsolidation window. *Neuropsychopharmacology* **44**, 1762–1768 (2019).
71. Gomperts, S. N., Kloosterman, F. & Wilson, M. A. VTA neurons coordinate with the hippocampal reactivation of spatial experience. *Elife* **4**, e05360 (2015).
72. Chu, H. Y., Ito, W., Li, J. & Morozov, A. Target-specific suppression of GABA release from parvalbumin interneurons in the basolateral amygdala by dopamine. *J. Neurosci.* **32**, 14815–14820 (2012).
73. Bissiere, S., Humeau, Y. & Luthi, A. Dopamine gates LTP induction in lateral amygdala by suppressing feedforward inhibition. *Nat. Neurosci.* **6**, 587–592 (2003).
74. De Bundel, D. et al. Dopamine D2 receptors gate generalization of conditioned threat responses through mTORC1 signaling in the extended amygdala. *Mol. Psychiatry* **21**, 1545–1553 (2016).
75. Pedraza, L. K. et al. Chronic fluoxetine prevents fear memory generalization and enhances subsequent extinction by remodeling hippocampal dendritic spines and slowing down systems consolidation. *Transl. Psychiatry* **9**, 53 (2019).
76. Rashid, A. J. et al. Competition between engrams influences fear memory formation and recall. *Science* **353**, 383–387 (2016).
77. Jiang, L. et al. Inhibition of Rac1 activity in the hippocampus impaired extinction of contextual fear. *Neuropharmacology* **109**, 216–222 (2016).
78. Morikawa, M., Tanaka, Y., Cho, H. S., Yoshihara, M. & Hirokawa, N. The molecular motor KIF21B mediates synaptic plasticity and fear extinction by terminating Rac1 activation. *Cell Rep.* **23**, 3864–3877 (2018).
79. Fenoy, A. J. et al. A longitudinal study on deep brain stimulation of the medial forebrain bundle for treatment-resistant depression. *Transl. Psychiatry* **8**, 111 (2018).
80. Figee, M. et al. Deep brain stimulation induces striatal dopamine release in obsessive-compulsive disorder. *Biol. Psychiatry* **75**, 647–652 (2014).
81. Varela, C. & Wilson, M. A. mPFC spindle cycles organize sparse thalamic activation and recently active CA1 cells during non-REM sleep. *Elife* **9**, e48881 (2020).
82. Clemens, Z. et al. Temporal coupling of parahippocampal ripples, sleep spindles and slow oscillations in humans. *Brain* **130**, 2868–2878 (2007).
83. Binette, A. N., Totty, M. S. & Maren, S. Sex differences in the immediate extinction deficit and renewal of extinguished fear in rats. *PLoS One* **17**, e0264797 (2022).
84. McLean, C. P., Asnaani, A., Litz, B. T. & Hofmann, S. G. Gender differences in anxiety disorders: prevalence, course of illness, comorbidity and burden of illness. *J. Psychiatr. Res.* **45**, 1027–1035 (2011).
85. Maeng, L. Y. & Milad, M. R. Sex differences in anxiety disorders: Interactions between fear, stress, and gonadal hormones. *Horm. Behav.* **76**, 106–117 (2015).
86. Haaker, J. et al. Single dose of L-dopa makes extinction memories context-independent and prevents the return of fear. *Proc. Natl. Acad. Sci. USA* **110**, E2428–E2436 (2013).
87. Esser, R., Korn, C. W., Ganzer, F. & Haaker, J. L-DOPA modulates activity in the vmPFC, nucleus accumbens, and VTA during threat extinction learning in humans. *Elife* **10**, e65280 (2021).
88. Kozak, G. & Berenyi, A. Sustained efficacy of closed loop electrical stimulation for long-term treatment of absence epilepsy in rats. *Sci. Rep.* **7**, 6300 (2017).
89. Vandecasteele, M. et al. Large-scale recording of neurons by movable silicon probes in behaving rodents. *J. Vis. Exp.* **4**, e3568 (2012).
90. Shumake, J., Jones, C., Auchter, A. & Monfils, M. H. Data-driven criteria to assess fear remission and phenotypic variability of extinction in rats. *Philos. Trans. R Soc. Lond. B Biol. Sci.* **373**, 20170035 (2018).
91. Russo, A. S., Lee, J. & Parsons, R. G. Individual variability in the recall of fear extinction is associated with phosphorylation of mitogen-activated protein kinase in the infralimbic cortex. *Psychopharmacology (Berl)* **236**, 2039–2048 (2019).
92. Monfils, M. H. et al. Predicting extinction phenotype to optimize fear reduction. *Psychopharmacology (Berl)* **236**, 99–110 (2019).

Acknowledgements

We thank Laura Herrera and Johanna Duran for their technical assistance. This work was supported by the Momentum program II of the Hungarian Academy of Sciences (A.B.), EFOP-3.6.1-16-2016-00008 (A.B.), EFOP-3.6.6-VEKOP-16-2017-00009 (A.B.), and KKP133871/KKP20 grants of the National Research, Development and Innovation Office, Hungary (A.B.), the 20391-3/2018/FEKUSTRAT of the Ministry of Human Capacities, Hungary (A.B.), and the EU Horizon 2020 Research and Innovation Program (No. 739593—HCEMM to A.B.), Ministry of Innovation and Technology of Hungary grant TKP2021-EGA-28 (A.B.), Hungarian Scientific Research Fund (Grants NN125601 and FK123831 to M.L.L.), the Hungarian Brain Research Program (grant KTIA_NAP_13-2-2014-0014 (M.L.L.) and NAP2022-I-7/2022 (A.B. & M.L.L.)), UNKP-20-5 New National Excellence Program of the Ministry for Innovation and Technology from the source of the National Research, Development and Innovation Fund (M.L.L.), Premium Postdoctoral Research Program of the Hungarian Academy of Sciences (ROS), Japan Agency for Medical Research and Development grant 22zf0127004h0002 and 22gm6510015h0001 (Y.T). M.L.L. was a grantee of the János Bolyai Fellowship.

Author contributions

R.O.S., L.K.P., and A.B. conceived the project. R.O.S., L.K.P., G.K., A.J.N., Y.T., and A.B. developed the methodology. R.O.S., L.K.P., L.B., Q.L., and A.P. performed the experiments and analyzed data. R.O.S., L.K.P., M.L.L., O.D., G.B., and A.B. wrote the manuscript. O.D., G.B. advised the project. A.B. supervised the project.

Funding

Open access funding provided by University of Szeged.

Competing interests

A.B. is the owner of Amplipex Llc. Szeged, Hungary a manufacturer of signal-multiplexed neuronal amplifiers. A.B. is a shareholder, chairman,

and CEO, O.D. is an advisor and director, and G.B. is a shareholder of Neunos Inc, a Boston, MA company, developing neurostimulator devices. The remaining authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41467-023-39546-7.

Correspondence and requests for materials should be addressed to Antal Berényi.

Peer review information *Nature Communications* thanks Stephen Maren and the other, anonymous, reviewer(s) for their contribution 2 to the peer review of this work. A peer review file is available.

II.

Pedraza et al. *Translational Psychiatry* (2019)9:53
https://doi.org/10.1038/s41398-019-0371-3

Translational Psychiatry

ARTICLE Open Access

Chronic fluoxetine prevents fear memory generalization and enhances subsequent extinction by remodeling hippocampal dendritic spines and slowing down systems consolidation

Lizeth K. Pedraza[1,2], Rodrigo O. Sierra[2,3], Marcelo Giachero[4], Walquiria Nunes-Souza[2], Fernanda N. Lotz[3] and Lucas de Oliveira Alvares[1,2]

Abstract

Fear memory overgeneralization contributes to the genesis and persistence of anxiety disorders and is a central hallmark in the pathophysiology of post-traumatic stress disorder (PTSD). Recent findings suggest that fear generalization is closely related to hippocampal dependency during retrieval. The selective serotonin reuptake inhibitor (SSRI) fluoxetine has been used as a first-line treatment for PTSD; however, how it exerts its therapeutic effect remains a matter of debate. Here, using contextual fear conditioning in rats, we show that chronic fluoxetine treatment prevents fear generalization and enhances subsequent extinction. Moreover, fluoxetine treatment after extinction prevents spontaneous recovery. The mechanism through which fluoxetine affects generalization and extinction seems to be through the postponement of systems consolidation, thereby maintaining hippocampal involvement during retrieval. Such an effect relies on a remodeling of dendritic spines in the hippocampus, as well as the number of mature, mushroom-type spines promoted by fluoxetine treatment. In order to further investigate whether fear generalization is a potential predictor of extinction effectiveness, we categorized a large naive population according to their generalization rate. We found that discriminator rats showed a better extinction profile compared to generalizers, suggesting that the generalization rate predicts extinction effectiveness. Hence, we propose that the therapeutic strategy of choice should take into account the extension of memory generalization, in which therapies based on extinction could induce a better outcome in patients who present less fear overgeneralization. These results open new avenues for the development of interventions that prevent fear generalization by maintaining memory dependency of the hippocampus.

Introduction

Memory generalization allows animals to extend behavioral repertories to similar situations, contributing to cognitive flexibility. Although generalization can be considered a highly adaptive response, overgeneralization of fear memories contributes to pathological states such as post-traumatic stress disorder (PTSD). In fact, fear overgeneralization is considered a hallmark of the diagnostic

Correspondence: Lizeth K. Pedraza (pedraza.lk@hotmail.com) or Lucas Oliveira Alvares (lucas.alvares@ufrgs.br)
[1]Laboratório de Neurobiologia da Memória, Biosciences Institute, Porto Alegre 91.501-970, Brazil
[2]Graduate Program in Neuroscience, Institute of Health Sciences, Universidade Federal do Rio Grande do Sul, Porto Alegre 90.046-900, Brazil
Full list of author information is available at the end of the article.
These authors contributed equally: Lizeth K. Pedraza, Rodrigo O. Sierra

criteria for PTSD[1,2] Accordingly, these patients are unable to restrict fear expression to appropriate predictors, causing fear and avoidance in response to harmless stimuli that are not directly related to trauma[3].

Behavioral therapies and pharmacological treatments are the most common interventions to attenuate these pathological memories[4]. In exposure extinction-based therapies, traumatic reminders are repeatedly presented in a safe environment, leading to a progressive reduction in fear expression. However, extinction does not erase the original memory but induces new learning that transiently inhibits fear expression. Thus fear memory eventually re-emerges by the passage of time (spontaneous recovery)[5,6].

Fluoxetine and citalopram are well-known selective serotonin reuptake inhibitor (SSRIs) antidepressant used as a first-line treatment for adult PTSD[7]. In the past decade, the mechanisms underlying the clinical improvement associated with fluoxetine have been thoroughly investigated[8,9]. It has been shown that fluoxetine induces neurogenesis, synaptic plasticity, and dendritic spine remodeling[10–12]. Indeed, the mood-improving effects of fluoxetine depend on dendritic spine remodeling in the hippocampus[12]. An interesting study has shown that 3 weeks of fluoxetine treatment combined with extinction training induces an enduring reduction in the conditioned fear response and prevents spontaneous recovery[13]. Interestingly, this behavioral outcome coincided with increase in synaptic plasticity in amygdala GABAergic neurons that control fear expression[13]. Importantly, other SSRIs such as citalopram have shown the opposite effect, disrupting acquisition and retention of fear extinction[14].

Surprisingly, few studies have been conducted to explore the effects of SSRIs on fear generalization[15]. Recently, we showed that fear generalization is closely related to hippocampal dependency during retrieval[16,17]; we found that this structure is crucial to orchestrate the reconstruction of detailed memories[16–18]. Environmental factors, such as sequential learning and training intensity, can accelerate hippocampal independency and memory generalization[17,19,20]. Evidence from animal studies shows that retrieval of recent contextual fear memory induces higher hippocampal activation than remote memories[21]. In contrast, several areas of the medial prefrontal cortex (mPFC) are more activated during retrieval of remote memories[22]. As opposed to the hippocampus, these cortical structures seem to be essential for the expression of fear generalized memories[23,24]. Thus the transition from hippocampus dependence to hippocampus independence renders memories into a more schematic, generalized state[25,26] (for a comprehensive review of these systems consolidation models, see refs. [27,28]).

Here we tested the effects of chronic treatment with the SSRI fluoxetine and citalopram on contextual fear memory generalization and subsequent performance during fear extinction. Additionally, we explored the close relationship between memory discrimination and fear extinction in naive animals, as a potential predictor of extinction outcome. Our results are discussed in light of the observation that systems consolidation is an important player in the pathophysiology of PTSD and may be a novel approach to be considered for pharmacological and behavioral treatments of fear-related disorders.

Materials and Methods

Subjects

Naive, adult male Wistar rats (270–320 g/3 months) from our breeding colony were used. Animals were housed in plastic cages, 4–5 per cage, under a 12 h light/dark cycle at a constant temperature of 24 °C, with water and food ad libitum. Sample size for each group (n = 8–15) was estimated based on previous studies of our laboratory[16,17,19,20]. Animals were randomly assigned to treatment groups. All experiments were conducted in accordance with local and national guidelines for animal care (Federal Law no 11.794/ 2008), and the project was approved by the Ethics Committee of the Federal University of Rio Grande do Sul, Brazil.

Stereotaxic surgery and cannulae placement

Rats were anesthetized with intraperitoneally (i.p.) ketamine/xylazine (75 and 10 mg/kg, respectively) and bilaterally implanted with 22-gauge guide cannulae aimed at dorsal hippocampus (AP −4.0 mm (from bregma), LL ±3.0 mm, DV −1.6 mm) positioned 1.0 mm above of each structure[29]. Following a 1-week recovery from surgery, animals were submitted to the behavioral procedures.

Drugs

Fluoxetine hydrochloride and Citalopram hydrobromide (Sigma-Aldrich) were dissolved in 0.9% sterile saline. Both drugs were administered in a dose of 10 mg/kg/ml based on previous studies[14,30,31] and injected i.p. Chronic treatment consisted of daily administration during 21 days after fear conditioning or extinction protocols depending on the experiment performed.

Muscimol (1 μg/μl; Sigma Aldrich) was dissolved in phosphate-buffered saline (PBS) and bilaterally infused (0.5 μl/side) into dorsal hippocampus 15 min before memory retrieval.

Intracerebral infusion

At the time of infusion, a 27-gauge infusion needle was inserted into the guide cannulae, with its tip protruding 1.0 mm beyond the tip of the cannula and aimed at the dorsal hippocampus. A volume of 0.5 μl was bilaterally infused at a slow rate (20 μl/h), and the needle was removed only after waiting for an additional 30 s.

Behavioral procedure

Contextual fear conditioning (CFC)

The conditioning chamber consisted of an illuminated Plexiglas box, $25 \times 25\,cm^2$ with a metallic grid floor. During training, rats were placed in the chamber for 3 min, received 4 footshocks (0.7 mA/2 s) separated by a 30 s interval; 30 s after the last shock, they were returned to their homecages.

Context apparatus

Conditioning context consisted of an illuminated Plexiglas box of $25 \times 25\,cm^2$, grid floor of parallel 0.1 cm caliber stainless steel bars spaced 1.0 cm apart, and fan background noise. The novel context was a rectangular box 2/3 the size of the conditioning context, smooth floor, vanilla essence, and without fan background noise.

Fear generalization test

Animals were tested for 4 min both in the novel context (Novel) and in the conditioning context (Ctx) without footshocks on days 22 and 23 after training, respectively.

Fear extinction session

Subjects were re-exposed to the training context on day 24 without footshocks for 30 min to induce memory extinction.

Fear extinction and spontaneous recovery test

Animals were tested for 4 min in the conditioning context (Ctx) 24 h and 22 days after the extinction session in order to evaluate early retention and spontaneous recovery response, respectively.

Open field (OF)

The OF chamber consisted of a 50 cm height, $60 \times 40\,cm^2$ plywood box and a linoleum floor divided into 12 equal rectangles or "sectors." In addition, floor was divided into two squares, which allowed the definition of central and peripheral areas. The behavior was recorded by video tracking and processed offline. During the 5-min test session, crossings between sectors (locomotor activity) and the time spent in the periphery and center of the apparatus were measured.

Behavioral scoring

Freezing behavior was registered in real time by an experienced observer who was blind to the treatments. Freezing was defined as the absence of all movements, except those related to breathing. In the OF test, the number of crossings was considered a measure of motor performance, while the time spent in the center or periphery of the field was considered anxiolytic or anxiogenic responses induced by the treatment, respectively.

Structural plasticity analysis

Dendritic spine visualization and analysis was performed as previously reported by other researchers[32–34]. Concisely, under deep anesthesia (chloral hydrate, 400 mg/kg i.p.), animals were transcardially perfused, first by ice-cold PBS (0.1 M, pH 7.4) and then fixed using ice-cold 4% paraformaldehyde (PFA) (in 0.1 M PBS, pH 7.4). After the brain was removed and postfixed (4% PFA, 24 h, 4 °C), coronal sections (200-µm thick) containing the dorsal hippocampus were obtained with a vibratome and collected in 0.1% PBS. The CA1 dorsal hippocampus was stained with small droplets (<10 µm) of a saturated solution of the lipophilic dye 1,1′-dioctadecyl-3,3,3′,3′-tetramethyl indocarbocyanine perchlorate (Invitrogen; Carlsbad, CA) in fish oil[35] by microinjection via a patch pipette and positive pressure application[34]. Using a Leica DMI6000 B laser scanning confocal microscope with a ×100× oil immersion from the Laboratório Central de Microscopia Eletrônica, Florianópolis, Brazil, stacks of labeled dendritic segments were collected. The images were deconvolved using LAS AF Lite software (Leica Microsystems, Wetzlar, Germany). A theoretical point spread function was used.

The dendritic spine analysis was achieved manually using the ImageJ software. Dendritic protrusions <3 µm in length and contacting with the parent dendrite were employed for the analysis[33,36,37]. Special consideration was taken to select a single dendritic segment, presumably from different neurons but from CA1 stratum radiatum, in light of the high density of labeled dendrites. Thus, from the *z*-section projection, both the total number and the number of each particular type of dendritic spine normalized to 10 µm of the dendritic segment length was counted with certainty that each spine was counted only once.

Spine types were classified as previously[32,38,39]: type I or "stubby"-shaped dendritic spines, type II or "mushroom"-shaped dendritic spines, and type III or "thin"-shaped dendritic spines. Different measurements were taken for each dendritic protrusion in order to classify them, in brief: the length (dimension from the base at the dendrite to the tip of its head, *L*), the diameter of the neck (measured as the maximum neck diameter, dn), and the diameter of the head (measured as the maximum head diameter, dh)[38]. Thus individual spines were classified into category based on the specific ratios L/dn and dh/dn[32–34,38].

Histology for cannulae placement

The position of the cannulae was verified at the end of the experiments. The brains were removed and immersed in a fixation solution of 30% sucrose and 4% PFA. Brains were then frozen and sliced (50-µm coronal sections) using a cryostat. Sections were stained with cresyl violet

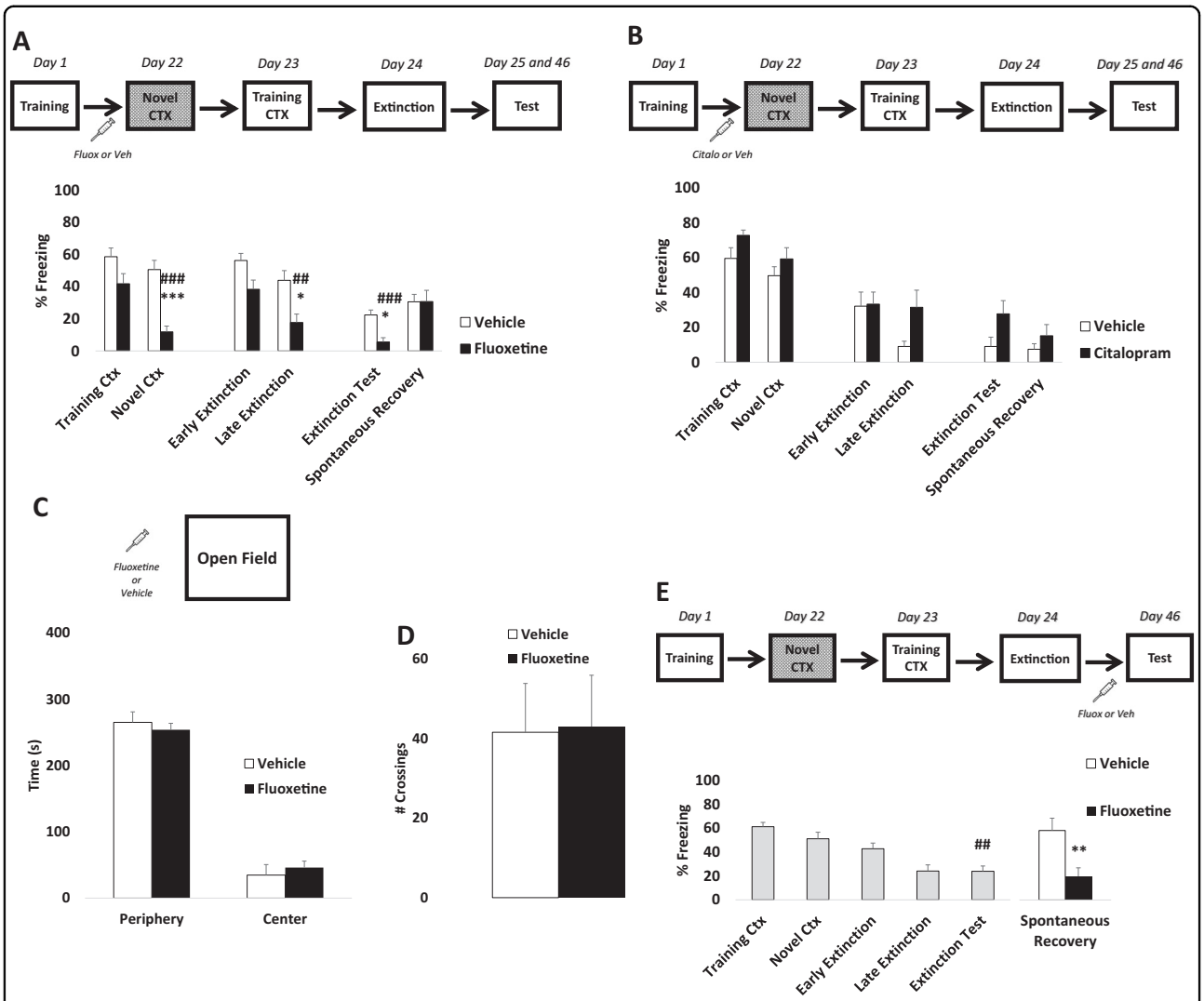


Fig. 1 Chronic fluoxetine prevents fear generalization and enhances subsequent fear extinction. The graphs show percentage of freezing time expressed as mean ± SEM, and experimental design is shown at the top of each panel. **a** Animals treated with fluoxetine are able to discriminate between novel and threat context compared to the control group. The same animals showed a better extinction profile (*Differences between groups, #Differences within group. ***$p < 0.001$, *$p < 0.05$, ###$p < 0.001$, ##$p < 0.01$, repeated-measures analysis of variance (ANOVA) followed by Student–Newman–Keuls post hoc test—Vehicle $n = 13$, Fluoxetine $n = 13$). **b** No differences were detected after chronic citalopram administration (ANOVA for repeated measures all factor $p > 0.05$, Vehicle $n = 9$, Citalopram $n = 10$). In addition, fluoxetine effects were not associated with **c** anxiety-like time effects (time spent in the periphery vs. center of the open field (t (8) = −0.602; $p = 0.563$, independent t test—Vehicle $n = 5$, Fluoxetine $n = 5$)) or **d** locomotion (number of crossings (t (8) = −0.078; $p = 0.939$, independent t test)). **e** Chronic fluoxetine was able to prevent fear recovery 21 days after the extinction protocol suggesting two potential pharmacological window to attenuate contextual fear memories (*Differences between groups, #Differences within group, **$p < 0.01$, ##$p < 0.01$, independent t-test—Vehicle $n = 12$, Fluoxetine $n = 12$)

and subsequently examined to verify the location of the cannulae (Fig. 2a). Statistical analysis considered only animals with correct cannulae placements.

Statistical analysis

After checking for normality (Kolmogorov–Smirnov (KS) test) and homoscedasticity (Levene test), each relevant phase of the experiment (generalization, extinction and test (extinction test and spontaneous recovery) was analyzed by two-way repeated-measures analysis of variance (ANOVA) with Student–Newman–Keuls (SNK) post hoc test. OF result were analyzed using independent Student's t test. Significance was set at $p < 0.05$. Simple linear regression was used to evaluate the relationship between discrimination index and freezing behavior during the extinction test. Cumulative distribution probabilities for total number, mushroom, stubby, and thin dendritic spines per 10 mm of dendritic segment were compared by KS test. Data were also expressed as median (quartile) and compared by Mann-Whitney U test. $p <$

0.05 was considered statistically significant. For behavioral profile classification, we used the unsupervised learning algorithm expectation-maximization (EM) to divide naive animals between generalizers and discriminators using as input the discrimination index between conditioning and neural context [training ctx/(training ctx + novel ctx)]. This method was used based on previous studies evaluating fear memory discrimination-based populations[40,41].

Results

Chronic fluoxetine prevents fear memory generalization and enhances subsequent extinction

First, in order to assess the effects of fluoxetine on fear generalization and subsequent extinction, animals were trained in CFC and received daily administration of fluoxetine or its vehicle i.p. for 21 days after training. Twenty-four hours after the last administration, animals were exposed first to a novel context (Novel Ctx) and, on the following day, to the training context (Training Ctx). Twenty-four hours later, animals were submitted to a fear extinction procedure in the training context and memory retrieval was evaluated 24 h and 21 days after extinction (spontaneous recovery). Repeated-measures ANOVA revealed that fluoxetine-treated animals expressed contextual discrimination; less freezing was expressed in the novel context compared to the training context (group × context interaction ($F_{1,24} = 6.006$, $p = 0.021$; SNK post hoc $p < 0.001$)). Moreover, fluoxetine-treated animals expressed less freezing in the novel context than vehicle-treated animals ($p < 0.001$). Vehicle-treated animals were unable to discriminate between the contexts, expressing fear generalization (training vs. novel context $p > 0.05$; Fig. 1a).

Fluoxetine-treated animals expressed a better extinction profile; less freezing was detected during the last 5 min of fear extinction (late extinction) compared to the control group (group × time interaction ($F_{1,24} = 0.664$, $p = 0.422$; SNK post hoc $p < 0.05$)). Additionally, fluoxetine enhanced fear extinction during the test performed 24 h after extinction but did not prevent spontaneous recovery (group × time interaction ($F_{1,24} = 6.573$, $p = 0.017$; SNK post hoc $p < 0.05$). Interestingly, the chronic administration of citalopram, another SSRI, did not prevent fear generalization or alter fear expression (ANOVA for repeated measures all factor $p > 0.05$; Fig. 1b).

These results suggest that chronic fluoxetine (but not citalopram) is able to prevent fear generalization and enhance subsequent extinction. Anxiety-like (time spent in the periphery or center of the OF between groups (t (8) = −0.602; $p = 0.563$, independent t test); Fig. 1c) or locomotion (number of crossings (t (8) = −0.078; $p = 0.939$, independent t test)) effects were not induced by the treatment (Fig. 1d).

Although fluoxetine enhanced fear extinction, it did not prevent spontaneous recovery. Prevention of fear relapse after extinction is considered a major challenge for pharmacological and exposure-based therapies[42]. Finally, to verify whether fluoxetine treatment after the extinction training would be able to postpone fear re-emergence, we administered fluoxetine after extinction for 21 days until the spontaneous recovery test. Repeated-measures ANOVA revealed that animals were not able to discriminate between the training and novel contexts. Moreover, a significant reduction in freezing levels was detected between early and late extinction and was maintained during the extinction test (time factor ($F_{4,92} = 17.670$, $p < 0.001$; SNK post hoc $p > 0.05$); Fig. 1e).

After that, animals were randomly assigned to two groups and treated with fluoxetine or vehicle. Our results showed that chronic fluoxetine is able to prevent spontaneous recovery (t (22) = 3.055, $p = 0.005$; independent t test). This result suggests a dual window of opportunity for pharmacological intervention with fluoxetine to enhance extinction, based on decreasing fear generalization (Fig. 1a) or preventing spontaneous recovery (Fig. 1e).

Chronic fluoxetine maintains hippocampal dependency during retrieval

Previous studies demonstrate that memory precision involves hippocampal processing during retrieval[17,18], whereas fear generalization has been associated with an increase in mPFC activity[23,24] but a decrease in the hippocampus[17,18]. These results are consistent with the systems memory consolidation hypothesis on progressive trace reorganization over time between hippocampal and cortical structures[21,43,44]. Since chronic fluoxetine induces memory precision, we reason that this treatment could promote hippocampal dependency as well. To address this issue, animals were treated with fluoxetine and infused intrahipocampally 15 min before the test in the training context with vehicle or muscimol, a selective agonist for GABA-A receptors able to suppress transiently neural activity. Four hours after the test, a drug-free re-test was performed in the same context. If fluoxetine slows down systems consolidation (keeping hippocampal dependency), then we would expect a strong effect in the test under muscimol.

Repeated-measures ANOVA revealed that fluoxetine-treated animals were more sensitive to intrahippocampal muscimol than the control group during the test (group × time (test vs. re-test) interaction ($F_{1,17} = 5.371$, $p = 0.033$; SNK post hoc $p < 0.05$); Fig. 2b). Thus fear generalization prevented by chronic fluoxetine seems to be strongly associated with hippocampal involvement during retrieval.

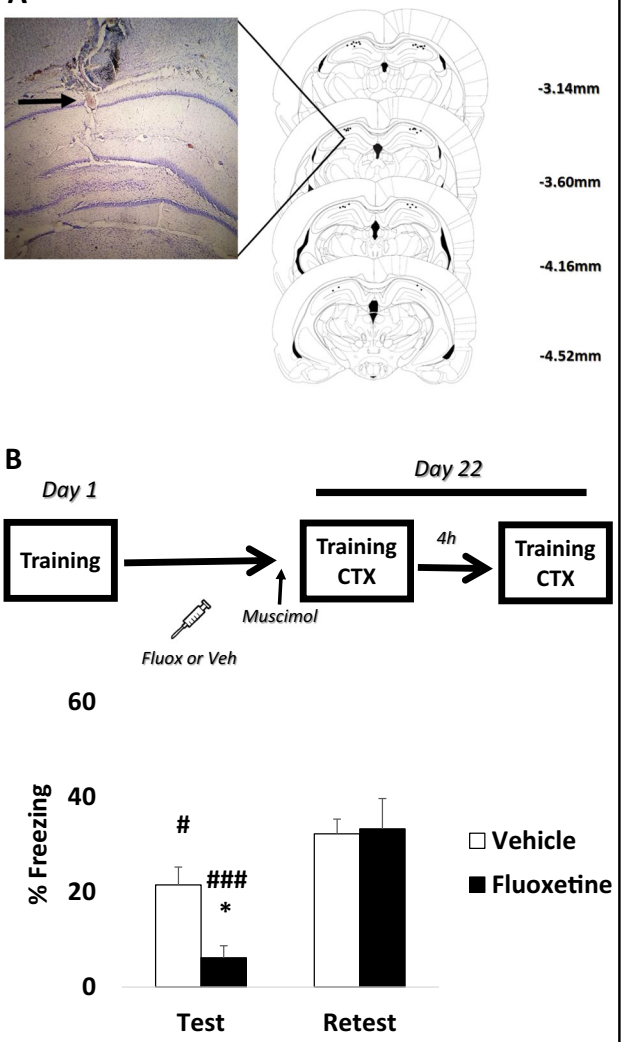


Fig. 2 Chronic fluoxetine increases hippocampal involvement during retrieval. The graphs show percentage of freezing time expressed as mean ± SEM, and experimental design is shown at the top of each panel. **a** Examples of Nissl-stained sections and summary of microinjection cannula placements in the dorsal hippocampus. The numbers on the right side of the individual sections indicate the distances from bregma. **b** Although fluoxetine- and vehicle-treated animals were sensitive to intrahippocampal muscimol infusion (test vs. re-test comparison), fluoxetine-treated animals expressed a more robust impairment during retrieval (between-group comparison during test). No differences were detected during the re-test (*Differences between groups, #Differences within group. $^{*}p < 0.05$, $^{***}p < 0.001$, $^{\#}p < 0.05$, repeated-measures analysis of variance followed by Student–Newman–Keuls post hoc test—Vehicle $n = 11$, Fluoxetine $n = 8$)

Chronic fluoxetine induces hippocampal structural rearrangement

Recent evidence suggests that systems consolidation involves an increase in dendritic spine density in the mPFC over time[45] together with a reduction in dendritic spines in the hippocampus[46]. We hypothesize that hippocampal dependency during retrieval is closely related to dendritic spine density and morphology. Animals treated with chronic fluoxetine or vehicle were perfused, and the brains removed for dendritic spine analysis in the dorsal hippocampus. Spine counts were performed on a total of 122 dendritic segments as follows: control ($n = 55$ segments, 1160.50 µm of total dendritic length analyzed, 3 rats), fluoxetine (67 segments, 1234.51 µm, 3 rats) (Fig. 3a).

Analysis of the cumulative probability distributions for the total density of dendritic spines reflected a significant rightward shift, toward higher numbers of dendritic spines in fluoxetine-treated animals compared to the control group ($p < 0.05$; KS test; Fig. 3b). Moreover, the fluoxetine group also showed a higher median (quartiles, total density/10 µm), 19.0 [13.3–21.4], with respect to the control group, 14.5 [12.1–16.4] (Mann–Whitney U test = 1162, $p < 0.001$). Similar to total dendritic spines, a significant rightward shift toward higher numbers of mushroom dendritic spines in the fluoxetine group compared to the control group was observed ($p < 0.05$; KS test; Fig. 3c). In parallel, a higher median (quartiles, mature spines/10 µm) in the fluoxetine group, 7.01 [4.67–8.85], was observed in comparison to the control group, 5.83 [4.38–7.08] (Mann–Whitney U test = 1394, $p < 0.001$). The analysis of stubby dendritic spines revealed no significant difference between the experimental groups ($p > 0.05$; KS test; Fig. 3d). There was a comparable median (quartiles, thin spines/10 µm) between the experimental groups, control 5.98 [4.93–7.90], fluoxetine 6.53 [4.58–9.07] (Mann–Whitney U test = 1394, $p < 0.001$). For thin dendritic spines, a significant rightward shift toward higher numbers of spines in the fluoxetine group compared to the control group was observed ($p < 0.05$; KS test; Fig. 3e). Similarly, a higher median (quartiles, mature spines/10 µm) in the fluoxetine group, 3.89 [2.45–5.22], was observed in comparison to the control group, 2.45 [1.48–3.41] (Mann–Whitney U test = 1043, $p < 0.001$). Our findings suggest that chronic fluoxetine induces a higher density of dendritic spines in the CA1 hippocampal area, indicating a potential mechanism underpinning the maintenance of hippocampal dependency during retrieval and consequently the prevention of memory generalization.

Discrimination between threat and safe context predicts subsequent fear extinction in naive animals

Our first experiment showed that chronic fluoxetine administration prevents generalization and enhances fear extinction. These results suggest that discrimination between the training and the novel context can be considered a potential predictor of the extinction outcome, in which memory generalization would impair fear extinction. To address this possibility, a large number of animals

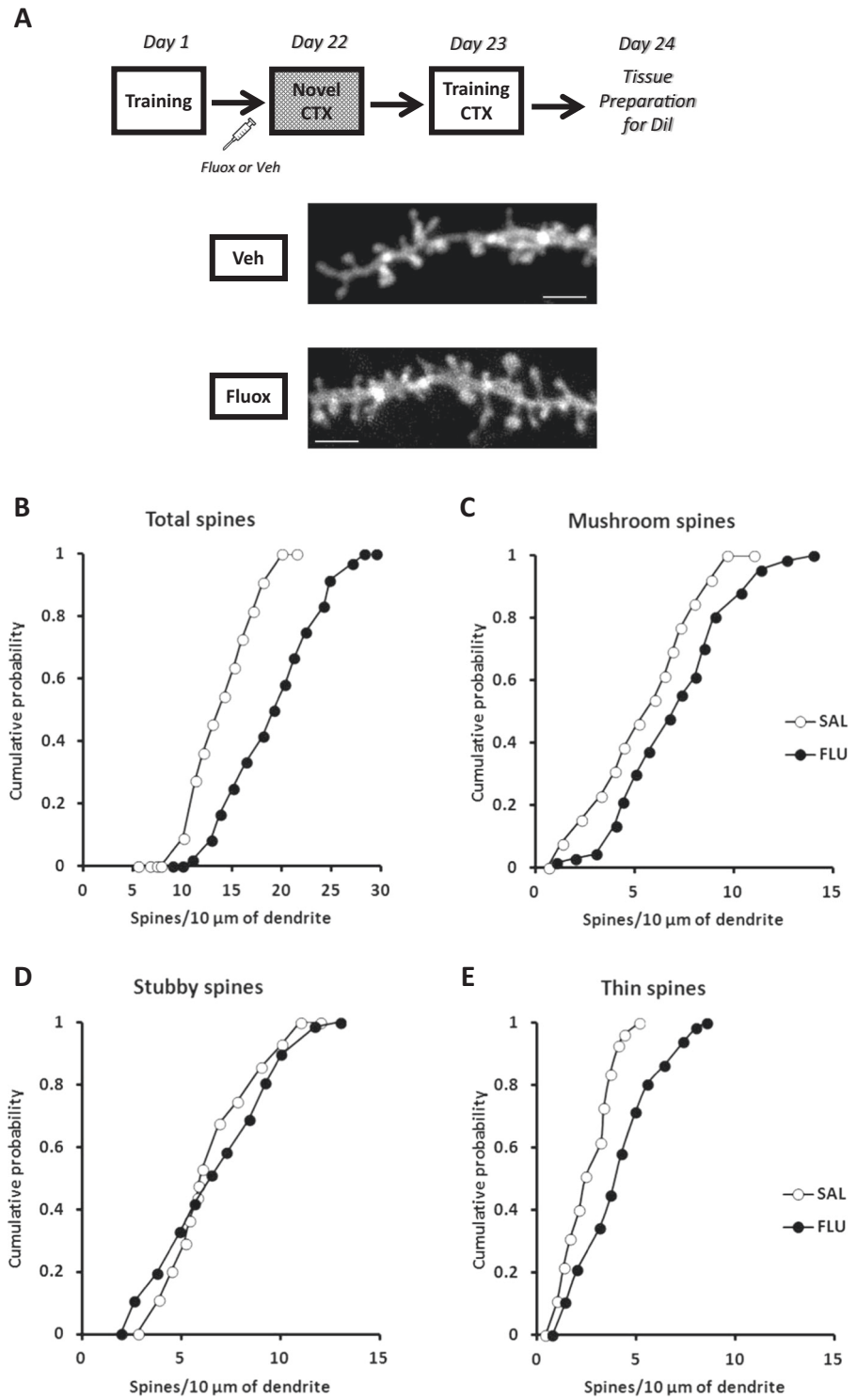


Fig. 3 Chronic fluoxetine induces hippocampal structural rearrangement. The graphs show cumulative probability of the spine density. Experimental design is shown at the top of the panel. **a** Representative examples of apical dendritic segments of CA1 dorsal hippocampal pyramidal neurons (stratum radiatum) selected for quantitative analysis of dendritic spines from animals of each experimental group. Bar scale: 2 mm. Cumulative frequency of total (**b**), mushroom (**c**), stubby (**d**), and thin (**e**) dendritic spine (Kolmogorov–Smirnov test—Vehicle $n = 55$ segments, Fluoxetine $n = 67$ segments; 3 rats per group)

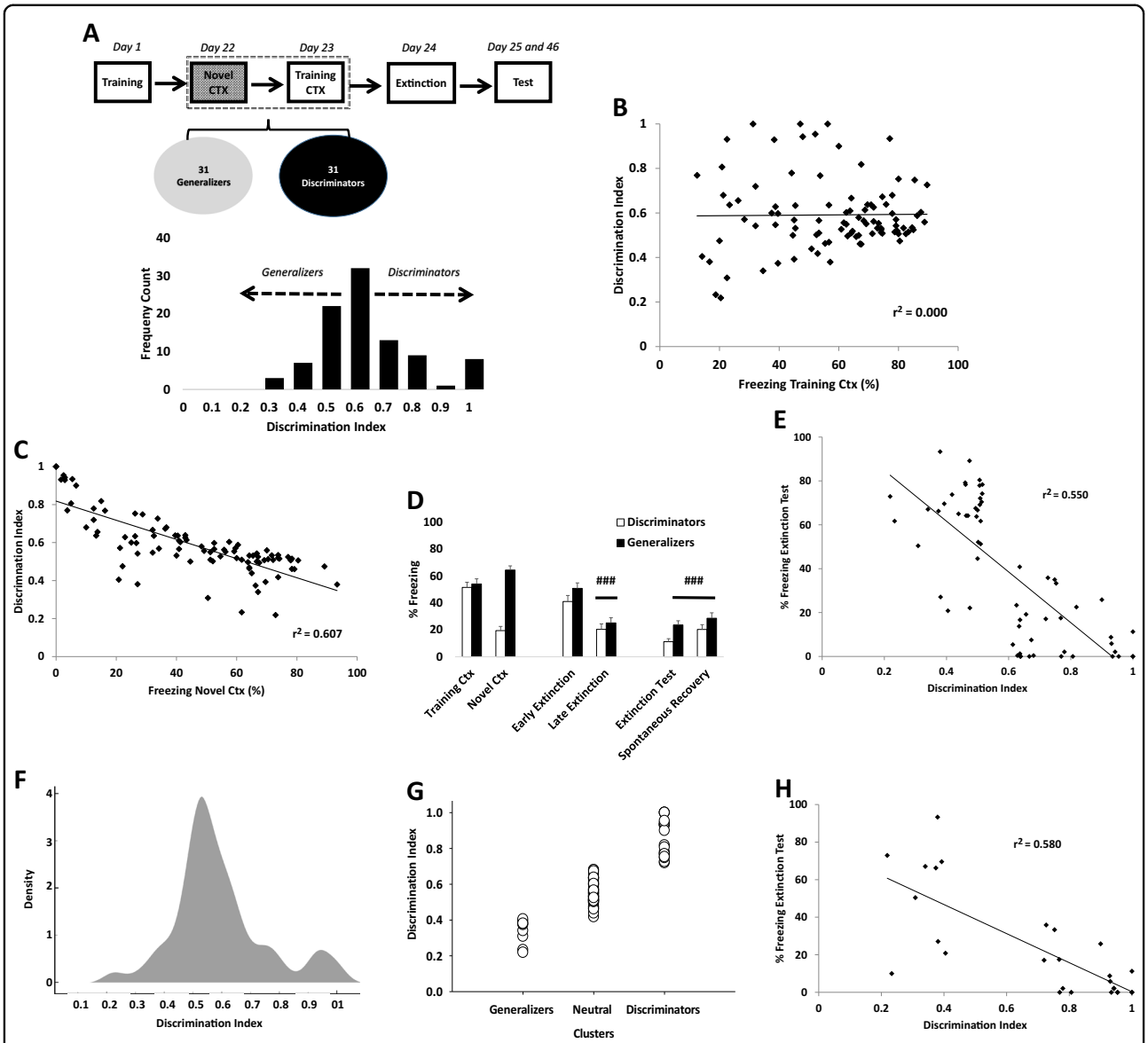


Fig. 4 Fear discrimination predicts extinction outcome in naive animals. The graphs show frequency count, percentage of freezing time (mean ± SEM), and linear regression between percentage of freezing time and discrimination index. Experimental design is shown at the top of the panel. **a** The means of higher discrimination were used as a cut-off value to divide total population ($n = 95$) between discriminators ($\bar{x} < 0.51$) ($n = 31$) and generalizers ($\bar{x} > 0.61$) ($n = 31$). **b** Regression analysis with the total naive population ($n = 95$) revealed no relationship between freezing in the training context and discrimination index (slope did not differ from zero ($F_{1,93} = 0.013$, $p = 0.909$, $r^2 = 0.000$). However, **c** the same analysis showed a strong relationship between novel context and the discrimination index (slope different from zero, $F_{1,93} = 144.084$, $p < 0.001$, $r^2 = 0.607$). **d** Animals classified as discriminators expressed less freezing during extinction and spontaneous recovery test compared to generalizers ($^{\#}$Differences within group. $^{\#\#\#}p < 0.001$, repeated-measures analysis of variance followed by Student–Newman–Keuls post hoc test—Discriminators $n = 31$, Generalizers $n = 31$). **e** Linear regression analysis using only discriminators and generalizer populations ($n = 62$) showed a strong relationship between discrimination index and freezing behavior during the extinction test (slope differed from zero, $p < 0.001$, $r^2 = 0.550$). **f** Gaussian mixture model showing the distribution of the discrimination index. **g** Expectation-maximization (EM) algorithm classified the total population in 3 potential profiles (cluster chosen a priori based on Fig. 5a): generalizers $n = 9$ (9%), neutral $n = 68$ (72%), and discriminators $n = 18$ (19%). **h** Linear regression analysis using only discriminators and generalizer populations ($n = 27$) after EM analysis showed the same strong relationship of Fig. 5e between discrimination index and freezing behavior during the extinction test (slope differed from zero, $p < 0.001$, $r^2 = 0.580$)

($n = 95$) were trained and tested in the same conditions as the first experiment, without any pharmacological treatment. First, we calculated a discrimination index [training ctx/(training ctx + novel ctx)] for each animal in order to determine a frequency distribution (Fig. 4a). This analysis allowed us to identify that the vast majority of animals

have a discrimination ratio between $\bar{x} = 0.51$ and 0.61 ($n = 33$). This range was used as a cut-off value to divide the total population into two specific profile: generalizers ($\bar{x} < 0.51$) and discriminators ($\bar{x} > 0.61$).

A regression analysis conducted on the freezing levels in the training context and the discrimination index revealed that there was no relationship between these factors, since the slope did not differ from zero ($F_{1,93} = 0.013$, $p = 0.909$, $r^2 = 0.000$; Fig. 4b). However, a strong relationship was found between the novel context and the discrimination index (slope different from zero; $F_{1,93} = 144.084$, $p < 0.001$, $r^2 = 0.607$; Fig. 4c). This result suggests that the freezing expressed in the novel context drives fear discrimination.

After analyzing the behavioral profile of the total population, 31 animals were classified as discriminators and 31 as generalizers. As expected, repeated-measures ANOVA revealed higher discrimination and generalization rates (group × context interaction ($F_{1,60} = 86.420$, $p < 0.001$; SNK post hoc $p < 0.001$). However, both groups expressed the same freezing levels in the training context (Fig. 4d).

Both groups were able to extinguish the fear response (time factor (early vs. late) ($F_{1,60} = 62.435$, $p < 0.001$). However, during the extinction and spontaneous recovery test, discriminators expressed less freezing levels than generalizers (group factor (test vs. spontaneous recovery) ($F_{1,60} = 4.278$, $p < 0.042$). This result suggests that discrimination between the training and the novel context is closely associated with a better extinction profile.

Surprisingly, regression analysis revealed a strong relationship between the discrimination index and freezing behavior during the extinction test (slope different from zero; $F_{1,60} = 73.389$, $p < 0.001$, $r^2 = 0.550$; Fig. 4e).

To further investigate whether these results could be influenced by the data selection method to generate discriminators and generalizers groups, we employed the unsupervised learning algorithm EM in the total population ($n = 95$). This algorithm estimates the maximum likelihood parameters from Gaussian mixture model[40,41] (Fig. 4f). Number of clusters were chosen a priori based on our previous result (Fig. 4a). This analysis reduced the number of generalizer assigned animals to 9 (9%) and 18 (19%) for discriminators (Fig. 4g). However, even after decreasing the number of animals per group we found the same strong correlation between the discrimination index and freezing behavior during the extinction test (simple linear regression, slope different from zero; $F_{1,25} = 34.581$, $p < 0.001$, $r^2 = 0.580$; Fig. 4h).

Taken together, these results demonstrate, for the first time, that memory discrimination can be considered as a potential predictor of the extinction outcome. Our results were supported by two different methods of behavioral categorization. We conclude that better fear extinction profile can be reached as a natural consequence of better discrimination between threat and safe.

Discussion

The current study showed that chronic fluoxetine administration after training, but not citalopram, prevents fear generalization and enhances subsequent fear extinction (Fig. 1a–b). Although fluoxetine after training did not prevent spontaneous recovery, a persistent fear reduction was reached when fluoxetine was chronically administered after extinction (Fig. 1e). Interestingly, fluoxetine increases hippocampal dependency, indicating that the treatment is able to delay the systems consolidation process (Fig. 2b). This result was accompanied by a higher density of dendritic spines in the CA1 region in animals treated with fluoxetine (Fig. 3a–e). Finally, in a large naive population, animals categorized as good discriminators showed a better extinction profile (similar to fluoxetine-treated animals), indicating that fear discrimination predicts extinction outcome (Fig. 4b–h).

Antidepressant drugs have been used for decades as the first-line pharmacological treatment for PTSD[47]. However, there are several conflicting reports on their efficacy with and without exposure therapies[48,49]. Our findings show that chronic fluoxetine, but not citalopram, prevents fear generalization and enhances fear extinction. This differential effect between antidepressant drugs of the same class in not entirely new. In fact, Burghardt et al.[14] have shown that chronic citalopram administration impaired the acquisition of fear extinction, an effect closely related to the inhibition of NR2B-NMDA down-regulation in lateral and basal nuclei of the amygdala. These results contrast with the fear reduction enhancement promoted by chronic administration of fluoxetine in similar experimental settings[13]. Moreover, the antidepressant escitalopram is able to attenuate mood and anxiety dysfunction in a rodent PTSD model; however, it is insufficient to revert the impaired fear extinction[50]. Taking together, SSRIs seem to modulate differentially fear-related responses, an observation currently validated in clinical population[49]. Importantly, fluoxetine changes the quality, rather than the strength, of fear memories, since no difference was detected in freezing behavior in the training context, but there was a difference in the novel context.

Prevention of spontaneous recovery after extinction is one of the main challenges of behavioral and pharmacological therapies[42]. In our protocol, post-conditioning chronic fluoxetine was able to enhance extinction of contextual fear memories, as previously reported[13,51]. Moreover, a persistent fear reduction was achieved when chronic fluoxetine was administered after extinction training. Altogether, these results indicate a dual window of opportunity to avoid fear memory: (i) by enhancing fear

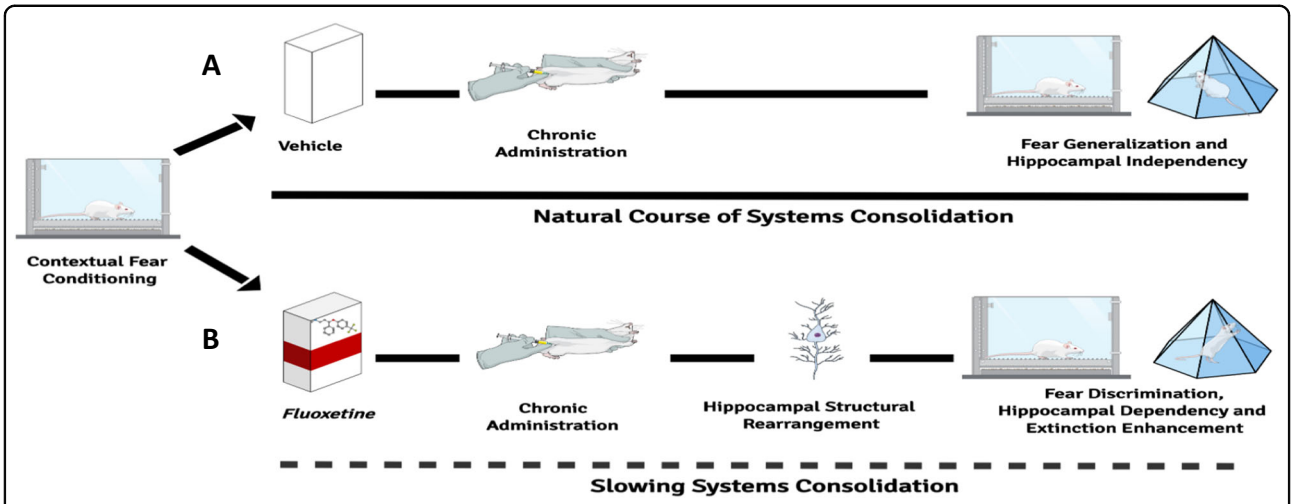


Fig. 5 Summary of our findings. a Fear learning is able to trigger structural reorganization over time between hippocampal and cortical structures. As a consequence of this systemic process, detailed fear memories are transformed into a more schematic state, inducing fear generalization (the inability to restrict the fear expression to appropriate predictors, causing fear and avoidance in response to harmless stimuli such as the novel context that is not directly associated with trauma). **b** Chronic fluoxetine treatment after contextual fear conditioning prevents fear generalization by maintaining fear memory dependency of the hippocampus. The mechanism underlying such process seems to be the remodeling of dendritic spines densities, especially the mushroom type in the CA1 region. Our findings suggest that manipulating the natural course of systems consolidation can be considered as a potential clinic strategy to treat fear-related disorders through its extinction facilitation

extinction when fluoxetine is administered after conditioning, via slowing of fear generalization, and (ii) by preventing fear recovery after extinction.

A previous study has shown that fear generalization can be prevented by pharmacological treatments targeting glucocorticoid (Metyrapone) and noradrenergic (Propranolol) systems during training[17]. Interestingly, in that study, fear discrimination was closely associated with hippocampal involvement during retrieval. Here chronic fluoxetine administration was able to increase hippocampal dependency and prevent fear generalization, suggesting that fluoxetine delays the systems consolidation rate. The transition from hippocampus dependence to hippocampus independence is thought to render memories into a more schematic, generalized state[25,26]. Additionally, remote memories submitted to this reorganization process seem to be less susceptible to modification or to update[52,53] and are largely influenced by fear incubation[54]. We suggest that manipulating the systems consolidation rate (i.e., by slowing it down) allows the expansion of the therapeutic window for treating fear-related disorders. This means that preserving hippocampal involvement during retrieval and fear discrimination could be a potential clinical approach to enhance subsequent fear extinction.

The hippocampal dependency induced by chronic fluoxetine was accompanied by a remarkable structural rearrangement in the CA1 region. Remodeling of dendritic spines seems to guide memory reorganization between hippocampal and cortical structures[46,55–57]. Recently, it has been shown that an immature engram is formed in the mPFC after training, before the process of systems consolidation occurs over time. This maturation process involves a substantial increase in dendritic spines in the mPFC, a structure previously associated with retrieval of generalized memories[23,24], and a decrease in the hippocampus[45,46]. In our study, fluoxetine treatment modulates remodeling of dendritic spines in the hippocampus, particularly the mature, mushroom type. We assume that the increase in dendritic spines in the hippocampus is one of the mechanisms underpinning the maintenance of hippocampal dependency over time (by slowing down systems consolidation) thus prevent fear generalization and ultimately facilitates extinction. That is, both systems consolidation and memory generalization are controlled by a switch of hippocampal–cortical spine remodeling susceptible to manipulation, which in turn affects subsequent fear extinction (see Fig. 5 for a summary of the findings).

The hypothesis that fear discrimination predicts the extinction outcome was confirmed in subsequent experiments in a large naive population; these studies showed a strong correlation between the discrimination rate and fear extinction using two different methods of behavioral categorization. Indeed, animals classified as good discriminators expressed a similar extinction profile to fluoxetine-treated animals. This result indicates that basal fear discrimination guides fear reduction during/after extinction. Therefore, interventions that keep memory precise and prevent fear generalization would be

a promising therapeutic strategy to enhance fear extinction in PTSD.

Although SSRIs such as fluoxetine have been widely used to treat psychiatric disorders, the mechanisms responsible for the clinical improvements are still a matter of debate[8,9]. This study shed new light on the neurobiological process involved in the therapeutic mechanisms of fluoxetine. In fact, it has been previously shown that the mood-improving effects of fluoxetine rely on hippocampus remodeling via dendritic spine remodeling[12]. In the current study, we confirmed this finding; we observed a spine remodeling induced by chronic fluoxetine treatment and extended those findings by revealing an association between dendritic spine morphology, fear generalization, and the maintenance of hippocampus-dependent memory. However, one remaining question after the experimental setting shown here and in other studies[13,14] are the molecular and structural difference induced by fluoxetine and citalopram treatment that induce different outcomes in fear conditioning experiments.

In conclusion, we have shown that (i) chronic fluoxetine but not citalopram enhances fear discrimination and subsequent fear extinction; (ii) fluoxetine is effective in preventing fear recovery when administered after extinction training; (iii) fluoxetine slows down systems consolidation; and (iv) fluoxetine increases dendritic spine density in the hippocampus. Finally, we showed, for the first time that (v) individual differences in fear discrimination predict extinction performance. These results offer a new strategy for the treatment of fear-related disorders based on maintaining fear discrimination and hippocampal dependency. Our findings could contribute to explaining variable responses to pharmacological and behavioral interventions.

Acknowledgements
We thank Dr. Yuichi Takeuchi for helpful comments on the manuscript and Zelma Regina for her competent and kind technical assistance. This research was supported by the Brazilian National Council for Scientific and Technological Development (CNPq) and The Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) grants and Universal-2014 (process number 440413/2014–1).

Author details
[1]Laboratório de Neurobiologia da Memória, Biosciences Institute, Porto Alegre 91.501-970, Brazil. [2]Graduate Program in Neuroscience, Institute of Health Sciences, Universidade Federal do Rio Grande do Sul, Porto Alegre 90.046-900, Brazil. [3]Laboratório de Psicobiologia e Neurocomputação, Biophysics Department, Biosciences Institute, Porto Alegre 91.501-970, Brazil. [4]Department of Pharmacology, Federal University of Santa Catarina, Florianópolis, Brazil

Authors' contributions
L.K.P. and L.d.O.A. designed the study. L.K.P., R.O.S., F.N.L., W.N.-S., and M.G. performed the experiments and analyzed the data. L.K.P., R.O.S., and L.d.O.A. wrote the manuscript. All authors have reviewed the manuscript.

Compliance with ethical standard
All experiments were performed in accordance to the national animal care legislation and guidelines (Brazilian Law 11.794/2008) and approved by the Ethics Committee of the Federal University of Rio Grande do Sul.

Competing interests
The authors declare no competing interests.

Publisher's note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Received: 1 September 2018 Revised: 16 November 2018 Accepted: 1 January 2019
Published online: 31 January 2019

References

1. Jovanovic, T., Kazama, A., Bachevalier, J. & Davis, M. Impaired safety signal learning may be a biomarker of PTSD. *Neuropharmacology* **62**, 695–704 (2012).
2. Lopresto, D., Schipper, P. & Homberg, J. R. Neural circuits and mechanisms involved in fear generalization: implications for the pathophysiology and treatment of posttraumatic stress disorder. *Neurosci. Biobehav.* **60**, 31–42 (2016).
3. Kaczkurkin, A. N. et al. Neural substrates of overgeneralized conditioned fear in PTSD. *Am. J. Psychiatry* **174**, 125–134 (2017).
4. Jonas, D. E. et al. *Psychological and Pharmacological Treatments for Adults with Posttraumatic Stress Disorder (PTSD)* (Agency for Healthcare Research and Quality, Rockville (MD), 2000).
5. Archbold, G. E., Bouton, M. E. & Nader, K. Evidence for the persistence of contextual fear memories following immediate extinction. *Eur. J. Neurosci.* **31**, 1303–1311 (2010).
6. Bouton, M. E. Context, ambiguity, and unlearning: sources of relapse after behavioral extinction. *Biol. Psychiatry* **52**, 976–986 (2002).
7. Ravindran, L. N. & Stein, M. B. Pharmacotherapy of PTSD: premises, principles, and priorities. *Brain Res.* **1293**, 24–39 (2009).
8. Berton, O. & Nestler, E. J. New approaches to antidepressant drug discovery: beyond monoamines. *Nat. Rev. Neurosci.* **7**, 137–151 (2006).
9. Castrén, E. & Hen, R. Neuronal plasticity and antidepressant actions. *Trends Neurosci.* **36**, 259–267 (2013).
10. Santarelli, L. et al. Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science* **301**, 805–809 (2003).
11. Wang, J. W., David, D. J., Monckton, J. E., Battaglia, F. & Hen, R. Chronic fluoxetine stimulates maturation and synaptic plasticity of adult-born hippocampal granule cells. *J. Neurosci.* **28**, 1374–1384 (2008).
12. Bessa, J. M. et al. The mood-improving actions of antidepressants do not depend on neurogenesis but are associated with neuronal remodeling. *Mol. Psychiatry* **14**, 764–773 (2009). 739.
13. Karpova, N. N. et al. Fear erasure in mice requires synergy between antidepressant drugs and extinction training. *Science* **334**, 1731–1734 (2011).
14. Burghardt, N. S., Sigurdsson, T., Gorman, J. M., McEwen, B. S. & LeDoux, J. E. Chronic antidepressant treatment impairs the acquisition of fear extinction. *Biol. Psychiatry* **73**, 1078–1086 (2013).
15. McAvoy, K., Russo, C., Kim, S., Rankin, G. & Sahay, A. Fluoxetine induces input-specific hippocampal dendritic spine remodeling along the septo-temporal axis in adulthood and middle age. *Hippocampus* **25**, 1429–1446 (2015).
16. de Oliveira Alvares, L. et al. Periodically reactivated context memory retains its precision and dependence on the hippocampus. *Hippocampus* **22**, 1092–1095 (2012).
17. Pedraza, L. K. et al. The dynamic nature of systems consolidation: stress during learning as a switch guiding the rate of the hippocampal dependency and memory quality. *Hippocampus* **26**, 362–371 (2016).
18. Wiltgen, B. J. et al. The hippocampus plays a selective role in the retrieval of detailed contextual memories. *Curr. Biol.* **20**, 1336–1344 (2010).
19. Haubrich, J. et al. Novel learning accelerates systems consolidation of a contextual fear memory. *Hippocampus* **26**, 924–932 (2016).

20. Pedraza, L. K. et al. Sequential learning during contextual fear conditioning guides the rate of systems consolidation: implications for consolidation of multiple memory traces. *Hippocampus* **27**, 518–528 (2017).
21. Frankland, P. W., Bontempi, B., Talton, L. E., Kaczmarek, L. & Silva, A. J. The involvement of the anterior cingulate cortex in remote contextual fear memory. *Science* **304**, 881–883 (2004).
22. Bontempi, B., Laurent-Demir, C., Destrade, C. & Jaffard, R. Time dependent reorganization of brain circuitry underlying long-term memory storage. *Nature* **400**, 671–675 (1999).
23. Einarsson, E. Ö., Pors, J. & Nader, K. Systems reconsolidation reveals a selective role for the anterior cingulate cortex in generalized contextual fear memory expression. *Neuropsychopharmacology* **40**, 480–487 (2015).
24. Cullen, P. K., Gilman, T. L., Winiecki, P., Riccio, D. C. & Jasnow, A. M. Activity of the anterior cingulate cortex and ventral hippocampus underlie increases in contextual fear generalization. *Neurobiol. Learn. Mem.* **124**, 19–27 (2015).
25. Moscovitch, M. et al. Functional neuroanatomy of remote episodic, semantic and spatial memory: a unified account based on multiple trace theory. *J. Anat.* **207**, 35–66 (2005).
26. Winocur, G., Moscovitch, M. & Sekeres, M. Memory consolidation or transformation: context manipulation and hippocampal representations of memory. *Nat. Neurosci.* **10**, 555–557 (2007).
27. Winocur, G. & Moscovitch, M. Memory transformation and systems consolidation. *J. Int. Neuropsychol. Soc.* **17**, 766–780 (2011).
28. Tonegawa, S., Morrissey, M. D. & Kitamura, T. The role of engram cells in the systems consolidation of memory. *Nat. Rev. Neurosci.* **19**, 485–498 (2018).
29. Paxinos, G. & Watson, C. *The Rat Brain in Stereotaxic Coordinates* 6th edn (Academic Press, San Diego, 2007).
30. Silva, R. C. & Brandão, M. L. Acute and chronic effects of gepirone and fluoxetine in rats tested in the elevated plus-maze: an ethological analysis. *Pharmacol. Biochem. Behav.* **65**, 209–216 (2000).
31. Burghardt, N. S., Bush, D. E., McEwen, B. S. & LeDoux, J. E. Acute selective serotonin reuptake inhibitors increase conditioned fear expression: blockade with a 5-HT(2C) receptor antagonist. *Biol. Psychiatry* **62**, 1111–1118 (2007).
32. Tyler, W. J. & Pozzo-Miller, L. Miniature synaptic transmission and BDNF modulate dendritic spine growth and form in rat CA1 neurones. *J. Physiol.* **1**, 497–509 (2003).
33. Calfa, G. et al. HDAC activity is required for BDNF to increase quantal neurotransmitter release and dendritic spine density in CA1 pyramidal neurons. *Hippocampus* **22**, 1493–1500 (2012).
34. Giachero, M., Calfa, G. D. & Molina, V. A. Hippocampal structural plasticity accompanies the resulting contextual fear memory following stress and fear conditioning. *Learn. Mem.* **20**, 611–616 (2013).
35. Pozzo-Miller, L. D., Inoue, T. & Murphy, D. D. Estradiol increases spine density and NMDA-dependent Ca2+ transients in spines of CA1 pyramidal neurons from hippocampal slices. *J. Neurophysiol.* **81**, 1404–1411 (1999).
36. Murphy, D. D. & Segal, M. Regulation of dendritic spine density in cultured rat hippocampal neurons by steroid hormones. *J. Neurosci.* **16**, 4059–4068 (1996).
37. Chapleau, C. A., Larimore, J. L., Theibert, A. & Pozzo-Miller, L. Modulation of dendritic spine development and plasticity by BDNF and vesicular trafficking: fundamental roles in neurodevelopmental disorders associated with mental retardation and autism. *J. Neurodev. Disord.* **1**, 185–196 (2009).
38. Koh, I. Y., Lindquist, W. B., Zito, K., Nimchinsky, E. A. & Svoboda, K. An image analysis algorithm for dendritic spines. *Neural Comput.* **14**, 1283–1310 (2002).
39. Boda, B. et al. The mental retardation protein PAK3 contributes to synapse formation and plasticity in hippocampus. *J. Neurosci.* **24**, 10816–10825 (2004).
40. Ciocchi, S. et al. Encoding of conditioned fear in central amygdala inhibitory circuits. *Nature* **468**, 277–282 (2010).
41. Grosso, A., Santoni, G., Manassero, E., Renna, A. & Sacchetti, B. A neuronal basis for fear discrimination in the lateral amygdala. *Nat. Commun.* **9**, 1214 (2018).
42. Vervliet, B., Craske, M. G. & Hermans, D. Fear extinction and relapse: state of the art. *Annu Rev. Clin. Psychol.* **9**, 215–248 (2013).
43. Markowitsch, H. J. et al. Searching for the anatomical basis of retrograde amnesia. *J. Clin. Exp. Neuropsychol.* **15**, 947–967 (1993).
44. Teng, E. & Squire, L. R. Memory for places learned long ago is intact after hippocampal damage. *Nature* **400**, 675–677 (1999).
45. Kitamura, T. et al. Engrams and circuits crucial for systems consolidation of a memory. *Science* **356**, 73–78 (2017).
46. Restivo, L., Vetere, G., Bontempi, B. & Ammassari-Teule, M. The formation of recent and remote memory is associated with time-dependent formation of dendritic spines in the hippocampus and anterior cingulate cortex. *J. Neurosci.* **29**, 8206–8214 (2009).
47. Ipser, J. C. & Stein, D. J. Evidence-based pharmacotherapy of post-traumatic stress disorder (PTSD). *Int. J. Neuropsychopharmacol.* **15**, 825–840 (2012).
48. Hoskins, M. et al. Pharmacotherapy for posttraumatic stress disorder: systematic review and meta-analysis. *Br. J. Psychiatry* **206**, 93–100 (2015).
49. Hertzberg, M. A., Feldman, M. E., Beckham, J. C., Kudler, H. S. & Davidson, J. R. Lack of efficacy for fluoxetine in PTSD: a placebo-controlled trial in combat veterans. *Ann. Clin. Psychiatry* **12**, 101–105 (2000).
50. Lin, C. C., Tung, C. S. & Liu, Y. P. Escitalopram reversed the traumatic stress-induced depressed and anxiety-like symptoms but not the deficits of fear memory. *Psychopharmacology (Berl.)* **233**, 1135–1146 (2016).
51. Deschaux, O., Spennato, G., Moreau, J. L. & Garcia, R. Chronic treatment with fluoxetine prevents the return of extinguished auditory-cued conditioned fear. *Psychopharmacology (Berl.)* **215**, 231–237 (2011).
52. Gräff, J. et al. Epigenetic priming of memory updating during reconsolidation to attenuate remote fear memories. *Cell* **156**, 261–276 (2014).
53. Clem, R. & Huganir, R. L. Calcium-permeable AMPA receptor dynamics mediate fear memory erasure. *Science* **330**, 1108–1112 (2010).
54. Pickens, C. L., Golden, S. A., Adams-Deutsch, T., Nair, S. G. & Shaham, Y. Long-lasting incubation of conditioned fear in rats. *Biol. Psychiatry* **65**, 881–886 (2009).
55. Lesburgueres, E. et al. Early tagging of cortical networks is required for the formation of enduring associative memory. *Science* **331**, 924–928 (2011).
56. Vetere, G. et al. Spine growth in the anterior cingulate cortex is necessary for the consolidation of contextual fear memory. *Proc. Natl. Acad. Sci. USA* **108**, 8456–8460 (2011).
57. Aceti, M., Vetere, G., Novembre, G., Restivo, L. & Ammassari-Teule, M. Progression of activity and structural changes in the anterior cingulate cortex during remote memory formation. *Neurobiol. Learn. Mem.* **123**, 67–71 (2015).

III.

The World Journal of Biological Psychiatry

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/iwbp20

Systems consolidation and fear memory generalisation as a potential target for trauma-related disorders

Lizeth K. Pedraza, Rodrigo O. Sierra & Lucas de Oliveira Alvares

To cite this article: Lizeth K. Pedraza, Rodrigo O. Sierra & Lucas de Oliveira Alvares (2022): Systems consolidation and fear memory generalisation as a potential target for trauma-related disorders, The World Journal of Biological Psychiatry, DOI: 10.1080/15622975.2022.2027010

To link to this article: https://doi.org/10.1080/15622975.2022.2027010

Published online: 21 Jan 2022.

THE WORLD JOURNAL OF BIOLOGICAL PSYCHIATRY
https://doi.org/10.1080/15622975.2022.2027010

REVIEW ARTICLE

Systems consolidation and fear memory generalisation as a potential target for trauma-related disorders

Lizeth K. Pedraza[a,b], Rodrigo O. Sierra[a,b] and Lucas de Oliveira Alvares[a,c]

[a]Laboratório de Neurobiologia da Memória, Biophysics Department, Biosciences Institute, 91.501-970, Federal University of Rio Grande do Sul, Porto Alegre, Brazil; [b]Department of Physiology, University of Szeged, Szeged, Hungary; [c]Graduate Program in Neuroscience, Institute of Health Sciences, Porto Alegre, Brazil

ABSTRACT

Fear memory generalisation is a central hallmark in the broad range of anxiety and trauma-related disorders. Recent findings suggest that fear generalisation is closely related to hippocampal dependency during retrieval. In this review, we describe the current understanding about memory generalisation and its potential influence in fear attenuation through pharmacological and behavioural interventions. In light of systems consolidation framework, we propose that keeping memory precision could be a key step to enhance therapeutic outcomes.

ARTICLE HISTORY
Received 12 October 2021
Revised 31 December 2021
Accepted 5 January 2022

KEYWORDS
Fear generalisation; systems consolidation; anxiety; extinction; hippocampus

Introduction

Exposure to traumatic events during the lifetime is highly common (approximately 90%) (Kilpatrick et al. 2013; Yehuda et al. 2015). Despite the worldwide exposure to aversive experiences, the prevalence of post-traumatic stress disorder (PTSD) is about 7–8% (Kilpatrick et al. 2013), showing that resilience is predominant after trauma (Rakesh et al. 2019). A key characteristic in the diagnostic criteria for PTSD, as well as the broad range of anxiety disorders, is the fear memory generalisation. Indeed, it is a putative transdiagnostic marker in anxiety (Lissek et al. 2005, 2014; American Psychiatric Association 2013; Dymond et al. 2015). This assumption has been supported by studies showing overgeneralisation in panic disorder (Lissek et al. 2010), generalised anxiety disorder (GAD) (Lissek et al. 2014), PTSD (Kaczkurkin et al. 2017), obsessive compulsive disorder (OCD) (Apergis-Schoute et al. 2017) and phobias (McTeague et al. 2009). Generalisation can be defined as the incapacity to discriminate between similar trauma-associated stimuli. Although a certain degree of memory generalisation allows animals to display appropriate defensive responses (Asok et al. 2018), fear overgeneralisation can disrupt behavioural flexibility (Moscarello and Maren 2018) and induce resistance to fear attenuation (Pedraza et al. 2018; Zinn et al. 2020).

A growing literature suggest the close relationship between the hippocampal involvement during retrieval and memory quality (Wiltgen and Silva 2007; Pedraza et al. 2016, 2017; Kitamura et al. 2017). The hippocampus seems to be critical for expressing a detailed memory, and the gradual loss of memory precision is accompanied by a reduced hippocampal activation (Frankland and Bontempi 2005; Teixeira et al. 2006; Restivo et al. 2009; Tzakis et al. 2020). Systems consolidation has been the theoretical framework to explain this memory trace reorganisation between the hippocampus and neocortical structures (Frankland and Bontempi 2005). However, if systems consolidation has an active role during the genesis and persistence of pathological fear memories remains elusive.

In this review, we describe a new perspective regarding the role of hippocampal dependency during retrieval as a critical factor for symptoms triggered by traumatic memories. Thus, we propose a pathological accelerated systems consolidation process contributing to the genesis and persistence of trauma-related disorders. Moreover, we suggest that interventions aimed to prevent generalisation by keeping the hippocampal dependency may be an important approach to

CONTACT Lucas de Oliveira Alvares lucas.alvares@ufrgs.br; Lizeth Katherine Pedraza pedraza.lk@hotmail.com Laboratório de Neurobiologia da Memória, Departamento de Biofísica, Instituto de Biociências, Universidade Federal do Rio Grande do Sul (UFRGS), Av. Bento Gonçalves 9500, Prédio 43422, Sala 216, CEP 91.501-970, Porto Alegre, Rio Grande do Sul, Brasil; Department of Physiology, University of Szeged, Szeged, Hungary; Graduate Program in Neuroscience, Institute of Health Sciences, Porto Alegre, Brazil

enhance the clinical efficacy of exposure treatments based on reconsolidation and extinction.

Systems consolidation

Early psychological studies have described that memory loss caused by brain injuries was inversely proportional to the age of memory (Ribot 1882). That is, recent memories were more affected than remote ones. Some decades later, Scoville and Milner provided the first evidence that bilateral medial temporal lobe lesion, including the hippocampus, produces retrograde amnesia, preferentially affecting recent, instead of remote memories (Scoville and Milner 1957). Over the years, these observations have been supported by studies in amnesic patients showing the same temporal gradient (Bayley and Squire 2005; Bayley et al. 2006).

Extensive research with animal models also corroborates these results since recent, but not remote memory, is affected by hippocampal lesion (Winocur 1990; Anagnostaras et al. 1999; Maviel et al. 2004). For instance, electrolytic lesions or pharmacological inactivation of the dorsal hippocampus impairs memory for contextual fear conditioning (CFC) when performed recently after training, but not in remote timepoints (Kim and Fanselow 1992; Winocur et al. 2005; Lee et al. 2016). This pattern of temporally retrograde amnesia has been consistently described in different studies (Anagnostaras et al. 1999, Winocur 1990; Winocur et al. 2001; Maviel et al. 2004), and suggest a decreasing involvement of hippocampal activity in memory retrieval over time. Based on these reports, different theories have been formulated pointing out the role of hippocampal/cortical interactions supporting memory.

The 'standard consolidation model' assumes that neural ensembles distributed across many areas of the neocortex and associated to memory representations are initially linked only weakly; however, they become more strongly connected as they are repeatedly co-activated by the corresponding index in the hippocampus. Once this reinforcement process has been completed, the hippocampus is no longer required for the retrieval of that memory because partial inputs can activate all corresponding neocortical representations through newly formed cortico-cortical connections (Squire and Alvarez 1995). The medial prefrontal cortex (mPFC) seems to be specially involved in remote memory retrieval, in particular the anterior cingulate cortex (ACC), infralimbic (IL) and prelimbic (PL) cortex (Lopez et al. 2012; Aceti et al. 2015; Barry et al. 2016; Silva et al. 2019). Even with this preferred recruitment during remote retrieval, cortical structures are critical for acquisition and cellular consolidation, since prefrontal disruption after acquisition compromises memory persistence (Lesburguères et al. 2011; Sierra et al. 2017). Therefore, neocortical/hippocampal interactions are formed during learning, generating a synaptic route submitted to maturation in order to ensure late memory expression (Kitamura et al. 2017).

An alternative view to the standard consolidation model is the Multiple Trace Theory (MTT) and its derivation, the Trace Transformation Theory (Nadel et al. 2000; Moscovitch et al. 2005; Sekeres et al. 2018). It has been proposed to explain some amnestic situations where hippocampal and parahippocampal lesions disrupt detailed episodic memory equally for recent and remote memories (without any retrograde gradient). This framework emphasis that the retrieval of episodic memories induces a subsequent re-encoding, building multiple traces that are mediated by ensembles of hippocampal–neocortical neurons (Nadel et al. 2000). As a consequence, the core prediction of this theory claims that detailed precise episodic memories remain hippocampus-dependent regardless of the memory age.

Memory corticalization triggered by systems consolidation correlates with less susceptibility to modification or updating (Clem and Huganir 2010; Gräff et al. 2014) and in the case of fear memories are largely influenced by fear incubation (Pickens et al. 2009). Extinction-based protocols used during PTSD treatments are highly dependent on the context (i.e. hippocampus-dependency) (Bouton et al. 2006). Moreover, hippocampal dependency is closely related to the extinction efficacy (Pedraza et al. 2019). In accordance with the MTT hypothesis, we suggest that the involvement of hippocampal activity during retrieval can be a critical condition for memory reprocessing and the subsequent fear attenuation. Consequently, slowing down systems consolidation can be considered as a potential target to extend the window opportunity to modify maladaptive fear memories.

Memory precision

Fear generalisation is a common symptom affecting the everyday life of people suffering anxiety and trauma-related disorders (Jovanovic and Ressler 2010; Lissek 2012; Pitman et al. 2012; Grupe and Nitschke 2013; Dunsmoor and Paz 2015; Dymond et al. 2015). It has been shown that the severity of PTSD symptoms

is positively correlated with fear generalisation (Kaczkurkin et al. 2017). Interestingly, studies in humans have found a consistent hippocampus reduction in PTSD patients compared to subjects exposed to trauma who do not meet the diagnostic criteria for PTSD (Woon and Hedges 2008; Veer et al. 2015; Ahmed-Leitao et al. 2016, 2019). Although this correlation is well established, it is still unknown whether the hippocampus size is a risk factor for developing PTSD or is actually a consequence of the trauma.

On the other hand, individuals submitted to early-life stress express a significant volume reduction in the dentate gyrus and CA3 region (Teicher et al. 2012) and stressful experiences decrease proliferation of neural stem cells (Schoenfeld and Gould 2013; Miller and Hen 2015). Similar hippocampal changes can be observed in PTSD patients (for instances; Wang et al. 2010).

In the last decade, many studies have shown that during retrieval of recent and precise memories, the hippocampus is preferentially recruited (McHugh et al. 2007; Wiltgen et al. 2010; De Oliveira Alvares et al. 2012, 2013; Pedraza et al. 2016, 2019; Lynch et al. 2017). The temporal gradient of the hippocampus dependency reported in different studies seems to correlate with the gradient loss of memory precision during retrieval (Kim and Fanselow 1992; Winocur et al. 2005; Zelikowsky et al. 2012; Lee et al. 2016). That is, animals are prone to discriminate between training and novel contexts some days after acquisition, but there is a gradual decay in memory quality, causing a generalised fear response (Figure 1(A,B)). Importantly, fear generalisation can be evaluated using both contextual and cued fear conditioning, however, these procedures differ in the transition from precision to generalisation. In fact, cued fear conditioning, a classical paradigm claimed to be a hippocampal-independent task, is generalised faster in particular under high training intensities while contextual fear conditioning shows a switch towards generalisation gradually over time (Wiltgen and Silva 2007; De Bundel et al. 2016). Although there are mixing results regarding the role of the hippocampus in cued fear conditioning (Kim and Fanselow 1992; Phillips and LeDoux 1992; Likhtik et al. 2014; Ressler et al. 2021) the hypothesis here raised may be broadly considered since memory contextualisation is a critical factor during learning for appropriate behavioural responses, then, some degree of hippocampal involvement is expected in both cued and contextual generalisation (Xu et al. 2016). Fear generalisation might be adaptive in order to avoid situations closely related with earlier dangerous experiences. However, if the generalisation is expanded to safe stimuli that do not predict danger, it becomes extremely adverse, and represents a cardinal feature of PTSD.

Considering (i) the close relationship between generalisation and PTSD, and (ii) that memory precision (and the flip side of the coin, memory generalisation) is an epiphenomenon of hippocampal involvement, we propose that the maintenance of accurate trauma representation could be a key element to avoid generalisation. Thus, it would enhance intervention such as extinction or reconsolidation-based therapy in order to reduce fear expression.

Pharmacological and behavioural approaches aimed to reduce fear generalisation and their subsequent effects on fear attenuation

Pharmacological and behavioural manipulations applied either during memory acquisition or the

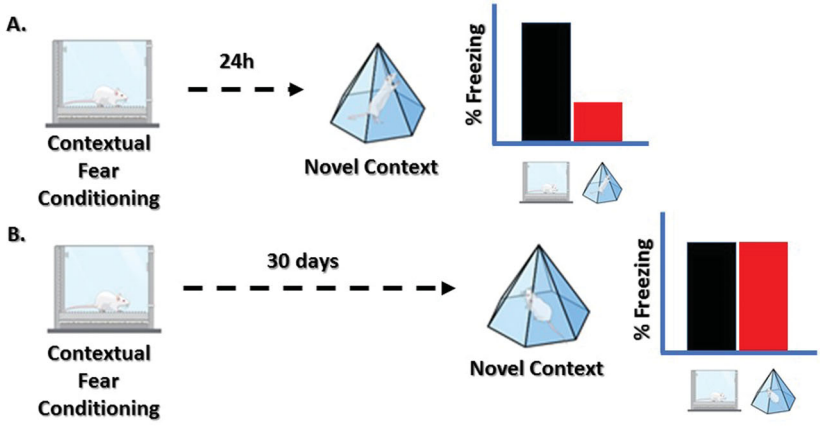


Figure 1. Fear generalisation over time. (A) Contextual fear discrimination is maintained during days and weeks after training. However, (B) generalisation take place gradually over time. This process is highly modulated by training intensity suggesting that the aversiveness of the experience determine the fear generalisation profile.

development of systems consolidation have been shown to modify fear generalisation.

Manipulating memory precision during learning

It has been shown that fear generalisation occurs gradually, with higher discrimination in recent timepoints after training (i.e. a few days) and higher generalisation a few weeks later. Is the generalisation rate constant or does it vary? In the last years, several studies have reported that the fear generalisation rate might be modulated. For instance, training intensity is an important factor determining the generalisation velocity and can be considered as a predictor of memory quality. Previous studies showed that CFC training using strong foot-shock (4×1.0 mA) induces fear generalisation 15 days after learning while low intensity training (4×0.4 mA) maintains memory precision even after 40 days (Pedraza et al. 2016). Further studies corroborated this result (Dos Santos Corrêa et al. 2019), suggesting that the conditions induced by the strong emotional training determine the fear generalisation profile. The accelerated fear generalisation can be prevented targeting the glucocorticoid system using Metyrapone before CFC or the noradrenergic transmission with the administration of propranolol after CFC (Pedraza et al. 2016). Also, the co-administration of the partial NMDA agonist D-cycloserine and the α2-adrenoceptor agonist clonidine is able to overcome the resistance to reconsolidation inhibition in generalised fear memories (Gazarini et al. 2015). In addition, fear generalisation is enhanced by acute restraint stress prior to CFC compared to unstressed animals (Bender et al. 2018).

Dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis is a well-known physiological marker after traumatic events (Roozendaal et al. 2009; Craske et al. 2017; De Quervain et al. 2017). Thus, glucocorticoid(GRs)-based treatments are currently claimed as a therapeutic option for PTSD (Schelling et al. 2006; Golier et al. 2012, 2016; Delahanty et al. 2013). Similar findings are reported in animal models where severe stress activate GRs triggering hippocampal cell apoptosis disrupting fear attenuation by extinction protocols (Araki et al. 2020). Of note, some studies in animals, including humans suggest that administration of cortisol might be target to reduce or prevent the development of PTSD (Zohar et al. 2011; Delahanty et al. 2013).

Taken together, these results indicate that the mechanisms underpinning the accelerated generalisation are triggered by the glucocorticoid and noradrenergic system activation induced by the training. Thus, manipulations surrounding the traumatic experience such as inhibiting glucocorticoid and noradrenergic transmission might prevent memory overgeneralisation and the development of trauma-related disorders.

Considering that behavioural factors such as training intensity and pharmacological manipulation early after training can change the fear generalisation rate and the hippocampal dependency, it is likely that the rate of systems consolidation itself is susceptible to manipulation, decreasing or speeding up cortical engagement and consequently the memory quality upon retrieval. Thus, pharmacological approaches surrounding the traumatic experience acting on preventing systems consolidation and fear generalisation might be an important strategy to prevent the establishment of PTSD.

Manipulating memory precision during the development of systems consolidation

Although interventions immediately after the traumatic event may be effective in avoiding generalisation, it seems difficult to use in real-life situations due to its limited window of efficacy time. Another window of opportunity to deal with generalisation is during systems consolidation. Recent studies suggests that systems consolidation is a dynamic process susceptible to manipulation. For instance, administration of the selective serotonin reuptake inhibitor (SSRI) fluoxetine for 21 days prevents fear generalisation and enhances the subsequent extinction (Pedraza et al. 2019). Interestingly, the chronic treatment with fluoxetine also maintains the hippocampal involvement during retrieval, suggesting a slow-down effect over systems consolidation. This effect was associated with a remodelling of dendritic spines in the hippocampus via increased density of mature, mushroom-type spines promoted by fluoxetine. We assume that this persistence of mature spines can be underpinning the maintenance of hippocampal involvement during retrieval delaying the maturation of neocortical memory engram cells critical for remote memories and systems consolidation (Kitamura et al. 2017). Importantly, fluoxetine treatment does not reduce fear expression itself, suggesting that it affects specifically the systems consolidation/generalisation process. However, a similar antidepressant, citalopram, fails to reproduce these effects.

Recently the non-competitive glutamate N-methyl-d-aspartate (NMDA) receptor antagonist ketamine has been shown to improve major depression (Brachman et al. 2016; Yang et al. 2018; Zanos and Gould 2018), Obsessive-Compulsive Disorder (OCD), (Davis et al.

2021) and trauma-related disorders (McGowan et al. 2017; Mastrodonato et al. 2018). Although the exact mechanism is not fully understood, ketamine administered 22 h after fear conditioning significantly decreases fear generalisation (Asim et al. 2020).

In behavioural terms, fear generalisation can be prevented by reactivating the original context (De Oliveira Alvares et al. 2012). Short-term retrieval sessions also maintain hippocampal dependency, delaying systems consolidation. A similar effect was observed by a single memory reactivation session in the original context before the exposition to a neutral environment (Pedraza et al. 2017) (Figure 2 summarises some of these recently reported findings). Memory reactivation sessions used in humans inhibits the forgetting of neutral image recognition and preserves the details of the learning experience (MacLeod et al. 2018).

Although memory reactivation is generally associated to direct exposition to any stimuli able to trigger memory retrieval, evidence suggests that imaginal-based reactivation can induce memory reconstruction successfully, allowing reconsolidation-based interventions (Agren et al. 2017; Grégoire and Greening 2019). Even though more studies are needed regarding this topic, a standardised imaginal memory reactivation may be sufficient to prevent fear generalisation and natural forgetting, facilitating clinical intervention and adherence to the treatment. Moreover, trauma reconstruction by imaginal reactivation could be less distressing compared to direct exposition.

Memory reactivation might also provide a window of opportunity to attenuate fear expression by inserting appetitive information in the background of the trauma-related memory through counterconditioning. For instance, the concomitant application during retrieval of rewarding stimulus such as morphine, caffeine, chocolate, cereal, methylphenidate or providing a very weak footshock, attenuates fear memory (Sierra et al. 2013; Haubrich et al. 2015; Pedraza et al. 2018;

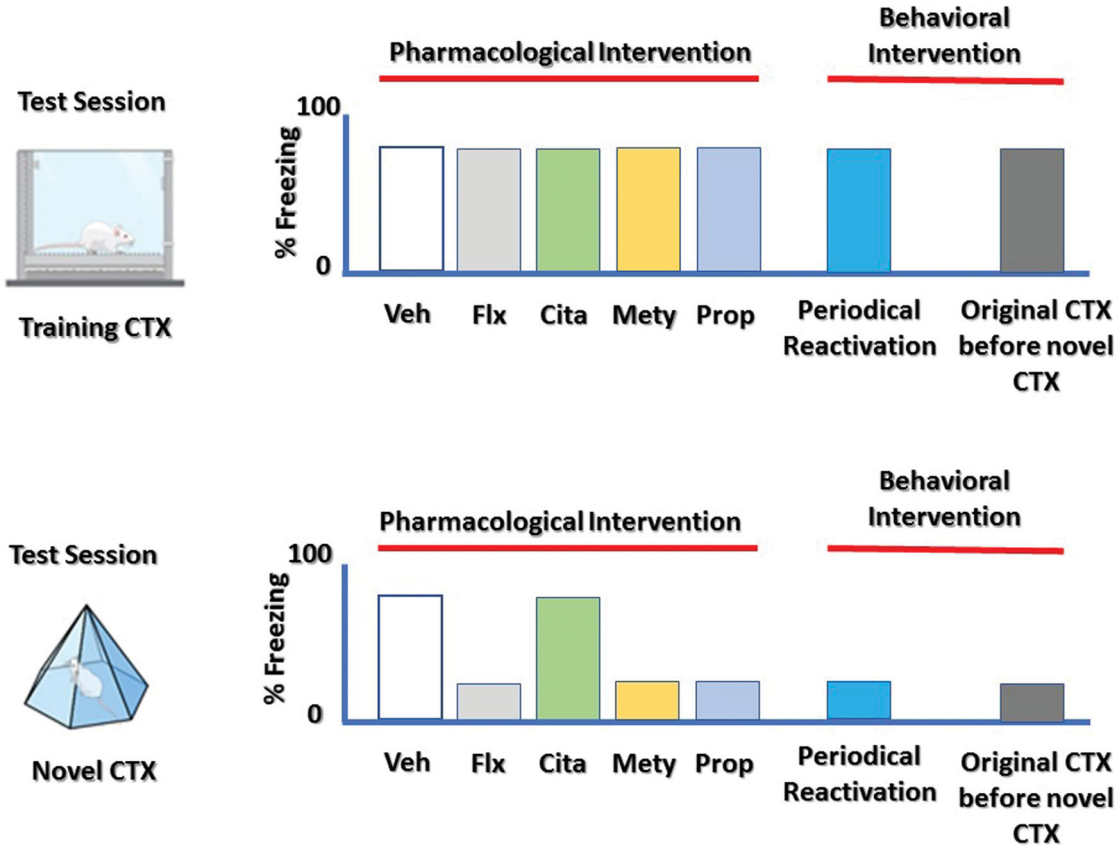


Figure 2. Pharmacological and behavioural strategies aimed to prevent memory generalisation. Studies over the last years in our lab have been shown that some treatments are able to prevent fear generalisation. In fact, the synthesis inhibitor of glucocorticoids metyrapone before CFC, the antagonist of ß adrenergic receptors propranolol after CFC and the serotonin reuptake inhibitors (SSRIs) fluoxetine over 21 days after CFC prolongs the time of contextual discrimination. Interestingly, other similar SSRIs, citalopram, was not effective to reproduce this effect. In behavioural terms, fear generalisation can be prevented by short-term retrieval sessions or using a single memory reactivation session in the original context before the exposition to a neutral environment (Veh: Vehicle; Flx: Fluoxetine; Cita: Citalopram; Mety: metyrapone; Prop: Propranolol).

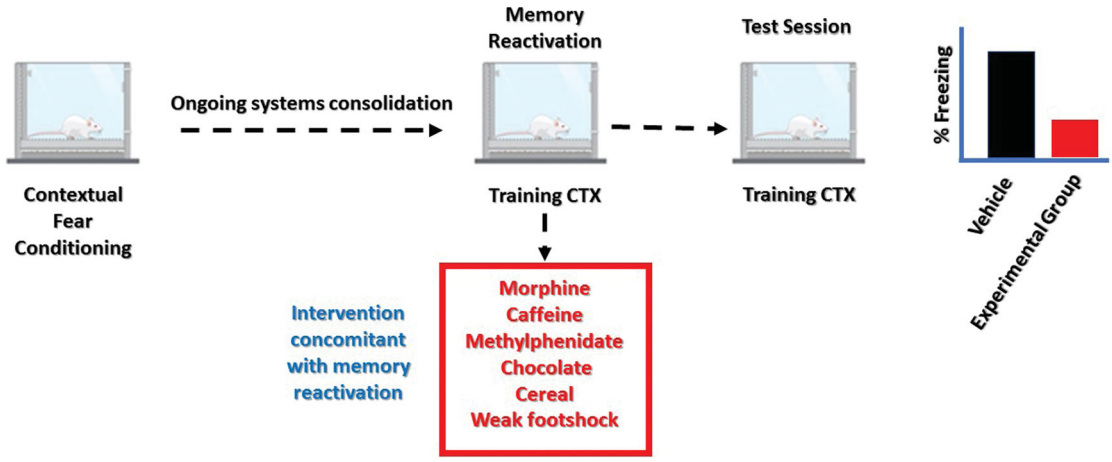


Figure 3. Memory reactivation during the ongoing systems consolidation allows emotional updating through rewarding stimuli. Rewarding stimuli such as morphine after reactivation, caffeine before reactivation, chocolate/cereal during reactivation or methylphenidate before reactivation are able to shift the emotional valence to a less aversive state through reconsolidation dependent mechanisms. The possibility to update the memory trace upon retrieval suggest that reconsolidation can change the systems consolidation itself.

Arellano Pérez et al. 2020; Popik et al. 2020) (Figure 3). All mentioned interventions were able to shift the emotional valence to a less aversive state through reconsolidation dependent mechanisms, resulting in a persistent suppression of the fear response. The experimental setting and treatments mentioned above are particularly successful when animals are tested in the same reactivation context (AAA). To note, reconsolidation interference can be also performed exposing animals to the generalised context, however, potential amnestic effects seem to be restricted to reactivation context since there is no disruption of memory retrieval performing the test in the original context (ABA) or in a different context (ABC) (Alfei et al. 2021). On the one hand, fear generalisation may promote a more extensive variety of stimuli and circumstances to reactivate fear memories independent on the original learning contingencies and apply intervention based on counter-conditioning, but on the other side, how to generate a broad therapeutic effect manipulating the stimuli presentation during reactivation remain a challenge.

Although pharmacological and behavioural interventions can prevent generalisation, the impact of these procedures during the extinction is poorly understood. Recently, Zinn et al. (2020) showed different conditions promoting fear generalisation. For instance, incomplete contextual encoding during learning induces generalisation and resistance to extinction in CFC. Notwithstanding this, a single memory reactivation session completes the contextual information, reverting fear generalisation and enhancing subsequent extinction. The authors argued that impoverished contextual representation leads to overgeneralisation and impairs fear attenuation through extinction. Likewise, it has been shown that the faster fear generalisation takes place, the worse the effectiveness of extinction (Pedraza et al. 2018).

These preclinical results suggest that different therapeutic strategies targeting cellular as well as systems consolidation can be used to modify the rate of fear generalisation with a subsequent benefit for fear attenuation by extinction or reconsolidation. This assumption is in agreement with different studies showing that early interventions following trauma may mitigate PTSD development (Rothbaum et al. 2012, 2014; Astill Wright et al. 2019; Maples-Keller et al. 2020). We propose that overcoming fear generalisation could be a boundary condition to increase the efficacy of current treatments.

Hippocampal dependency and fear generalisation as interindividual predictors of fear reduction

There is a huge heterogeneity response to pharmacological and behavioural treatments in anxiety and trauma-related disorders. It has been reported that no more than 60% of patients treated with selective serotonin reuptake inhibitors show positive responses and less than 20-30% achieve full remission (for a systematic review see Berger et al. 2009; Brady et al. 2000; Davidson et al. 2001). Beyond the urgent necessity for further research into more effective agents and based-evidence psychotherapy, these data highlight the individual variability observed in the genesis, course and treatment of neuropsychiatric illnesses that

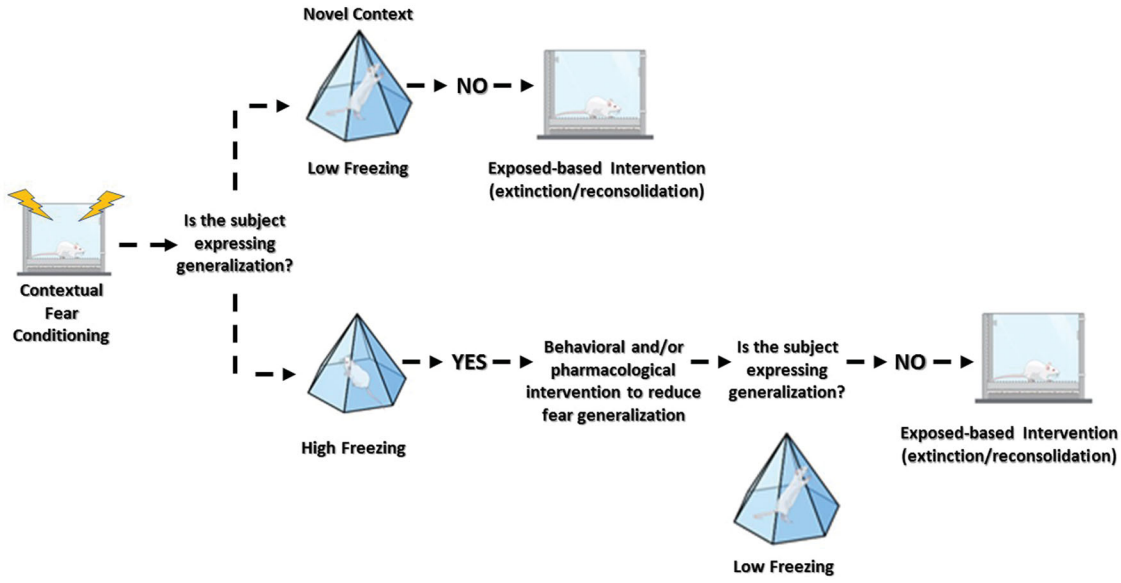


Figure 4. Fear generalisation as a putative marker to predict fear reduction under exposed-based interventions. After the initial fear conditioning, verification of lower freezing levels in a neutral context can be predictor of a subsequent succefully fear attenuation through reconsolidation or extinction. In case of persistent fear expression, pharmacological and behavioural strategies as depicted in Figure 3 can be applied to recover memory precision. Under this model, memory precision is a critical step for fear attenuation. Translated to a clinical context, protocols based on mnemonic reconstruction of the trauma can consider memory distortions such as generalisation as potential factors influencing the efficacy of therapeutical interventions.

must be taken into account in order to choose a treatment.

The assumption about the influences of individual factors is not different in animal research. Shumake et al. (2018) developed a data-driven approach in order to define a standard criterion for remission after extinction and identify individual differences in the rate of fear attenuation. The remission was achieved when the animals behaved as if they had never been conditioned (low fear responses). The criterion was based on a logistic regression analysis applied to freezing data from a large sample of rats that either underwent fear conditioning or did not. As evidenced in humans, animals can be clustered according to phenotypic extinction, highlighting the individual responses in rodents to the same training and extinction protocol. This result matches with our recent findings demonstrating that naïve animals are differentially susceptible to discrimination or generalisation after contextual fear conditioning. This heterogeneity affects the subsequent fear extinction outcome (Pedraza et al. 2019). Similar correlative individualities were found by Monfils et al. (2019) showing that CO2 reactivity is able to predict extinction phenotype in rats.

In clinical terms, brain activity and behavioural biomarkers able to predict extinction outcome could be used to guide the most appropriate treatment (e.g. pharmacotherapy, psychotherapy neuromodulation therapies or mixed approaches). Undoubtedly, the ultimate goal is the life quality improvement, reducing time intervention and increasing the adherence to the treatment. Some of these attempts to predict clinical outcome comes from machine learning algorithms trained to identify subgroups of patients. Trajectories of patients during treatment and network analysis can be used to differentiate individual factors contributing to symptoms development and prognostic (Gutner et al. 2016; Grisanzio et al. 2018; Ramos-Lima et al. 2020). Among several factors contributing to heterogeneous fear behaviour, a special attention arises around epigenetic mechanisms (Widagdo et al. 2016; Siddiqui et al. 2017; Marshall and Bredy 2019), inter and transgenerational inheritance (Arai et al. 2009; Debiec and Sullivan 2014), social fear transmission (Twining et al. 2017) and sex differences (Bangasser and Wicks 2017; Maeng et al. 2017; Velasco et al. 2019). Together, individual differences in clinical and pre-clinical research are emerging as a new framework to enhance neuropsychiatric treatments encouraging the development of a precision medicine for mental illness (van den Oord et al. 2018; Figee and Mayberg 2021; Stoyanov and Maes 2021). In this general scenario, we suggest that the rate of fear generalisation and hippocampal activation during retrieval are also inter subject differences that can predict the clinical outcome. For instances, reducing fear generalisation by pharmacological or behavioural approaches during early

intervention could enhance the subsequent effect of exposed-based therapies using reconsolidation or extinction protocols (Figure 4). In a clinical context, intervention based on mnemonic components of the trauma such as the narrative reconstruction (Mørkved et al. 2014; Sloan et al. 2018), narrative exposure therapy (Pabst et al. 2012) and written exposure therapy (Sloan et al. 2020) have shown promising results on symptoms reduction. In these approaches, patients are exposed gradually to detailed self-reconstruction of the trauma (verbal and/or written) in order to organise information about the experience in a coherent narrative (Gofman et al. 2020). Memory reconsolidation has been proposed as potential neurobiological mechanism underpinning the efficacy of narrative interventions where original memory can be overridden through reprocessing over sessions (Gofman et al. 2020) or creating competitive information (Craske et al. 2008). We hypothesise that individual trauma reconstruction could be a candidate to maintain memory precision as coadjutant of cognitive-behavioural therapy.

Remember vs forget: Are systems consolidation and memory generalisation product of natural forgetting?

Forgetting is a feature of how our brain works, allowing us to remove unnecessary information. It is thought that forgetting allows animals to express flexible behaviours, responding properly to the environment (Richards and Frankland 2017). Similarly, generalisation extends previously acquired knowledge to novel (but related) learning experiences. Interestingly, it has been suggested the generalisation of contextual fear memories reflects the forgetting of the detailed characteristics or attributes of stimuli involved in the initial learning (Riccio et al. 1992). This assumption is also based on hippocampal involvement during retrieval; that is, the hippocampus becomes less responsible to stimulus attributes during retrieval when the interval between training and testing increases (Jasnow et al. 2012). Migues et al. (2016) showed that contextual fear generalisation can be prevented by blocking mechanisms responsible for memory forgetting. In the last decade, some of the neurobiological basis of memory forgetting have been described (for a review, please see De Oliveira Alvares and Do-Monte 2021), allowing the modulation of this process in order to prevent memory generalisation.

Generalisation can be a product of memory decay and reorganisation of neural ensembles supporting memory retrieval. In other words, forgetting and generalisation may be the price to pay during systems consolidation. In contrast with the increased tendency to search for forgetting enhancers in order to attenuate the traumatic experience, we suggest that keeping memory precision by avoiding forgetting would prevent memory generalisation. Therefore, keeping memory precision during recollection of the original trauma can facilitate emotional reprocessing in a clinical context.

Conclusions and emerging questions

Several studies in the last decade have shown the close relationship between memory generalisation and hippocampal dependency. Understanding the mechanisms underpinning fear generalisation and exploring strategies to mitigate this phenomenon is crucial to developing new therapeutical approaches in anxiety and trauma-related disorders. Fear generalisation can be considered as a critical hallmark rather than collateral effect of exposure to aversive events, since pharmacological and behavioural interventions promoting memory discrimination enhance fear extinction. Natural processes governing memory trace organisation in the brain such as systems consolidation and forgetting seem to modulate fear generalisation. We hypothesise that interventions using either forgetting enhancers or modifying the hippocampal involvement during retrieval (accelerating systems consolidation) could be counterproductive in a clinical context. Instead, keeping memory precision or rebuilding the correct stimuli associated with the trauma experience will reduce the extension of fear reaction to non-related stimuli. Thus, avoiding generalisation seems to be a critical step for the positive outcome of pharmacotherapy and psychotherapy of trauma-related psychopathologies.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Author statement

All authors read and approved the final manuscript. The authors are entirely responsible for the scientific content of this paper.

Funding

This work was supported by a CNPq Universal-2018 grant to L.d.O.A. Sierra R.O is funded by the Premium Postdoctoral Research Program of the Hungarian Academy of Sciences (MTA) - Magyar Tudományos Akadémia.

ORCID

Lizeth K. Pedraza http://orcid.org/0000-0001-6132-068X

References

Aceti M, Vetere G, Novembre G, Restivo L, Ammassari-Teule M. 2015. Progression of activity and structural changes in the anterior cingulate cortex during remote memory formation. Neurobiol Learn Mem. 123:67–71.

Agren T, Björkstrand J, Fredrikson M. 2017. Disruption of human fear reconsolidation using imaginal and in vivo extinction. Behav Brain Res. 319(319):9–15.

Ahmed-Leitao F, Rosenstein D, Marx M, Young S, Korte K, Seedat S. 2019. Posttraumatic stress disorder, social anxiety disorder and childhood trauma: differences in hippocampal subfield volume. Psychiatry Res Neuroimaging. 284:45–52.

Ahmed-Leitao F, Spies G, van den Heuvel L, Seedat S. 2016. Hippocampal and amygdala volumes in adults with posttraumatic stress disorder secondary to childhood abuse or maltreatment: a systematic review. Psychiatry Res Neuroimaging. 256:33–43.

Alfei JM, De Gruy H, De Bundel D, Luyten L, Beckers T. 2021. Apparent reconsolidation interference without generalized amnesia. Prog Neuropsychopharmacol Biol Psychiatry. 108(108):110161.

American Psychiatric Association. 2013. Diagnostic and statistical manual of mental disorders. 5th ed. Arlington (VA): American Psychiatric Publishing.

Anagnostaras SG, Maren S, Fanselow MS. 1999. Temporally graded retrograde amnesia of contextual fear after hippocampal damage in rats: within-subjects examination. J Neurosci. 19(3):1106–1114.

Apergis-Schoute AM, Gillan CM, Fineberg NA, Fernandez-Egea E, Sahakian BJ, Robbins TW. 2017. Neural basis of impaired safety signaling in Obsessive Compulsive Disorder. Proc Natl Acad Sci U S A. 114(12):3216–3221.

Arai JA, Li S, Hartley DM, Feig LA. 2009. Transgenerational rescue of a genetic defect in long-term potentiation and memory formation by juvenile enrichment. J Neurosci. 29(5):1496–1502.

Araki M, Fuchikami M, Omura J, Miyagi T, Nagashima N, Okamoto Y, Morinobu S. 2020. The role of glucocorticoid receptors in the induction and prevention of hippocampal abnormalities in an animal model of posttraumatic stress disorder. Psychopharmacology (Berl). 237(7):2125–2137.

Arellano Pérez AD, Popik B, de Oliveira Alvares L. 2020. Rewarding information presented during reactivation attenuates fear memory: methylphenidate and fear memory updating. Neuropharmacology. 171:108107. https://nrdp.2015.57/nrdp.2015.57.

Asim M, Hao B, Yang YH, Fan BF, Xue L, Shi YW, Wang XG, Zhao H. 2020. Ketamine alleviates fear generalization through GluN2B-BDNF signaling in mice. Neurosci Bull. 36(2):153–164.

Asok A, Kandel ER, Rayman JB. 2018. The neurobiology of fear generalization. Front Behav Neurosci. 12:329.

Astill Wright L, Sijbrandij M, Sinnerton R, Lewis C, Roberts NP, Bisson JI. 2019. Pharmacological prevention and early treatment of post-traumatic stress disorder and acute stress disorder: a systematic review and meta-analysis. Transl Psychiatry. 9(1):334.

Bangasser DA, Wicks B. 2017. Sex-specific mechanisms for responding to stress. J Neurosci Res. 95(1-2):75–82.

Barry DN, Coogan AN, Commins S. 2016. The time course of systems consolidation of spatial memory from recent to remote retention: a comparison of the Immediate Early Genes Zif268, c-Fos and Arc. Neurobiol Learn Mem. 128:46–55.

Bayley PJ, Hopkins RO, Squire LR. 2006. The fate of old memories after medial temporal lobe damage. J Neurosci. 26(51):13311–13317.

Bayley PJ, Squire LR. 2005. Failure to acquire new semantic knowledge in patients with large medial temporal lobe lesions. Hippocampus. 15(2):273–280.

Bender CL, Otamendi A, Calfa GD, Molina VA. 2018. Prior stress promotes the generalization of contextual fear memories: involvement of the gabaergic signaling within the basolateral amygdala complex. Prog Neuropsychopharmacol Biol Psychiatry. 83:18–26.

Berger W, Mendlowicz MV, Marques-Portella C, Kinrys G, Fontenelle LF, Marmar CR, Figueira I. 2009. Pharmacologic alternatives to antidepressants in posttraumatic stress disorder: a systematic review. Prog Neuropsychopharmacol Biol Psychiatry. 33(2):169–180.

Bouton ME, Westbrook RF, Corcoran KA, Maren S. 2006. Contextual and temporal modulation of extinction: behavioral and biological mechanisms. Biol Psychiatry. 60(4):352–360.

Brachman RA, McGowan JC, Perusini JN, Lim SC, Pham TH, Faye C, Gardier AM, Mendez-David I, David DJ, Hen R, Denny CA. 2016. Ketamine as a prophylactic against stress-induced depressive-like behavior. Biol Psychiatry. 79(9):776–786.

Brady K, Pearlstein T, Asnis GM, Baker D, Rothbaum B, Sikes CR, Farfel GM. 2000. Efficacy and safety of sertraline treatment of posttraumatic stress disorder: a randomized controlled trial. JAMA. 283(14):1837–1844.

Clem RL, Huganir RL. 2010. Calcium-permeable AMPA receptor dynamics mediate fear memory erasure. Science. 330(6007):1108–1112.

Craske MG, Stein MB, Eley TC, Milad MR, Holmes A, Rapee RM, Wittchen HU. 2017. Correction: anxiety disorders. Nat Rev Dis Primers. 3:17100.

Craske MG, Kircanski K, Zelikowsky M, Mystkowski J, Chowdhury N, Baker A. 2008. Optimizing inhibitory learning during exposure therapy. Behav Res Ther. 46(1):5–27.

Davidson JR, Rothbaum BO, van der Kolk BA, Sikes CR, Farfel GM. 2001. Multicenter, double-blind comparison of sertraline and placebo in the treatment of posttraumatic stress disorder. Arch Gen Psychiatry. 58(5):485–492.

Davis GL, Minerva AR, Lario A, Simmler LD, Rodriguez CI, Gunaydin LA. 2021. Ketamine increases activity of a

fronto-striatal projection that regulates compulsive behavior in SAPAP3 knockout mice. Nat Commun. 12(1):6040.
De Bundel D, Zussy C, Espallergues J, Gerfen CR, Girault JA, Valjent E. 2016. Dopamine D2 receptors gate generalization of conditioned threat responses through mTORC1 signaling in the extended amygdala. Mol Psychiatry. 21(11):1545–1553.
De Oliveira Alvares L, Crestani AP, Cassini LF, Haubrich J, Santana F, Quillfeldt JA. 2013. Reactivation enables memory updating, precision-keeping and strengthening: exploring the possible biological roles of reconsolidation. Neuroscience. 244:42–48.
De Oliveira Alvares L, Do-Monte FH. 2021. Understanding the dynamic and destiny of memories. Neurosci Biobehav Rev. 125:592–607.
De Oliveira Alvares L, Einarsson EÖ, Santana F, Crestani AP, Haubrich J, Cassini LF, Nader K, Quillfeldt JA. 2012. Periodically reactivated context memory retains its precision and dependence on the hippocampus. Hippocampus. 22(5):1092–1095.
De Quervain D, Schwabe L, Roozendaal B. 2017. Stress, glucocorticoids and memory: implications for treating fear-related disorders. Nat Rev Neurosci. 18(1):7–19.
Debiec J, Sullivan RM. 2014. Intergenerational transmission of emotional trauma through amygdala-dependent mother-to-infant transfer of specific fear. Proc Natl Acad Sci U S A. 111(33):12222–12227.
Delahanty DL, Gabert-Quillen C, Ostrowski SA, Nugent NR, Fischer B, Morris A, Pitman RK, Bon J, Fallon W. 2013. The efficacy of initial hydrocortisone administration at preventing posttraumatic distress in adult trauma patients: a randomized trial. CNS Spectr. 18(2):103–111.
Dos Santos Corrêa M, Vaz BDS, Grisanti GDV, de Paiva JPQ, Tiba PA, Fornari RV. 2019. Relationship between footshock intensity, post-training corticosterone release and contextual fear memory specificity over time. Psychoneuroendocrinology. 110:104447.
Dunsmoor JE, Paz R. 2015. Fear generalization and anxiety: behavioral and neural mechanisms. Biol Psychiatry. 78(5): 336–343.
Dymond S, Dunsmoor JE, Vervliet B, Roche B, Hermans D. 2015. Fear generalization in humans: systematic review and implications for anxiety disorder research. Behav Ther. 46(5):561–582.
Figee M, Mayberg H. 2021. The future of personalized brain stimulation. Nat Med. 27(2):196–197.
Frankland PW, Bontempi B. 2005. The organization of recent and remote memories. Nat Rev Neurosci. 6(2):119–130.
Gazarini L, Stern CA, Piornedo RR, Takahashi RN, Bertoglio LJ. 2015. PTSD-like memory generated through enhanced noradrenergic activity is mitigated by a dual step pharmacological intervention targeting its reconsolidation. Int J Neuropsychopharmacol. 18(1):pyu026–pyu026.
Gofman M, Kivity Y, Bar-Kalifa E, Vidan Z, Hasson Ohayon I, Tuval-Mashiach R, Peri T. 2020. Narrative reconstruction as an intervention for posttraumatic stress disorder: a pilot delayed intervention quasi-randomized controlled trial. J Trauma Stress. 34:92–103.
Golier JA, Caramanica K, Demaria R, Yehuda R. 2012. A pilot study of mifepristone in combat-related PTSD. Depress Res Treat. 2012:393251.
Golier JA, Caramanica K, Michaelides AC, Makotkine I, Schmeidler J, Harvey PD, Yehuda R. 2016. A randomized, double-blind, placebo-controlled, crossover trial of mifepristone in Gulf War veterans with chronic multisymptom illness. Psychoneuroendocrinology. 64:22–30.
Gräff J, Joseph NF, Horn ME, Samiei A, Meng J, Seo J, Rei D, Bero AW, Phan TX, Wagner F, et al. 2014. Epigenetic priming of memory updating during reconsolidation to attenuate remote fear memories. Cell. 156(1–2):261–276..
Grégoire L, Greening SG. 2019. Opening the reconsolidation window using the mind's eye: extinction training during reconsolidation disrupts fear memory expression following mental imagery reactivation. Cognition. 183:277–281.
Grisanzio KA, Goldstein-Piekarski AN, Wang MY, Rashed Ahmed AP, Samara Z, Williams LM. 2018. Transdiagnostic symptom clusters and associations with brain, behavior, and daily function in mood, anxiety, and trauma disorders. JAMA Psychiatry. 75(2):201–209.
Grupe DW, Nitschke JB. 2013. Uncertainty and anticipation in anxiety: an integrated neurobiological and psychological perspective. Nat Rev Neurosci. 14(7):488–501.
Gutner CA, Galovski T, Bovin MJ, Schnurr PP. 2016. Emergence of transdiagnostic treatments for PTSD and posttraumatic distress. Curr Psychiatry Rep. 18(10):95.
Haubrich J, Crestani AP, Cassini LF, Santana F, Sierra RO, Alvares LDO, Quillfeldt JA. 2015. Reconsolidation allows fear memory to be updated to a less aversive level through the incorporation of appetitive information. Neuropsychopharmacology. 40(2):315–326.
Jasnow AM, Cullen PK, Riccio DC. 2012. Remembering another aspect of forgetting. Front Psychol. 3:175.
Jovanovic T, Ressler KJ. 2010. How the neurocircuitry and genetics of fear inhibition may inform our understanding of PTSD. AJP. 167(6):648–662.
Kaczkurkin AN, Burton PC, Chazin SM, Manbeck AB, Espensen-Sturges T, Cooper SE, Sponheim SR, Lissek S. 2017. Neural substrates of overgeneralized conditioned fear in PTSD. Am J Psychiatry. 174(2):125–134.
Kilpatrick DG, Resnick HS, Milanak ME, Miller MW, Keyes KM, Friedman MJ. 2013. National estimates of exposure to traumatic events and PTSD prevalence using DSM-IV and DSM-5 criteria. J Trauma Stress. 26(5):537–547.
Kim JJ, Fanselow MS. 1992. Modality-specific retrograde amnesia of fear. Science. 256(5057):675–677. PMID: 1585183.
Kitamura T, Ogawa SK, Roy DS, Okuyama T, Morrissey MD, Smith LM, Redondo RL, Tonegawa S. 2017. Engrams and circuits crucial for systems consolidation of a memory. Science. 356(6333):73–78.
Lee JQ, Zelinski EL, McDonald RJ, Sutherland RJ. 2016. Heterarchic reinstatement of long-term memory: a concept on hippocampal amnesia in rodent memory research. Neurosci Biobehav Rev. 71:154–166.
Lesburguères E, Gobbo OL, Alaux-Cantin S, Hambucken A, Trifilieff P, Bontempi B. 2011. Early tagging of cortical networks is required for the formation of enduring associative memory. Science. 331(6019):924–928.
Likhtik E, Stujenske JM, Topiwala MA, Harris AZ, Gordon JA. 2014. Prefrontal entrainment of amygdala activity signals safety in learned fear and innate anxiety. Nat Neurosci. 17(1):106–113.

Lissek S, Kaczkurkin AN, Rabin S, Geraci M, Pine DS, Grillon C. 2014. Generalized anxiety disorder is associated with overgeneralization of classically conditioned fear. Biol Psychiatry. 75(11):909–915.

Lissek S, Powers AS, McClure EB, Phelps EA, Woldehawariat G, Grillon C, Pine DS. 2005. Classical fear conditioning in the anxiety disorders: a meta-analysis. Behav Res Ther. 43(11):1391–1424.

Lissek S, Rabin S, Heller RE, Lukenbaugh D, Geraci M, Pine DS, Grillon C. 2010. Overgeneralization of conditioned fear as a pathogenic marker of panic disorder. Am J Psychiatry. 167(1):47–55.

Lissek S. 2012. Toward an account of clinical anxiety predicated on basic, neurally mapped mechanisms of Pavlovian fear-learning: the case for conditioned overgeneralization. Depress Anxiety. 29(4):257–263.

Lopez J, Herbeaux K, Cosquer B, Engeln M, Muller C, Lazarus C, Kelche C, Bontempi B, Cassel JC, de Vasconcelos AP. 2012. Context-dependent modulation of hippocampal and cortical recruitment during remote spatial memory retrieval. Hippocampus. 22(4):827–841.

Lynch JF, Winiecki P, Gilman TL, Adkins JM, Jasnow AM. 2017. Hippocampal GABAB(1a) receptors constrain generalized contextual fear. Neuropsychopharmacology. 42(4): 914–924.

MacLeod S, Reynolds MG, Lehmann H. 2018. The mitigating effect of repeated memory reactivations on forgetting. NPJ Sci Learn. 3:9. 018-0025-x.

Maeng LY, Cover KK, Taha MB, Landau AJ, Milad MR, Lebrón-Milad K. 2017. Estradiol shifts interactions between the infralimbic cortex and central amygdala to enhance fear extinction memory in female rats. J Neurosci Res. 95(1–2): 163–175.

Maples-Keller JL, Post LM, Price M, Goodnight JM, Burton MS, Yasinski CW, Michopoulos V, Stevens JS, Hinrichs R, Rothbaum AO, et al. 2020. Investigation of optimal dose of early intervention to prevent posttraumatic stress disorder: a multiarm randomized trial of one and three sessions of modified prolonged exposure. Depress Anxiety. 37(5):429–437.

Marshall PR, Bredy TW. 2019. Neuroepigenetic mechanisms underlying fear extinction: emerging concepts. Psychopharmacology (Berl). 236(1):133–142.

Mastrodonato A, Martinez R, Pavlova IP, LaGamma CT, Brachman RA, Robison AJ, Denny CA. 2018. Ventral CA3 activation mediates prophylactic ketamine efficacy against stress-induced depressive-like behavior. Biol Psychiatry. 84(11):846–856.

Maviel T, Durkin TP, Menzaghi F, Bontempi B. 2004. Sites of neocortical reorganization critical for remote spatial memory. Science. 305(5680):96–99.

McGowan JC, LaGamma CT, Lim SC, Tsitsiklis M, Neria Y, Brachman RA, Denny CA. 2017. Prophylactic ketamine attenuates learned fear. Neuropsychopharmacology. 42(8): 1577–1589.

McHugh TJ, Jones MW, Quinn JJ, Balthasar N, Coppari R, Elmquist JK, Lowell BB, Fanselow MS, Wilson MA, Tonegawa S. 2007. Dentate gyrus NMDA receptors mediate rapid pattern separation in the hippocampal network. Science. 317(5834):94–99.

McTeague LM, Lang PJ, Laplante MC, Cuthbert BN, Strauss CC, Bradley MM. 2009. Fearful imagery in social phobia: generalization, comorbidity, and physiological reactivity. Biol Psychiatry. 65(5):374–382.

Migues PV, Liu L, Archbold GE, Einarsson EÖ, Wong J, Bonasia K, Ko SH, Wang YT, Hardt O. 2016. Blocking synaptic removal of GluA2-containing AMPA receptors prevents the natural forgetting of long-term memories. J Neurosci. 36(12):3481–3494.

Miller BR, Hen R. 2015. The current state of the neurogenic theory of depression and anxiety. Curr Opin Neurobiol. 30:51–58.

Monfils MH, Lee HJ, Keller NE, Roquet RF, Quevedo S, Agee L, Cofresi R, Shumake J. 2019. Predicting extinction phenotype to optimize fear reduction. Psychopharmacology (Berl). 236(1):99–110.

Mørkved N, Hartmann K, Aarsheim LM, Holen D, Milde AM, Bomyea J, Thorp SR. 2014. A comparison of narrative exposure therapy and prolonged exposure therapy for PTSD. Clin Psychol Rev. 34(6):453–467.

Moscarello JM, Maren S. 2018. Flexibility in the face of fear: hippocampal-prefrontal regulation of fear and avoidance. Curr Opin Behav Sci. 19:44–49.

Moscovitch M, Rosenbaum RS, Gilboa A, Addis DR, Westmacott R, Grady C, McAndrews MP, Levine B, Black S, Winocur G, et al. 2005. Functional neuroanatomy of remote episodic, semantic and spatial memory: a unified account based on multiple trace theory. J Anat. 207(1): 35–66.

Nadel L, Samsonovich A, Ryan L, Moscovitch M. 2000. Multiple trace theory of human memory: computational, neuroimaging, and neuropsychological results. Hippocampus. 10(4):352–368.

Pabst A, Schauer M, Bernhardt K, Ruf M, Goder R, Rosentraeger R, Elbert T, Aldenhoff J, Seeck-Hirschner M. 2012. Treatment of patients with borderline personality disorder and comorbid posttraumatic stress disorder using narrative exposure therapy: a feasibility study. Psychother Psychosom. 81(1):61–63.

Pedraza LK, Sierra RO, Boos FZ, Haubrich J, Quillfeldt JA, Alvares LDO. 2016. The dynamic nature of systems consolidation: stress during learning as a switch guiding the rate of the hippocampal dependency and memory quality. Hippocampus. 26(3):362–371.

Pedraza LK, Sierra RO, Crestani AP, Quillfeldt JA, de Oliveira Alvares L. 2017. Sequential learning during contextual fear conditioning guides the rate of systems consolidation: implications for consolidation of multiple memory traces. Hippocampus. 27(5):518–528.

Pedraza LK, Sierra RO, Giachero M, Nunes-Souza W, Lotz FN, de Oliveira Alvares L. 2019. Chronic fluoxetine prevents fear memory generalization and enhances subsequent extinction by remodeling hippocampal dendritic spines and slowing down systems consolidation. Transl Psychiatry. 9(1):53.

Pedraza LK, Sierra RO, Lotz FN, de Oliveira Alvares L. 2018. Periodical reactivation under the effect of caffeine attenuates fear memory expression in rats. Sci Rep. 8(1):7260. Erratum in: Sci Rep. 2018. 8(1), 11944.

Phillips RG, LeDoux JE. 1992. Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. Behav Neurosci. 106(2):274–285.

Pickens CL, Golden SA, Adams-Deutsch T, Nair SG, Shaham Y. 2009. Long-lasting incubation of conditioned fear in rats. Biol Psychiatry. 65(10):881–886.

Pitman RK, Rasmusson AM, Koenen KC, Shin LM, Orr SP, Gilbertson MW, Milad MR, Liberzon I. 2012. Biological studies of post-traumatic stress disorder. Nat Rev Neurosci. 13(11):769–787.

Popik B, Amorim FE, Amaral OB, De Oliveira Alvares L. 2020. Shifting from fear to safety through deconditioning-update. Elife. 9:e51207.

Rakesh G, Morey RA, Zannas AS, Malik Z, Marx CE, Clausen AN, Kritzer MD, Szabo ST. 2019. Resilience as a translational endpoint in the treatment of PTSD. Mol Psychiatry. 24(9):1268–1283.

Ramos-Lima LF, Waikamp V, Antonelli-Salgado T, Passos IC, Freitas LHM. 2020. The use of machine learning techniques in trauma-related disorders: a systematic review. J Psychiatr Res. 121:159–172.

Ressler RL, Goode TD, Kim S, Ramanathan KR, Maren S. 2021. Covert capture and attenuation of a hippocampus-dependent fear memory. Nat Neurosci. 24(5):677–684.

Restivo L, Vetere G, Bontempi B, Ammassari-Teule M. 2009. The formation of recent and remote memory is associated with time-dependent formation of dendritic spines in the hippocampus and anterior cingulate cortex. J Neurosci. 29(25):8206–8214.

Ribot T. 1882. The diseases of memory: An essay in the positive psychology. Smith WH, Translator. London: Kegan Paul, Trench, & Co.

Riccio DC, Ackil J, Burch-Vernon A. 1992. Forgetting of stimulus attributes: methodological implications for assessing associative phenomena. Psychol Bull. 112(3):433–445.

Richards BA, Frankland PW. 2017. The persistence and transience of memory. Neuron. 94(6):1071–1084.

Roozendaal B, McEwen BS, Chattarji S. 2009. Stress, memory and the amygdala. Nat Rev Neurosci. 10 (6):423–433.

Rothbaum BO, Kearns MC, Price M, Malcoun E, Davis M, Ressler KJ, Lang D, Houry D. 2012. Early intervention may prevent the development of posttraumatic stress disorder: a randomized pilot civilian study with modified prolonged exposure. Biol Psychiatry. 72(11):957–963.

Rothbaum BO, Kearns MC, Reiser E, Davis JS, Kerley KA, Rothbaum AO, Mercer KB, Price M, Houry D, Ressler KJ. 2014. Early intervention following trauma may mitigate genetic risk for PTSD in civilians: a pilot prospective emergency department study. J Clin Psychiatry. 75(12): 1380–1387.

Schelling G, Roozendaal B, Krauseneck T, Schmoelz M, DE Quervain D, Briegel J. 2006. Efficacy of hydrocortisone in preventing posttraumatic stress disorder following critical illness and major surgery. Ann N Y Acad Sci. 1071:46–53.

Schoenfeld TJ, Gould E. 2013. Differential effects of stress and glucocorticoids on adult neurogenesis. Curr Top Behav Neurosci. 15:139–164.

Scoville WB, Milner B. 1957. Loss of recent memory after bilateral hippocampal lesions. J Neurol Neurosurg Psychiatry. 20(1):11–21.

Sekeres MJ, Winocur G, Moscovitch M. 2018. The hippocampus and related neocortical structures in memory transformation. Neurosci Lett. 680:39–53.

Shumake J, Jones C, Auchter A, Monfils MH. 2018. Data-driven criteria to assess fear remission and phenotypic variability of extinction in rats. Phil Trans R Soc B. 373(1742):20170035.

Siddiqui AS, Singh S, Ranjan V, Ugale R, Saha S, Prakash A. 2017. Enhanced histone acetylation in the infralimbic prefrontal cortex is associated with fear extinction. Cell Mol Neurobiol. 37(7):1287–1301.

Sierra RO, Cassini LF, Santana F, Crestani AP, Duran JM, Haubrich J, de Oliveira Alvares L, Quillfeldt JA. 2013. Reconsolidation may incorporate state-dependency into previously consolidated memories. Learn Mem. 20(7): 379–387.

Sierra RO, Pedraza LK, Zanona QK, Santana F, Boos FZ, Crestani AP, Haubrich J, de Oliveira Alvares L, Calcagnotto ME, Quillfeldt JA. 2017. Reconsolidation-induced rescue of a remote fear memory blocked by an early cortical inhibition: involvement of the anterior cingulate cortex and the mediation by the thalamic nucleus reuniens. Hippocampus. 27(5):596–607.

Silva BA, Burns AM, Gräff J. 2019. A cFos activation map of remote fear memory attenuation. Psychopharmacology (Berl). 236(1):369–381.

Sloan DM, Marx BP, Lee DJ. 2018. Written exposure therapy vs cognitive processing therapy-reply. JAMA Psychiatry. 75(7):758–759.

Sloan DM, Marx BP, Resick PA, Young-McCaughan S, Dondanville KA, Mintz J, Litz BT, Peterson AL, STRONG STAR Consortium. 2020. Study design comparing written exposure therapy to cognitive processing therapy for PTSD among military service members: a noninferiority trial. Contemp Clin Trials Commun. 17:100507.

Squire LR, Alvarez P. 1995. Retrograde amnesia and memory consolidation: a neurobiological perspective. Curr Opin Neurobiol. 5(2):169–177.

Stoyanov D, Maes MH. 2021. How to construct neuroscience-informed psychiatric classification? Towards nomothetic networks psychiatry. World J Psychiatry. 11(1):1–12.

Teicher MH, Anderson CM, Polcari A. 2012. Childhood maltreatment is associated with reduced volume in the hippocampal subfields CA3, dentate gyrus, and subiculum. Proc Natl Acad Sci U S A. 109(9):E563–E572.

Teixeira CM, Pomedli SR, Maei HR, Kee N, Frankland PW. 2006. Involvement of the anterior cingulate cortex in the expression of remote spatial memory. J Neurosci. 26(29): 7555–7564. https://doi.org/10.1523/JNEUROSCI.1068-06.2006.

Twining RC, Vantrease JE, Love S, Padival M, Rosenkranz JA. 2017. An intra-amygdala circuit specifically regulates social fear learning. Nat Neurosci. 20(3):459–469.

Tzakis N, Bosnic T, Holahan MR. 2020. Hippocampal and anterior cingulate cortex contribution to the processing of recently-acquired and remotely stored spatial memories in rats trained during preadolescence. Neurobiol Learn Mem. 173:107271.

van den Oord EJCG, Chan RF, Aberg KA. 2018. Successes and challenges in precision medicine in psychiatry. JAMA Psychiatry. 75(12):1269–1270.

Veer IM, Oei NY, van Buchem MA, Spinhoven P, Elzinga BM, Rombouts SA. 2015. Evidence for smaller right amygdala volumes in posttraumatic stress disorder following childhood trauma. Psychiatry Res. 233(3):436–442.

Velasco ER, Florido A, Milad MR, Andero R. 2019. Sex differences in fear extinction. Neurosci Biobehav Rev. 103: 81–108.

Wang Z, Neylan TC, Mueller SG, Lenoci M, Truran D, Marmar CR, Weiner MW, Schuff N. 2010. Magnetic resonance imaging of hippocampal subfields in posttraumatic stress disorder. Arch Gen Psychiatry. 67(3):296–303.

Widagdo J, Zhao QY, Kempen MJ, Tan MC, Ratnu VS, Wei W, Leighton L, Spadaro PA, Edson J, Anggono V, et al. 2016. Experience-dependent accumulation of N6-methyladenosine in the prefrontal cortex is associated with memory processes in mice. J Neurosci. 36(25):6771–6777.

Wiltgen BJ, Silva AJ. 2007. Memory for context becomes less specific with time. Learn Mem. 14(4):313–317.

Wiltgen BJ, Zhou M, Cai Y, Balaji J, Karlsson MG, Parivash SN, Li W, Silva AJ. 2010. The hippocampus plays a selective role in the retrieval of detailed contextual memories. Curr Biol. 20(15):1336–1344.

Winocur G, McDonald RM, Moscovitch M. 2001. Anterograde and retrograde amnesia in rats with large hippocampal lesions. Hippocampus. 11(1):18–26.

Winocur G, Moscovitch M, Caruana DA, Binns MA. 2005. Retrograde amnesia in rats with lesions to the hippocampus on a test of spatial memory. Neuropsychologia. 43(11):1580–1590.

Winocur G. 1990. Anterograde and retrograde amnesia in rats with dorsal hippocampal or dorsomedial thalamic lesions. Behav Brain Res. 38(2):145–154.

Woon FL, Hedges DW. 2008. Hippocampal and amygdala volumes in children and adults with childhood maltreatment-related posttraumatic stress disorder: a meta-analysis. Hippocampus. 18(8):729–736.

Xu C, Krabbe S, Gründemann J, Botta P, Fadok JP, Osakada F, Saur D, Grewe BF, Schnitzer MJ, Callaway EM, et al. 2016. Distinct hippocampal pathways mediate dissociable roles of context in memory retrieval. Cell. 167(4): 961–972.e16.

Yang Y, Cui Y, Sang K, Dong Y, Ni Z, Ma S, Hu H. 2018. Ketamine blocks bursting in the lateral habenula to rapidly relieve depression. Nature. 554(7692):317–322.

Yehuda R, Hoge CW, McFarlane AC, Vermetten E, Lanius RA, Nievergelt CM, Hobfoll SE, Koenen KC, Neylan TC, Hyman SE. 2015. Post-traumatic stress disorder. Nat Rev Dis Primers. 1:15057. https://doi.org/10.1038/nrdp.2015.57.

Zanos P, Gould TD. 2018. Mechanisms of ketamine action as an antidepressant. Mol Psychiatry. 23(4):801–811.

Zelikowsky M, Bissiere S, Fanselow MS. 2012. Contextual fear memories formed in the absence of the dorsal hippocampus decay across time. J Neurosci. 32(10):3393–3397.

Zinn R, Leake J, Krasne FB, Corbit LH, Fanselow MS, Vissel B. 2020. Maladaptive properties of context-impoverished memories. Curr Biol. 30(12):2300–2311.e6.

Zohar J, Juven-Wetzler A, Sonnino R, Cwikel-Hamzany S, Balaban E, Cohen H. 2011. New insights into secondary prevention in post-traumatic stress disorder. Dialogues Clin Neurosci. 13(3):301–309.