Noradrenergic interactions via autonomic nervous system: a promising target for extinction-based exposure therapy?

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Noradrenergic interactions via autonomic nervous system: a promising target for extinction-based exposure therapy? J Neurophysiol 110: 2507–2510, 2013. First published August 14, 2013; doi:10.1152/jn.00502.2013.—Fearful associations can be replaced by neutral associations through repetitive exposure of an individual to the fearful situation without the aversive component. Recently, Peña and colleagues (Peña DF, Engineer ND, McIntyre CK. Biol Psychiatry 73: 1071–1077, 2013) demonstrated that pairing activation of noradrenergic (NA) pathways through vagus nerve stimulation (VNS) with extinction learning accelerates consolidation of extinction memories in rats. Their findings stress the importance of activating the NA system through VNS in treatment of anxiety disorders such as PTSD or phobia.

FEAR MEMORY MIGHT BE SEEN as a learned adaptive evolutionary mechanism crucial for organism survival. However, when fear becomes disproportionate to that necessary to cope with a given stimulus, or whether it begins to occur in inappropriate situations, a fear or anxiety disorder exists.

Classical fear conditioning is a widely used tool to model anxiety disorders in rodents. In this paradigm, a previously neutral stimulus, like a tone (conditioned stimulus; CS), is presented together with an aversive stimulus, such as foot shock (unconditioned stimulus; US). Subsequent presentation of the CS, but also the environment of the training, the context, will result in fear in anticipation of the US due to formation of associative memory between CS and US. Importantly, the retrieved fear memory can be extinguished upon repetitive exposures to CS without US (Quirk and Mueller 2008). Therefore, extinction of a conditioned fear memory is an active process in which an individual has to learn that the once fear-eliciting stimulus is now neutral again. It has been shown that fear conditioning involves excessive autonomic nervous system activation that leads to release of noradrenaline (NA) in various brain regions required for fear memory formation (Holmes and Quirk 2010; McGaugh 2004; Mueller and Cahill 2010).

Administration of NA enhances initial fear memory consolidation in both rodents and humans, but also extinction learning, while blockade of their β-type receptors reverses these effects (McGaugh 2004; Mueller and Cahill 2010). These memory enhancing effects of NA provide an interesting tool in treatment of anxiety disorders that involve a strong memory component, such as phobias or posttraumatic stress disorder (PTSD). PTSD is characterized by intrusive recollections of the traumatic event, accompanied with increased anxiety and hyperarousal (Parsons and Ressler 2013). Interestingly, PTSD is characterized by a long-lasting symptomatology, and the diagnosis is stated at least 1 mo after the initial event where the trauma-associated fear memories were formed. Therefore, the attenuation of an already established fear memory trace provides an expedient entry point for PTSD treatment. However, a successful extinction therapy in PTSD appears limited due to impaired extinction learning in these patients (Parsons and Ressler 2013). Employing the memory enhancing effects of NA might thereby allow for increasing the efficiency of extinction (Mueller and Cahill 2010).

In a recent publication, Peña and colleagues (2013) used vagus nerve stimulation (VNS) to promote NA release during extinction learning. By VNS, the authors could elegantly demonstrate accelerated consolidation of extinction memories in rats. In this article, we will discuss the results of Peña et al. (2013) in the context of NA effects on extinction of fear memory. Furthermore, we will focus on NA agents as a treatment option for PTSD and why VNS would be advantageous for this purpose. Extinction of fear memory relies in part on neural pathways similar to fear memory acquisition, but recruits also additional structures to the network. Extinction learning involves development of a new inhibitory memory in a context-dependent manner rather than deleting the original fear memory (Mueller and Cahill 2010). As for the initial fear memory formation, three different phases take place during extinction learning: 1) acquisition: the early stage during which conditioned fear response (CFR) decreases; 2) consolidation: the stage where extinction memory is stabilized through physiological and molecular alterations; and 3) retrieval: at a later time point when CS is represented. In their recent study, Peña et al. (2013) focused on the consolidation phase of extinction. For that, fear conditioning was used as explained before, pairing a tone (CS) with a footshock (US). Then, CS exposures were temporally paired with VNS during the extinction trials, hypothesizing it will enhance extinction learning. Indeed, the CFR significantly decreased 1 day after VNS in a 3-day extinction learning paradigm (Fig. 1). However, in real life, exposure therapy is given until the CFR decreases to a certain level. Thus in a second experiment, rats underwent extinction in multiple trials until their CFR was observed only in 10% of the test time. Peña and colleagues clearly showed that VNS paired to CS presentations accelerated extinction learning. Additionally, an unpaired group was employed where VNS was delivered in the home cage, after the extinction session had finished. This group showed no difference to extinction levels...
that the amygdala, especially the basolateral complex (BLA), and Cahill 2010; Ruffoli et al. 2011). It has been suggested that various brain areas, including key structures of emotional systems such as the amygdala, hippocampus, and medial prefrontal cortex (mPFC). Stimulation of the vagus nerve afferents results in activation of the locus coeruleus (LC) via the nucleus tractus solitaries. The LC gives rise to the main noradrenergic inputs into various brain areas, including key structures of emotional memory formation: the amygdala, hippocampus, and mPFC (Mueller and Cahill 2010; Ruffoli et al. 2011). It has been suggested that the amygdala, especially the basolateral complex (BLA), integrates and stores information about the CS and interacts with the hippocampus, which processes contextual information. While both regions play a key role in the consolidation of the initial fear conditioning as well as extinction, the infralimbic (IL) region of the mPFC is specifically involved in consolidation of extinction by integrating contextual information. During extinction retrieval, the IL-mPFC reduces the fear by inhibiting the amygdala output only in the extinction context (Quirk and Mueller 2008).

Next to NA, other neuromodulators and neurotransmitters also contribute to the consolidation of extinction memory. For example, reduced levels of GABA can enhance consolidation of fear memory and its extinction (Berlau and McGaugh 2006). Indeed, studies in epileptic patients describe an increase of GABA levels in the cerebrospinal fluid after VNS (Ben-Menachem et al. 1995; see also Carpenter et al. 2004: no effect of VNS in depressive patients). However, modulating effects of GABAergic neurotransmission on extinction memory consolidation occur in concert with the noradrenergic system, since blocking of β-adrenergic receptors omits the GABA effects (Berlau and McGaugh 2006). Therefore, the NA system appears crucial for extinction memory formation, although it is still under intensive discussion how NA takes part in these processes. Several lines of evidence indicate that NA enhances the formation of emotional memories: 1) systemic administration of NA enhances extinction learning in a β-receptor-dependent manner while NA depletion in the central nervous system (CNS) via lesions of the LC impairs extinction learning; 2) enhancement of memory consolidation through glucocorticoids is mediated by β-adrenergic receptors (McGaugh 2004); and 3) regions that are crucial for learning and memory such as the hippocampus and IL-mPFC are modulated by BLA activity and receive NA signaling. In that context, it was shown that systemic or local infusions of β-adrenergic receptor antagonists into the BLA can block memory enhancement (Mueller and Cahill 2010).

Systemic application of β-receptor blocker propranolol before extinction training impairs retrieval of extinction of context-dependent fear without any effect on extinction retrieval of cue-dependent fear. Additionally, it decreases CFR during cue-conditioned extinction training (Mueller and Cahill 2010). Furthermore, local infusions of propranolol into the IL-mPFC prior to the extinction training impaired retrieval of the extinction memory. Whole cell recordings revealed increased excitability in the IL-mPFC neurons after NA bath application measured by increased action potential number evoked by a depolarizing pulse. This effect was prevented by the blockers of both β-receptor and cAMP-dependent protein kinase A, which indicate that β-receptor activation during extinction is crucial for augmenting fear extinction memories (Mueller et al. 2008). Furthermore, increase in intrinsic excitability was suggested as a factor contributing to fear extinction memory in the hippocampus as well as in the amygdala. This increase in excitability sets the stage for subsequent synaptic plasticity. Indeed, β-receptor activation alone is not able to induce long-term potentiation (LTP) in the hippocampus. However, β-receptors mediate phosphorylation of GluR1 subunits, which results in the insertion of AMPA receptors. Thereby, the threshold for both LTP induction and memory formation is lowered (Mueller and Cahill 2010). Interestingly, when propranolol was infused into the IL-mPFC after extinction train-

Fig. 1. Vagus nerve stimulation (VNS) during fear extinction leads to noradrenaline (NA) release in the amygdala, hippocampus, and medial prefrontal cortex (mPFC). Catecholamines usually cannot cross the blood brain barrier. The peripheral hormone adrenaline, which is released during an emotionally charged experience, can communicate with the central nervous system (CNS) via activation of the adrenergic β-receptors on the vagus nerve resulting in activation of locus coeruleus (LC) neurons with NA. VNS provides a tool to increase the efficiency of this communication between peripheral nervous system and CNS. The LC-evoked NA release during extinction learning, where a conditioned stimulus (CS) is presented without the unconditioned stimulus, results in a sustained adrenergic activation of the infralimbic (IL) of the mPFC (thick arrows). Thereby, plasticity in this area is enhanced (Mueller et al. 2008), leading to increased inhibition of the basolateral amygdala (BLA). Upon retrieval, decreased activity in the amygdala and its output structure, the central nucleus (CeA), results in a reduced conditioned fear response (CFR). The mPFC integrates context-dependent information from the hippocampus and amygdala (thin arrows) to execute appropriate behavior. [Adapted from Quirk and Mueller (2008); Mueller and Cahill (2010); and Ruffoli et al. (2011) with permission.]
ing, no effect on the retrieval of extinction memory was observed (Mueller et al. 2008). One possible explanation of the time-dependent effect of β-receptor blockade on extinction consolidation would be a decrease in prominence and relevance of the CS as the extinction training progresses. At the early stage of extinction acquisition, presentation of the CS promotes LC neurons to fire prominently, resulting in NA input into the IL-mPFC. At the later stages of extinction learning, when the CFR decreases, the firing of LC neurons also decreases and results in decline in NA level in the IL-mPFC. NA release evoked by CS during the initial phase of extinction within regions related with extinction may lay the physiological and molecular groundwork for consecutive consolidation of extinction memories. During later phases of extinction, the impact of NA release on these processes may be reduced.

An alternative way through which extinction-related structures may communicate can be local field potential (LFP) network oscillations that provide a time window during which cells and target neurons may fire synchronously. Cells in the BLA fire in theta range frequency and participate in LFP oscillations. Theta synchrony in hippocampal CA1 and the BLA increases at relatively early stages of fear memory formation while theta synchrony decreases at remote memory stages. As expected, PFC-amygdala synchrony is prominent during extinction of fear memories. Thus, synchronized activity of the amygdala and hippocampus is increased during early stages of fear learning while amygdala-PFC synchronization shows a reverse trend towards retrieval and extinction (Pape and Pare 2010). To our knowledge, how NA system contributes to network synchronization between structures involved in fear memory formation and extinction has not been studied directly. NA release during fear conditioning should facilitate the network synchronization between the hippocampus and amygdala resulting in formation of fear memory, while during fear extinction, NA-elicited augmentation of IL-mPFC activity may cause increased theta synchronization in the amygdala-hippocampus-PFC network. It would be interesting to see how NA drugs modulate network synchronization in these extinction-related structures.

Timing, genetic background, or coexisting competing behaviors are some of the key factors determining diverging effects of NA drugs on fear extinction. One of the well-studied NA drugs concerning the effects on fear extinction is yohimbine, a competitive antagonist of α2-adrenoceptors, which acts on both postsynaptic and presynaptic sites. It increases extracellular concentration of NA in extinction-related structures via blocking autoreceptor inhibition of NA release (Holmes and Quirk 2010). As already discussed, increase in NA levels prior to extinction training facilitates the consolidation of extinction memories. This was also the case for yohimbine as systemic treatment prior to the extinction training resulted in reduction in both the conditional and contextual fear. However, yohimbine exhibited its improving effects on extinction learning only in partial extinction training. As the extinction training time prolongs, yohimbine loses its efficacy. Improvement in extinction retrieval was also observed when the CS presentations were temporally spaced (Cain et al. 2004). These findings support the idea that timing of NA drug administration and experimental design are important deciding factors in extinction learning.

Considering the different stages of fear conditioning and its extinction, timing is all that matters for an expedient use of NA modulation in treatment of anxiety disorders such as PTSD. Increased NA levels during a traumatic event may facilitate the formation of traumatic memory by enhancing initial fear memory consolidation. Therefore, applying β-receptor blockers like propranolol shortly after trauma should impair consolidation of the traumatic memory. Indeed, evidence from emergency room patients receiving propranolol shortly after a traumatic experience supports this hypothesis (Vaiva et al. 2003). However, during consolidation of extinction memory, elevated levels of NA appear beneficial, and preclinical studies indicate that yohimbine is a promising extinction facilitator (Cain et al. 2004). Conversely, it has been shown that yohimbine might trigger anxiety attacks in PTSD patients (Mueller and Cahill 2010), which would have devastating effects in extinction-based exposure therapy. While benzodiazepines and β-receptor blockers act as anxiolytics, they would impair memory consolidation. Therefore, a combination of exposure therapy with additional treatments that provide memory-enhancing and anxiolytic properties are required, and VNS has been shown to possess both of these qualities. First, VNS immediately after training enhances memory consolidation via eliciting release of NA in the CNS both in rats and humans. Second, use of VNS has been shown to have anxiolytic effects both in patients with resistant anxiety disorder and rodents (Mueller and Cahill 2010; Peña et al. 2013). Therefore, Peña and colleagues employed pairing VNS with extinction learning to accelerate consolidation of extinction memories in rats. Here, they used a temporally spaced extinction training. It would be interesting to investigate whether the facilitating effects of VNS on extinction would still be present in an extended extinction training. One advantage of this approach would be the possibility to isolate the initial phase of the extinction training, where NA release is still present, from the subsequent phase where NA release is decreased. Molecular and physiological processes involved in NA release in the later phase of extinction learning through VNS might underlie the facilitating effects on consolidation of fear memory extinction, and it would be interesting to dissect these components in future studies.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

G.C. and A.A. conception and design of research; G.C. and A.A. prepared figures; G.C. and A.A. drafted manuscript; G.C. and A.A. edited and revised manuscript; G.C. and A.A. approved final version of manuscript.
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