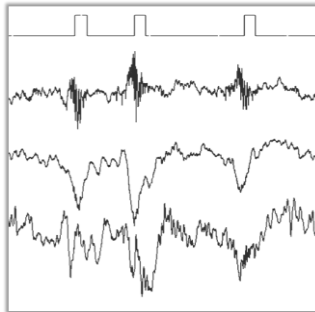University of Szeged

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

Summary of the Ph.D. thesis

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Introduction

Neuroscience faces the significant challenge of understanding the neurobiology of neuropsychiatric disorders to develop safer, more effective, and precise treatments. This study aimed to create a closed-loop neuromodulation method, using oscillatory markers of memory consolidation, to reduce fear memories.

Posttraumatic stress disorder (PTSD), is an incapacitating chronic disorder that results from exposure to stressful or life-threatening events, exhibiting significant resistance to both psychotherapy and pharmacotherapy, leading to low remission rates. Fear conditioning is used to model core aspects of PTSD, such as long-lasting and generalized fear expression.

PTSD is thought to be a memory-based disorder, characterized by either hypermnesia or explicit amnesia for trauma-related stimuli and fear generalization to non-trauma-related stimuli. Those memories that are crucial for survival and adaptation are resistant to forgetting but can be suppressed through extinction learning. PTSD is marked by impaired extinction, promoting memory intrusions in inappropriate contexts.

It is suggested that fear memory traces can be updated post-reactivation or during extinction with emotional content, promoting fear reduction. Despite the success of deep-brain stimulation (DBS) in

treating movement disorders, open-loop (OL) approaches used in psychiatric conditions often lack specificity. The recent use of on-demand closed-loop electrical stimulation (CL) in treating "oscillopathies", is a promising approach for psychiatric conditions.

This study presents the first demonstration of a closed-loop intervention to update the emotional information of fear memory traces through intracranial electrical stimulation, suggesting new directions for developing CL neuromodulation technologies for anxiety, trauma- and stressor-related disorders.

Aims

The aim of my doctoral work was to create a CL neuromodulation method, guided by oscillatory markers of memory consolidation and electrically stimulate reward and anxiety responses related brain regions. I evaluated the effects of this method on the resistance to extinction and fear generalization of cued fear conditioning.

Specific aims

- To develop a CL neuromodulation system detecting real time Sharp Wave Ripples (SWRs) and deliver an intracranial anxiolytic signal through infralimbic cortex (IL) stimulation and evaluate its effects during the consolidation of fear conditioning.

- To develop a CL neuromodulation system detecting real time SWRs and deliver basolateral amygdala (BLA) stimulation and evaluate its effects during the reconsolidation of fear conditioning.

- To develop a CL neuromodulation system detecting real time SWRs and deliver an intracranial reward signal through the medial forebrain bundle (MFB) stimulation and evaluate its effects during the consolidation of fear extinction.

Material and methods

Surgery

Animals were anesthetized with 2% isoflurane and had electrodes implanted in the IL, Anterior cingulate cortex (ACC), BLA, and bilateral CA1 of the dorsal hippocampus. Depending on the experiment, a bipolar stimulation electrode was placed in the IL, BLA, MFB or ventral hippocampal commissure (VHC). A Faraday cage was created using copper mesh and dental acrylic. In experiments involving pharmacological infusion, 25-gauge guide cannulas were implanted above the BLA.

Electrophysiological recordings and stimulation

The multiplexed signals were acquired at 500 Hz per channel for CL neuromodulation experiments. The neuronal signals were preamplified (total gain 400X), multiplexed on head and stored after digitalization at 20-kHz sampling rate per channel. During stimulation, preamplified signals were analyzed on-line by a programmable digital signal processor (RX-8, TDT) using a custom-made SWRs detection algorithm.

SWRs detection triggered train stimulation (10 pulses, 0.2-ms width at 100 µA, 50 Hz) in the IL or BLA, single pulse (5-15V) in VHC or stimulation train (lasting 100 ms and composed of fourteen 1-ms long, 100µA square-wave pulses at 140 Hz) in the MFB. Stimulation were performed after training, after reactivation or after extinction depending on the experiment.

Auditory fear conditioning

In the fear conditioning apparatus, animals underwent multiple sessions in four different contexts (A-D). Day 1 was habituation with tone presentations (CS+ and CS-) in context 'A'. Day 2 involved cue fear conditioning with CS+ footshock pairings in context 'B'. Two days post-training, reactivation occurred in context 'A' with 4 CS+ presentations without footshocks. Tests were performed on days 3-6 in context 'A' with 5 CS+ or CS- presentations. Extinction was performed in context

'C', one session or multiple sessions until achieve fear reduction to ≤ 20%, consisting of 20 CS+ presentations without footshocks. Renewal or remote tests were performed 24 hours or 25 days after extinction in context 'D'. Fear recovery involved a footshock in a neutral environment. A reinstatement test occurred in context 'D' 24 hours post-shock. Freezing behavior, analyzed off-line, served as a memory index. After transcardiac perfusion and postfixation, coronal sections were prepared for histological verification. Population results are presented as median and interquartile range.

Results

<u>Real-time detection of SWRs guiding closed-loop stimulation of the infralimbic cortex during memory consolidation prevents fear generalization</u>

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. Providing IL stimulation during the precise time of memory replay via SWRs, a positive emotional valence can 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 48h after training, a group received jittered stimulation (OL; OL), and a control group received no stimulation.

Average online detection rate of SWRs was 80.38 ± 1.349%.

Animals exposed to CL immediately after training expressed less fear responses to the CS- compared with all groups during test (CL 10min = median: 36.54, IQR 13.08 to 60; NS = median: 65.78, IQR 61.07 to 70.5; OL = median: 72.6, IQR 67.44 to 77.76; CL 48 h = median: 64.27, IQR 57.11 to 71.42). This facilitation was confirmed in the discrimination index. In the renewal, animals exposed immediately to CL expressed lower fear reaction compared to the other groups (CL 10min = median: 12.74, IQR 7.433 to 29.67; NS = median: 50.4, IQR 24.4 to 51.33; OL = median: 53.8, IQR 41.8 to 75.03; CL 48 h = median: 36.87, IQR 23.8 to 61.9). Finally, during reinstatement, CL animals kept low fear expression even after footshock exposition.

A relationship between memory precision and fear extinction enhancement was confirmed by significant negative correlation between the discrimination index and fear behavior during renewal (slope differed from zero, $p < 0.01$, $r^2 = 0.2824$).

We found that CL stimulation increases gamma incidence in BLA between pre and after stimulation   . However, this effect was absent in OL animals. There is a significant positive correlation between gamma incidence and fear discrimination (slope differed from zero, $p < 0.05$, $r^2 = 0.4158$).

Real-time detection of SWRs guiding closed-loop stimulation of the basolateral amygdala during memory reconsolidation reverts fear generalization

Anatomically connection between IL and BLA provides inhibitory circuits in amygdala implicated in the reduction of fear and anxiety responses. We decide to test whether direct CL-BLA stimulation could modify fear memory intensity or generalization when applied after reactivation, following the rationale of emotional updating.

After the stimulation, CL animals expressed less fear behavior compared OL during the CS- test (CL = median: 32.9, IQR 20.84 to 44.97; OL = median: 47.89, IQR 45 to 50.78). This facilitation was confirmed in the discrimination index   . During the renewal test, CL animals expressed less fear reactions compared to OL(CL = median: 17.33, IQR 12.67 to 23.47; OL = median: 41.47, IQR 33.13 to 52.73) but not during test after reinstatement.

Real-time detection of SWRs guiding closed-loop stimulation of the medial forebrain bundle enhance fear extinction

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.

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. Recording and stimulation session was performed one hour following each extinction session.

Animals exposed to CL stimulation required fewer extinction sessions to achieve the remission criterion compared to all groups (CL = median: 2, IQR 2 to 4; NS = median: 5, IQR 4 to 7; OL = median: 6, IQR 5 to 7) 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 (CL = median: 8.373, IQR 3.967 to 11.4; NS = median: 34, IQR 27.33 to 47.33; OL = median: 36.8, IQR 32.33 to 57.73).

To assess the persistence of the effects, animals were exposed to a remote test 25 days following the renewal. Freezing in CL stimulated animals kept low levels compared to the other groups (CL = median: 10.93, IQR 6.667 to 25.53; NS = median: 39.33, IQR 26.8 to 58.53; OL =

median: 48.27, IQR 25.47 to 51), suggesting that fear attenuation induced by CL-MFB stimulation was persistent.

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 (CL = median: -62.07, IQR -79.27 to -51.93; NS = median: -17.2, IQR -37.2 to -12.67; OL = median: -30.27, IQR -49.27 to -9.467), supporting the hypothesis that neuromodulation of the reward system accelerates fear extinction.

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

Next, we decided to test the causal role of SWRs. Following the experiment with extinction criterion, after each extinction session, online SWRs triggered a single-pulse (0.5 ms) stimulation of the VHC.

Animals that experienced SWRs disruption required more extinction sessions to achieve an 80% reduction in freezing compared the OL group (CL = median: 4, IQR 3 to 6; OL = median: 3, IQR 2 to 3). Additionally, CL animals expressed elevated levels of fear reaction during the renewal test (CL = median: 43.47, IQR 23.6 to 60.93; OL = median: 10.67, IQR 5.2 to 20.13). These results suggest that hippocampal SWRs are essential for consolidating fear extinction.

Discussion

Our study demonstrates the potential of SWRs, a memory consolidation marker, for triggering CL brain stimulation to attenuate fear. We revealed the applicability 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.

Memory is a continuous process vulnerable to interference after learning and retrieval. Animal models suppressing fear memories disrupt memory consolidation or impair reconsolidation post-retrieval. Fear attenuation during extinction emerge as a new inhibitory learning, which fades over time, allowing fear recovery. Thus, enhancing extinction and disrupting reconsolidation are primary strategies for treating fear and anxiety disorders.

Modifying the emotional content of memories can decrease maladaptive fear responses. For instance, reactivating memory with rewarding stimuli reduces the fear from conditioning. Here, stimuli predicting punishment are associated with reward. This counterconditioning process has been used in therapies like systematic desensitization.

DBS-IL has been linked to enhanced fear extinction and anxiolytic, antidepressant responses. Our findings suggest that IL stimulation during SWRs can mitigate fear conditioning aversiveness via precise counterconditioning.

Our study suggests that the role of SWRs extends beyond contextual encoding (hippocampal-dependent) to non-hippocampal dependent memories like cued fear conditioning, despite the hippocampus being viewed primarily as a navigation system. Evidence from other studies supports this perspective. Lesions or inactivation of the IL result in memory generalization and resistance to extinction. IL activity right after acquisition, but not six hours later, controls memory generalization, aligning with our findings where CL-IL stimulation only affected fear generalization.

Importantly, only CL animals demonstrated this effect, showing the dependence of SWRs and IL stimulation pairing. Despite similar stimulation in OL animals, no differences were detected in fear expression and generalization, confirming our hypothesis that SWRs mediate fear generalization but not memory consolidation in non-hippocampal dependent tasks.

Preventing fear generalization enhanced fear extinction, correlating with previous reports. Overgeneralization and resistance to extinction are hallmarks in PTSD, and studies have shown that overcoming fear generalization is critical for successful extinction.

The results replication using CL-BLA stimulation confirmed that the inhibitory role of IL is achieved through increased gamma incidence in the BLA. Amygdala hyperactivity is prominent in PTSD, while amygdala-compromising brain lesions act as a protective factor. DBS stimulation reduces PTSD symptoms in human trials.

The hypothesis that high-frequency electrical stimulation generates suppressive effects in targeted brain areas could explain some of our results. Both IL and BLA stimulation resulted in increased BLA gamma activity during CL stimulation, leading us to propose gamma oscillation as the neurobiological scaffolding of the results reported in this study. Besides fear generalization, gamma oscillations in the BLA may be critical to achieve fear extinction. Further research should explore whether selective gamma suppression in the BLA could induce fear generalization and impair fear extinction.

Despite several behavioral and cellular differences between consolidation and reconsolidation, the similarity between the results of CL-IL and CL-BLA stimulation supports our interpretation that emotional updating applies to both. Like consolidation, reconsolidation requires sleep to prevent interference and promote long-term retrieval, potentially involving similar oscillatory coupled activity.

In the case of extinction, we used the same principles of CL stimulation, changing potential anxiolysis induced by IL stimulation to a reward

signal. For this, SWRs detection triggered electrical stimulation of the MFB, selected biomarker due to its well-known rewarding properties and its translational value recognized by FDA-approved clinical trials.

We showed that SWRs-triggered MFB stimulation provides a reward safety signal during fear extinction enhancing fear attenuation and reducing persistently fear expression over time. Thus, the proposed mechanism could be summarized as follows: 1) SWRs reactivate the memory trace in the BLA, 2) CL-MFB stimulation promotes concurrent dopamine release, and 3) this dopamine release in "extinction cells" enhances fear reduction.

We also explore the role of SWRs in the consolidation of emotional memories. While SWRs were crucial for updating emotional content of cued fear memories during extinction, SWRs disruption is not sufficient to completely block this process. Thus, SWRs are partially required for extinction.

Our findings open several questions for future studies: Can this approach be generalized to both genders? How do SWRs mediate the consolidation of non-hippocampal dependent memories, such as cued-fear conditioning? Can these results be generalized to a hypothetical spindle/slow wave CL modulation approach?

In summary, our fear-related memory study and mitigation framework relies on CL stimulation guided by classical memory consolidation

biomarkers. We propose that disorders such as PTSD could be conceptualized as memory-based rather than anxiety-based. The modification of memory engrams related to these memories could lead to an immediate decrease in stress and anxiety-related symptomatology. This is especially promising considering the current attempts to enhance reconsolidation protocols in humans, capable of reactivating specific memories.

Acknowledgement

Publications related to the subject of the thesis

1. **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. *** Equal contribution**

2. **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: the official journal of the World Federation of Societies of Biological Psychiatry, 23(9), 653–665.

3. **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.