On the resilience of remote traumatic memories against exposure therapy-mediated attenuation

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Abstract

How to attenuate traumatic memories has long been the focus of intensive research efforts, as traumatic memories are extremely persistent and heavily impinge on the quality of life. Despite the fact that traumatic memories are often not readily amenable to immediate intervention, surprisingly few studies have investigated treatment options for remote traumata in animal models. The few that have unanimously concluded that exposure therapy-based approaches, the most successful behavioral intervention for the attenuation of recent forms of traumata in humans, fail to effectively reduce remote fear memories. Here, we provide an overview of these animal studies with an emphasis on why remote traumatic memories might be refractory to behavioral interventions: A lack of neuroplasticity in brain areas relevant for learning and memory emerges as a common denominator of such resilience. We then outline the findings of a recent study in mice showing that by combining exposure therapy-like approaches with small molecule inhibitors of histone deacetylases (HDACis), even remote memories can be persistently attenuated. This pharmacological intervention reinstated neuroplasticity to levels comparable to those found upon successful attenuation of recent memories. Thus, HDACis—or any other agent capable of heightening neuroplasticity—in conjunction with exposure therapy-based treatments might constitute a promising approach to overcome remote traumata.

Keywords epigenetic; extinction; fear; histone acetylation; remote memories

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See also Glossary for abbreviations used in this article.

Introduction

Post-traumatic stress and other anxiety disorders range among the most enduring forms of memories: Remembrances of traumata months later in rodents [1,2] and years after the original insult in humans are commonplace [3,4]. The lifetime prevalence of

post-traumatic stress disorder (PTSD) in the general population is estimated at 7% [5], a number that at least quadruples among persons having suffered severe traumata such as war or sexual assault [6,7]. Because of the persistent nature of traumatic memories, early interventions are considered of prime importance [6,8,9]. Yet, early interventions are oftentimes not readily available, placing equal or even greater emphasis on finding effective treatment options for remote traumata [10,11].

As memories mature, they are thought to undergo a gradual stabilization process termed memory consolidation [12]. Such stabilization occurs on two levels [13]. First, on a cellular level, where older memories become independent of new protein synthesis and gene expression, as evidenced by the administration of translation and transcription blockers at different intervals post-training [14]. Second, on a systems level, where older memories become increasingly independent of anatomical structures subserving memory formation, such as the hippocampus, but progressively dependent on brain regions subserving memory storage, such as different cortical areas [15]. This second level of consolidation is accounted for by cases of temporally graded retrograde amnesia, in which recently acquired memories are more affected by damage to the brain area of memory formation, for example the hippocampus, than memories acquired a long time ago [16,17]. Owing to this dual stabilization process, older memories are considered to be more stable and, consequently, more difficult to erase. By extension, such stability represents a second line of argument to investigate treatment options for remote traumata.

Once stabilized or consolidated, however, memories are not etched in stone. Instead, they undergo a second phase of protein synthesis dependency after being reactivated [18,19]. This process of memory reconsolidation is thought to allow the memory to incorporate new information pertinent to the current environmental contingencies that might no longer be the same as at the time of memory formation [20–24]. Thereby, memory reconsolidation is postulated to promote memory maintenance—in the case of a status quo between the situations encountered at learning and recall; memory strengthening—in the case of a higher valence encountered at recall; or memory weakening—in the case of a lower valence encountered at recall [21]. This third scenario is ideally suited to integrate neutral or non-fear-eliciting information into a fearful

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Glossary

ACC	anterior cingulate cortex
CP-AMPA	calcium-permeable AMPA receptor
CR	conditioned response
CS	conditioned stimulus
Cys	cysteine
DCS	D-cycloserine
fMRI	functional magnetic resonance imaging
HDAC1	histone deacetylase 1
HDAC2	histone deacetylase 2
HDACi	histone deacetylase inhibitor
NO	nitric oxide
PNNs	perineuronal nets
PTSD	post-traumatic stress disorder
SR	spontaneous recovery
US	unconditioned stimulus
VEH	vehicle

memory so that the fear component of a memory is updated toward one of safety and no longer persists in its original form [25].

Reconsolidation-updating mechanisms are a fundamental principle of exposure-based therapies, the most efficient treatment for anxiety-related disorders in humans [26,27]. In exposure-based therapy, patients undergo a repetitive re-exposure to the fear-eliciting stimulus in a safe environment, with the premise that the fear will eventually subside. To be fully effective, the repetitive exposure needs to be administered between 10 min and 6 h post-memory recall, during the so-called reconsolidation window, in which reactivated memories were found to be prone to behavioral disruption. When doing so, recent fear memories could be successfully and permanently attenuated in both rodents and humans alike [28,29]. However, it remained until recently unclear whether these approaches would also efficiently attenuate remote, that is, month-old fear memories. Indeed, several lines of evidence suggest that despite memory reactivation, remote memories do not enter a period of protein synthesis dependency [2,30–34], or only under certain conditions [2,35]. Other reports indicate that reconsolidation-updating paradigms are not unanimously effective in attenuating the fear response [36–38], casting doubt on the efficacy of such purely behavioral approaches for remote fear memories.

Purely behavioral reconsolidation-updating approaches fail to attenuate remote fear memories

Since the seminal discovery that repeated exposure to a fear-eliciting stimulus in a defined time period after memory recall can lead to the persistent attenuation of the fear response [28,29], two studies have attempted to employ this time-sensitive malleability of a memory to attenuate remote traumatic memories [39,40]. Both used Pavlovian fear conditioning in rodents as a model of PTSD and exposure-based therapies [41]. In fear conditioning, an unconditioned stimulus (US)—an electrical footshock—is paired with a conditioned stimulus (CS)—a specific tone or environment for cued and contextual fear conditioning, respectively. When the animals are later tested for their remembrance of the association CS–US by exposure to the CS alone, the CS will elicit the conditioned response (CR)—fear, which manifests itself as freezing in rodents.

The first study employed contextual fear conditioning and a so-called massed extinction protocol, in which fear-conditioned mice were presented 29 days after training with a 3-min exposure to the CS to recall the memory, followed by an uninterrupted 30 min CS exposure 1 h post-recall to extinguish it [40]. Although this procedure was effective in attenuating the fear response over the course of the extinction procedure itself, the original fear response spontaneously recovered 1 month later, indicating incomplete memory erasure [42,43]. Increasing the recall duration to 15 min also failed to reduce the spontaneous recovery (SR) of the fear [40]. Intriguingly, the same paradigm successfully attenuated recent fear memories without evidence of SR [44]. Thus, despite using a paradigm that permanently diminishes recent traumata, and despite being situated within the reconsolidation window of memory malleability upon recall [29], remote memories appear to be resilient to reconsolidation-updating paradigms.

Comparable results were observed in a recent study investigating reconsolidation-updating procedures for the attenuation of both remote cued and contextual fear memories [39]. Although using a massed extinction paradigm for 6 × 3 min tone and context exposure 1 h post-recall 30 days after training proved efficient in diminishing the fear response at the end of extinction as well as 1 day thereafter, the fear spontaneously recovered 30 days later. For cued fear extinction, there were also signs of fear reinstatement, another indicator of incomplete fear attenuation [45,46]. When a more spaced extinction paradigm was used—confronting the animals with two context exposures per day with an intertrial interval of 2 h for a total of 4 days—the fear response of remote memories showed no signs of attenuation at any given time, even though the same paradigm successfully attenuated recent fear memories without signs of SR. Combining the results of these two studies, five different behavioral interventions proved ineffective in attenuating remote traumata, providing sufficient grounds to ask why remote memories are refractory to behavioral attenuation.

Molecular correlates of ineffective attenuation of remote memories

According to the classical model of system-level consolidation [13], contextual memories become independent of the hippocampus but represented in cortical areas such as the anterior cingulate cortex (ACC) over time, as testified to by gene expression, metabolic, and structural differences between these brain regions upon recent and remote memory recall [47–50]. In addition, an fMRI study in humans indicated that people with PTSD show lower hippocampal activity at the time of memory recall than healthy control subjects [51]. Since the hippocampal formation is crucial for the learning of new contextual information [52] and since learning is an integral component of extinction [42], these findings indicate that a lack of, or insufficient, hippocampal activation might prevent remote memories from being updated with new information.

As we were able to recently reveal, such lack of hippocampal activation could reside on the epigenetic regulation of gene expression, specifically on the level of histone acetylation [39]. By increasing the repulsive force between histone proteins and the DNA, the core components of the chromatin, histone acetylation imposes a three-dimensional chromatin structure that is more permissive for

gene transcription [53], which, in turn, is a prerequisite for long-term synaptic plasticity and memory [54]. Increments in histone acetylation occur in response to neuronal activity [55], and one pathway shown to mediate these changes in neuronal culture involves the dissociation of histone deacetylase 2 (HDAC2) from the chromatin following its nitrosylation on Cys262 and Cys274 [56]. As HDAC2 is also major constraint on synaptic plasticity and memory [57], we reasoned that the inaptitude of remote memories to be updated might have its basis in this epigenetic signaling pathway.

We observed that whereas recent memory recall led to hippocampal histone hyperacetylation of the neuroplasticity-related immediate-early gene *cFos* by triggering HDAC2 nitrosylation and its dissociation from the promoter region of *cFos*, remote memory recall did not induce this signaling cascade, and *cFos* expression was not increased (Fig 1). Importantly, pharmacologically reducing HDAC2 nitrosylation by nitric oxide (NO) synthase inhibitors resulted in SR of recent memories, while exogenously elevating HDAC2 nitrosylation by NO donors prevented SR of remote memories. To boot this correlative evidence of a role of HDAC2 nitrosylation in memory updating, viral-mediated overexpression of a non-nitrosylatable form of HDAC2, HDAC2^{C262/274A}, reinstated SR of remote memories even in the presence of the NO donor [39]. Interestingly, learning itself is accompanied by increased NO signaling in the hippocampus [58], whereas mice lacking the enzyme neuronal NO synthase display contextual fear memory deficits [59]. Taken together, these findings depict a critical involvement of the NO–HDAC2 pathway in the lack of hippocampal learning capacities upon remote memory recall. Nevertheless, it remains to be determined by which mechanisms neural activity leads to the nitrosylation of HDAC2 in the context of memory recall.

Histone acetylation upon memory recall might also originate from another pathway: The retrieval of recent contextual memories was found to lead to the phosphorylation and acetylation of hippocampal histones in general, and in particular in the promoter region of the immediate-early gene *Zif268*, via activation of the transcription factor NF- κ B [60]. Thus, it is conceivable that this pathway might be inhibited for the recall of remote contextual fear memories, but such scenario remains to be tested.

Outside the context of histone acetylation, the resilience of older memories to be updated by behavioral interventions alone may also be driven by other mechanisms. One such mechanism includes the redistribution of calcium-permeable AMPA receptors (CP-AMPA) as a memory matures [38]: 7-day-old auditory fear memories resistant to reconsolidation-updating extinction paradigms were no longer associated with CP-AMPA-mediated neuroplasticity at thalamic afferents to lateral amygdala neurons, in contrast to 1-day-old fear memories amenable to reconsolidation-updating fear extinction. Furthermore, pharmacologically blocking the activity of CP-AMPA after memory retrieval impaired memory reconsolidation of only 1-day-old fear memories [61,62]. Here, it would be highly interesting to investigate a convergent effect of a lack of histone acetylation and CP-AMPA-mediated neuroplasticity on the resilience of remote memories to be updated during reconsolidation.

Another highly intriguing mechanism promoting the resistance of remote memories to extinction could be the presence of extracellular perineuronal nets (PNNs) [63]. PNNs are composed of extracellular matrix chondroitin proteoglycans and found in the amygdala of adult animals, for which regular extinction approaches

are ineffective in reducing cued fear memories. Fascinatingly, the same PNNs are absent in this brain area of juvenile animals, for which the same approaches efficiently diminish the fear response. Abolishing PNNs by pharmacological means rendered fear memories of adult animals again amenable to disruption. Similarly, PNN depletion was also found to enhance the extinction of morphine and cocaine-induced drug memories (i.e. conditioned place preferences) several days after training [64]. In future studies, it will be important to determine whether PNNs also play a role in protecting remote fear memories from being efficiently extinguished.

Pharmacological means to overcome extinction-resilient remote memories

In an attempt to overcome the lack of hippocampal neuroplasticity upon remote memory, we found that HDAC2-targeting HDAC inhibitors (HDACi) might constitute effective adjuncts to exposure-based interventions [39]. Applying the HDACi CI-994 in combination with extinction training increased the promoter acetylation of *cFos* despite the persistent binding of HDAC2 to the chromatin and thereby reinstated *cFos* expression (Fig 2A). Behaviorally, CI-994 was found to abolish the incidence of SR in both the massed and spaced contextual extinction paradigm (Fig 2B and C) and to even lower the animals' freezing response after extinction using the spaced paradigm (Fig 2C). Thus, the absence of the endogenously occurring NO–HDAC2 cascade that prevented the reactivated memory from being updated with new information could be overturned by exogenous application of an HDAC2-targeting HDACi. This finding is consistent with a report showing that a knockout of HDAC2 accelerated extinction learning in a taste-aversion task [65].

The usefulness of HDACis as adjuncts for remote extinction learning had been pioneered by reports about their beneficial role in attenuating recent fear memories in both rodents [59,66–70] and humans [71,72]. This therefore constitutes a promising area for further investigation. A caveat is, however, in place: As a recent study found that overexpressing HDAC1, another class I HDAC, facilitated recent fear memory extinction [73], any HDACi foreseen for the usage in memory extinction should avoid inhibiting HDAC1 (or conversely, activate it). As HDAC1 and 2 are structurally highly similar [74], this might define a significant challenge.

Another candidate with great promise for improving the learning process during extinction to attenuate long-term fear memories is the partial NMDA receptor agonist D-cycloserine (DCS). For example, DCS was effective in attenuating the fear response of lifelong human acrophobia, the fear of heights, in a virtual reality experiment of exposure-based therapy [75], and in recently completed clinical trials in patients with chronic PTSD [76,77]. However, depending on the precise nature of the exposure-based therapy, DCS was also found to lead to an unwanted strengthening in the original fear response and not to its extinction [22]. Furthermore, DCS was found to induce generalized extinction of two different cues in rats, while only one cue was subjected to the extinction procedure [78]. This is in contrast to the effect of the HDACi CI-994, which left a cued fear memory formed simultaneously to the context fear intact, when only the contextual fear was targeted by extinction [39]. These limitations notwithstanding, DCS might hold great promise as an adjunct to exposure-based therapies for traumatic memories.

Figure 1. Schematic representation depicting the physiological and molecular differences between recent and remote memory recall.

Data from Gräff *et al* [39] showing that for the recall of recent memories (left), which are primarily characterized by elevated hippocampal neuronal activity, hippocampal HDAC2 was nitrosylated (NO) on two cystein residues (C262/274), which was accompanied by its dissociation from the chromatin and an increase in histone acetylation at the promoter of the immediate-early gene *cFos*. *cFos*, in turn, showed a concomitant increase in gene and protein expression, which presumably allowed for memory updating. For the recall of remote memories (right), which are primarily stored in cortical areas such as the anterior cingulate cortex (ACC), hippocampal neuronal activity was low, hippocampal HDAC2 showed no increase in nitrosylation and remained chromatin bound. No *cFos* promoter acetylation or gene transcription was detected, and memories could not be updated.

Physiological correlates of successful remote memory extinction

While little is known about the molecular pathways underlying successful remote memory extinction with DCS, it has been

postulated that such treatment might facilitate neuroplasticity [79]. For HDACi treatment in combination with extinction training, enhanced neuroplasticity upon successful memory extinction was indeed observed on multiple levels. First, on a functional level, where hippocampal metabolic activity and synaptic plasticity at Schaffer

Figure 2. Combining HDACi with extinction training allows for the persistent attenuation of remote fear memories.

(A) Schematic representation depicting the physiological and molecular events occurring when extinction training following remote memory recall is combined with HDACi treatment. Despite the absence of hippocampal neuronal activity and HDAC2 being chromatin bound, HDACi treatment reinstated histone acetylation, which led to increased *cFos* expression, enhancement of neuroplasticity, and successful memory updating. (B) HDACi CI-994-treated animals, but not their vehicle (VEH)-treated counterparts, displayed significant long-term extinction of 30-day-old fear memories after a massed extinction protocol, as there was no spontaneous recovery of the fear. Note that although VEH-treated animals show a decreased fear response 1 h, 24 h after the extinction protocol, this effect was only transient. (C) HDACi CI-994-treated animals show significant and persistent fear reduction 1 h, 24 h, and 30 days after a spaced extinction paradigm, whereas the same paradigm failed to even temporarily attenuate the fear response in VEH-treated animals. (B, C) reproduced, with permission, from [39].

collaterals were enhanced [39]. This is similar to findings in humans showing that successful extinction memory correlates with higher hippocampal activity as assessed by fMRI [51]. These observations

are further reminiscent of electrophysiological studies in rats demonstrating that high-frequency stimulation in the hippocampus facilitates long-term extinction without the occurrence of SR [80].

Second, neuroplasticity was enhanced on a structural level, where increased synaptic density, dendritic branching and an elevated number of spines accompanied successful memory extinction [39]. These observations are consistent with the description of increased spine numbers following efficient extinction of recent memories [81] and with the detection of increased synaptic density and dendritic branching by HDACi treatment in an animal model of neurodegeneration [82].

Third, neuroplasticity was also evident on the level of gene transcription. HDACi treatment in conjunction with behavioral training was found to trigger the upregulation of a key set of neuroplasticity-related genes, which was accompanied by increased histone acetylation in their promoter region [39]. Of those, the immediate-early genes *Arc* and *cFos*, which are both implicated in coordinating synaptic plasticity and memory-related processes [83,84], *Adcy6*, an adenylate cyclase regulating neurite extension [85], *Npas4*, a transcription factor critical for contextual memory formation [86], and *Igf2*, a growth factor known to facilitate memory consolidation [87], might be particularly promising candidates to explain successful memory extinction. Taken together, the enhanced neuroplasticity observed by combining HDACi treatment with extinction training encompasses the molecular, structural and functional level and thus clearly speaks in favor of a facilitated memory updating process.

A gene expression signature for effective fear attenuation?

Intriguingly, several of the differentially expressed genes upon remote memory attenuation are also confirmed regulators of effective fear extinction of recent memories. In a microarray study conducted in the hippocampus following successful extinction of recent contextual fear memories, the expression of *cFos*, *Arc* and *Igf2* was increased [88]. Conversely, in another microarray study of hippocampal gene expression changes in animals with impaired recent contextual fear extinction, *cFos*, *Arc*, and *Npas4* were down-regulated [89]. Based on the results from these unbiased approaches, it appears that *cFos* and *Arc* are certainly, and *Igf2* and *Npas4* likely critical regulators of remote contextual fear extinction. Moreover, conclusive evidence exists that—at least for recent memories—hippocampal *Igf2* and amygdalar *Npas4* are necessary for successful contextual and cued fear extinction, respectively [88,90]. Thus, one would assume that the activation of *cFos*, *Arc*, and *Igf2* is also necessary for the extinction of remote contextual memories, but this remains to be experimentally addressed.

In addition to genome-wide approaches, several studies focused on the contribution of individual genes to memory extinction. One of these is *Bdnf* [91], which was not only shown to be increased following recent memory extinction in association with an increase in its promoter acetylation [68], but also to be causally implicated in successful memory extinction: Heterozygous knockout mice for *Bdnf* showed impaired fear extinction [92], whereas exogenous infusion of *Bdnf* ameliorated fear extinction [93]. Other genes with a role in recent memory extinction are summarized elsewhere [94] and include *Zif268*, *CREB*, *Cdk5*, and *CB1*, to name just a few. However, for all of these genes, their role, if any, in remote memory extinction awaits to be determined.

Recent insights into remote fear attenuation

Traditionally, remote memories have been considered being stored in higher cortical areas such as the medial prefrontal cortex, and in particular the ACC [14], since recalling remote memories led to gene expression, metabolic and structural changes in cortical areas, while hippocampal subfields were not engaged [47–49,95,96]. However, early studies blocking hippocampal activity after remote memory recall by means of protein synthesis inhibitors or lesions had already alluded to the fact that recalling a remote memory renders it hippocampus-dependent again [1]. Interestingly, such hippocampal engagement in remote memory recall has recently been confirmed in an elegant study using transgenic mice that allow for the identification of contextual fear conditioning-induced neuronal subpopulations: CA1 neurons active during memory formation were found to be also re-activated during remote memory recall [97]. As an optogenetic study further revealed excitatory (CaMKII α -positive) neurons in hippocampal area CA1 are not only active, but also necessary for remote memory recall. When the activity of these neurons was abolished precisely during recall, animals could no longer remember the context in which they had been trained [98]. Together, these findings point to a critical involvement of the hippocampus in remote memory recall.

Nevertheless, it is still not clear what the precise contribution of hippocampal neurons to the recall, let alone the extinction of remote memories, really is. For instance, although temporally restricted inhibition of CA1 neurons abolished the capacity to recall remote memories, prolonged inhibition did not [98]. This observation was paralleled by an increased engagement of other brain areas such as the ACC (in terms of *cFos* expression), suggesting that cortical areas might be able to compensate for prolonged CA1 inhibition and to steer remote memory recall. Furthermore, our own findings indicated a lack of histone acetylation-mediated neuroplasticity in the hippocampus upon remote memory recall [39]. As our approach did not distinguish between different subpopulations of neurons, it is possible that the observed reactivation of the hippocampus upon remote memory recall [97,98] only occurs in a spatially and temporally restricted manner, which *per se* is seemingly insufficient to induce the required changes in neuroplasticity for the successful attenuation of remote memories.

Instead, successful extinction of remote memories critically depends on the support of pharmacological adjuncts that can enhance neuroplasticity, and in particular learning-related processes, as exemplified by the HDACi CI-994 [39] or the NMDA receptor agonist DCS [75–77,99]. Intriguingly, these pharmacological enhancers of memory extinction were administered systemically and are therefore likely to affect not only the recall-reactivated hippocampus, but also other brain areas. This lack of precision, which is usually considered a shortcoming of any pharmaceutical, could in this case inadvertently be advantageous: As long as the precise contribution of the hippocampus and/or cortical areas to remote memory recall and extinction is not known and could indeed involve both, it might probably be best to stimulate neuronal activity indiscriminately in order to overcome remote traumatic memories. In the long run, however, it is evident that more studies are needed not only to precisely decipher the individual and joint contribution of cortical and hippocampal—and perhaps other—brain areas to successful remote memory attenuation, but also to

Sidebar: In need of answers

- (i) How safe are systemically administered pharmacological enhancers of memory attenuation such as CI-994 or DCS?
- (ii) What is the precise mode of action of these enhancers?
- (iii) What is the consequence of administering these enhancers on explicit, that is, narrative components of human fear memories? Otherwise asked: How general an amnesia will these enhancers induce?
- (iv) PKM ζ inhibition has been shown to permanently attenuate taste-aversion memories without the need for reconsolidation-updating behavioral paradigms [100]. Is PKM ζ inhibition also effective in attenuating remote fear memories?
- (v) A gene expression signature for successful memory extinction might have been identified [39,89] and consists of at least three genes. What is their individual role in remote memory attenuation?
- (vi) Do PNNs play a role in the resilience of remote fear memories against reconsolidation-updating behavioral interventions?
- (vii) Remote memories in rodents are most often defined as 30-day-old memories. Do the mechanisms of memory storage and attenuation described in this article also apply for even older memories?

determine the safety of neuroplasticity-promoting agents used for this purpose.

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Author contributions

This review was written by L-HT and J.G.

Conflict of interest

The authors declare that they have no conflict of interest.

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