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Towards new approaches to disorders of fear and anxiety

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Fear and anxiety are debilitating conditions that affect a significant number of individuals in their lifetimes. Understanding underlying mechanisms of these disorders affords us the possibility of therapeutic intervention. Such clarity in terms of mechanism and intervention can only come from an amalgamation of research from human to animal studies that attempt to mimic the human condition, both of which are discussed in this review. We begin by presenting an outline of our current understanding of the neurobiological basis of fear and anxiety. This outline spans various levels of organization that include the circuitry, molecular pathways, genetic and epigenetic components of fear and anxiety. Using these organizational levels as a scaffold, we then discuss strategies that are currently used to ameliorate these disorders, and forecast future interventions that hold therapeutic promise. Among these newer promising treatments, we include, optogenetic, pharmacological, and extinction-based approaches, as well as lifestyle modifications, with combinatorial treatment regimens of these holding the most promise.

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Towards new therapies for disorders of fear and anxiety

By recent estimates, 28% of the U.S. population suffers from some form of anxiety-related disorders during their lifetime [1]. These illnesses result in numerous adverse effects that extend from the impoverishment of the individual's quality of life to the high financial cost incurred to treat them. Understanding the functioning of the nervous system in a healthy and affected state will aid in the development of treatments for these disorders

[2]. While the eventual goal of such investigation is to ameliorate these conditions in humans, a productive and complementary strategy that can pave the way for intervention in humans uses animal models, wherein the etiology and treatment of anxiety-related disorders is studied [3]. Focusing on anxiety-related and fear-related disorders, this brief review attempts to synergize the existing state of knowledge in terms of their neural underpinnings, currently used therapeutic intervention, and potential treatments that hold promise for the future, using studies that focus on both animals and humans.

Anxiety and fear: operational definitions

While the physical and psychological manifestations of anxiety and fear appear to share commonalities, they are in fact two independent entities that merit operational definitions. *Anxiety* is characterized as a state of being that arises from general and non-specific stimuli that are perceived as being potentially threatening in the future. This perception often results in an apprehensive mood accompanied by increased arousal and vigilance, which when taken to an extreme, persist for extended periods of time. In contrast, *fear* is stimulated by specific stimuli and results in active defensive responses that gradually subside when the specific stimulus is no longer present [4]. Clinically, fear can be thought of as mirroring the response to a specific cue (e.g. the fear of snakes), while anxiety is a more long-lasting phenomenon that is not specific to overt cues.

Anxiety in laboratory rodents is often measured using crude behavioral assays such as the Elevated Plus Maze, while fear to specific cues is modeled by employing Pavlovian conditioning to cues such as tones or lights [5]. Recently, nuanced experimental paradigms that more faithfully represent the aforementioned operational distinctions between anxiety and fear have been employed in rats. Anxiety can be modeled using both light-enhanced and dark-enhanced startle paradigms, context conditioning, and by exploiting the unpredictability of aversive events (such as mild shock) [6,7]. In contrast, fear can be elicited and measured using paradigms that include but are not limited to cue-specific conditioning, and verbal threat in humans [8]. Further discussion of details about how best to model anxiety and fear in rodents and humans is outside the scope of this review, and there are other excellent comprehensive reviews [9–12]. The need to operationally define anxiety versus fear arises due to the fact that these two behavioral states are mediated by shared as well as independent subsystems in the brain, and can be treated with independent therapeutic strategies [13].

Anxiety and fear in the brain—from the connectome to the epigenome

Neural circuitry underlying anxiety and fear

As with any neuropsychiatric disorder, a constellation of brain regions underlie anxiety-like and fear-like states [14^{••},15[•],16–18]. At the core of this is thought to be the ‘extended amygdala’ which includes the central (CeA) and medial (MeA) nuclei of the amygdala and the bed nucleus of the stria terminalis (BNST) (Figure 1). The extended amygdala is responsive to afferent input from the Basolateral Amygdala (BLA), and cortical regions such as the insular cortex. Divisions of the prefrontal cortex (PFC)—medial PFC (mPFC) and infralimbic PFC (iPFC) are pivotal sites of consolidation and extinction of fear [19–21]. A review of this nature would not do justice to the intricacies of the neural circuitry underlying anxiety and fear, and we recommend [22,23] for those interested in the same.

Molecular underpinnings of anxiety and fear

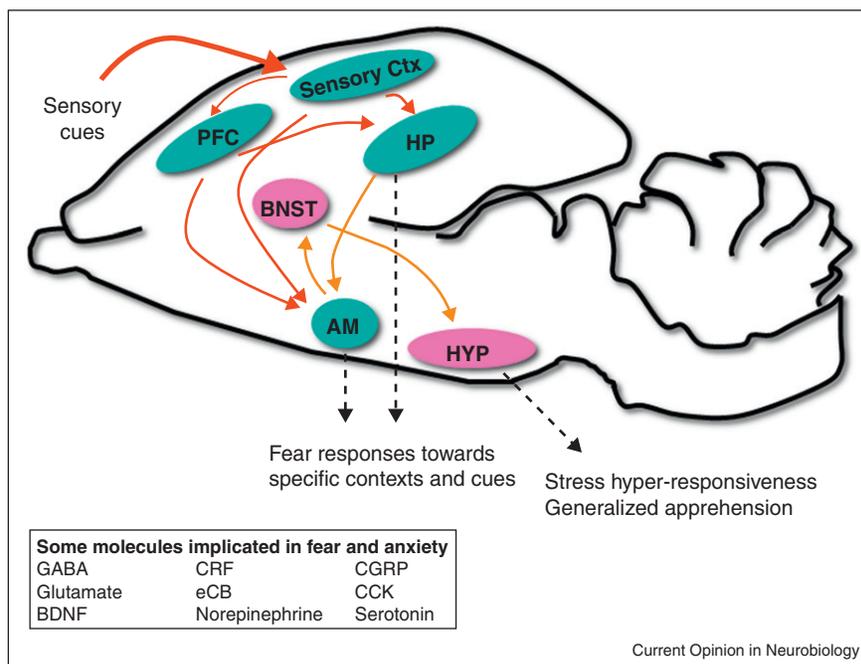
One of the most exciting observations in understanding the translation of mammalian fear to human anxiety-related and fear-related disorders is the shared conservation of the ‘fear reflex’ across all mammals (Figure 2). Seeing that the triggering of anxious and fearful behavior are stimuli that are perceived as stressful and threatening, it should come as no surprise that Corticotropin Releasing Factor (CRF) at the apex of the Hypothalamic–Pituitary–Adrenal axis plays a pivotal role in the manifestation of these states [24,25].

The successful treatment of anxiety-related and fear-related disorders using benzodiazepines and D-cycloserine make a compelling case for the involvement of neurotransmitters such as GABA and glutamate in these behavioral states [26–29]. Similarly neuromodulators such as serotonin, dopamine, and norepinephrine are involved, based on the use of antidepressant medication to treat anxious and fearful phenotypes. Small molecules like calcitonin gene-related peptide (CGRP), cholecystokinin (CCK), and the endocannabinoid system are receiving recent attention for their roles in these disorders [30–32]. We reference [33,34] for a more exhaustive treatment of the molecules implicated in anxiety and fear.

Genetic contribution to anxiety and fear

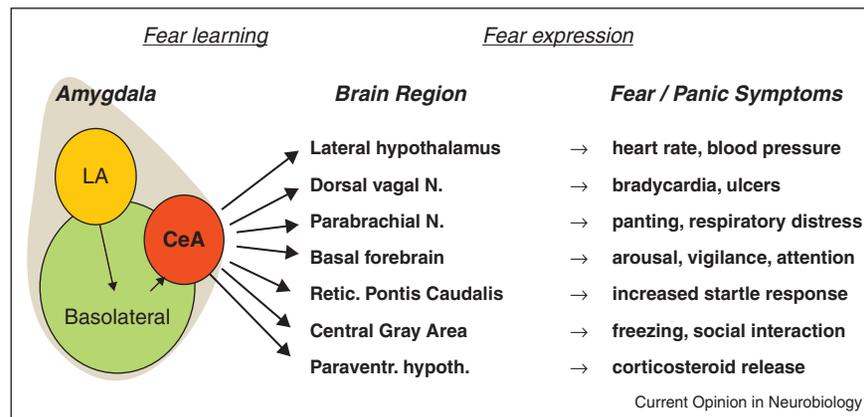
Twin studies suggest that the heritable risk of anxiety-related disorders is of the same order of magnitude as depression and other moderately heritable psychiatric disorders [35–37]. The field of anxiety genetics is in the process of gathering sufficiently large sample sizes for properly powered, hypothesis-neutral, genome-wide association studies (GWAS) of anxiety and its specific subtypes. However, several ongoing large studies of traumatized civilian and military cohorts are beginning to reveal hopeful results that await replication [38,39]. Several studies have investigated the genetic basis of these disorders mostly using SNP analysis of candidate genes. For example, the BDNF (Val66Met) SNP has been associated with anxiety and fear-learning in both humans

Figure 1



Structural and molecular pathways involved in fear and anxiety. A schematic mammalian brain is shown in which prefrontal cortex (PFC) and sensory cortex interact with hippocampal (HP), bed nucleus of the stria terminalis (BNST), amygdala (AM) and hypothalamic (HYP) circuits to mediate stress, anxiety, and fear responses.

Figure 2



The hard-wired 'fear reflex' underlies the symptoms of fear and panic responses. Neural connectivity between the Central Amygdala outputs (CeA) to brainstem and other subcortical areas activates a known and increasingly well-understood series of pathways which mediate the differential fear reflex patterns that are experienced as a fear or panic-attack in humans with anxiety and fear-related disorders. Modified from [80].

and rodents [40^{••},41]. A recent study from our laboratory emphasized a role for the PACAP-PAC1 receptor in PTSD in females but not males [42^{••}]. The observation that the SNP in the PAC1 receptor most strongly associated with PTSD symptoms resides in a predicted Estrogen Response Element (ERE) points to one potential mechanism for the predominance of PTSD in women. This finding was recently replicated in a separate analysis of physiological responses in children of traumatized patients [43], as well as in a separate cohort examining PTSD symptoms interacting with level of trauma exposure [44]. Notably, a separate independent study [45] conducted with sample populations completely different from those used in [42] did not find an association between the PAC1 gene and PTSD. Such experimental discrepancies emphasize how parameters like population samples, demographics, trauma exposure, and social conditions of the population profoundly influence GWAS. Findings such as these caution us to interpret these GWAS studies strictly within the context of the sample population studied. Some other candidate genes implicated in anxiety-related and fear-related disorders are *FKBP5*, *COMT*, *CCK*, and genes associated with the serotonergic system (reviewed in [46]). Given the complexity of anxiety-related and fear-related disorders, it would be naïve to assume that a single or only a handful of genes might be involved in their etiology.

An epigenetic basis for anxiety and fear

Epigenetic marking of the genome that includes modification of histone proteins and DNA methylation, as well as non-coding RNA based gene targeting, all allow for post-transcriptional regulation of gene expression [47]. Post-natal stress in rats alters the epigenetic signature of the BDNF gene with this effect appearing to persist in subsequent generations [48[•]]. Non-coding RNAs such as

miRNA have been shown to be involved in the extinction of learned fear in rodent models [49[•]]. While the importance of such epigenetic regulation of gene expression was first elucidated for its role in cancer, more studies are implicating this mechanism in neuropsychiatric disorders [50].

While the information provided thus far does not even begin to delve into the complexities of anxiety-related and fear-related disorders, it serves as a skeleton for us to describe how currently used treatments for these disorders, as well as future treatments target biological entities that span from the connectome to the epigenome.

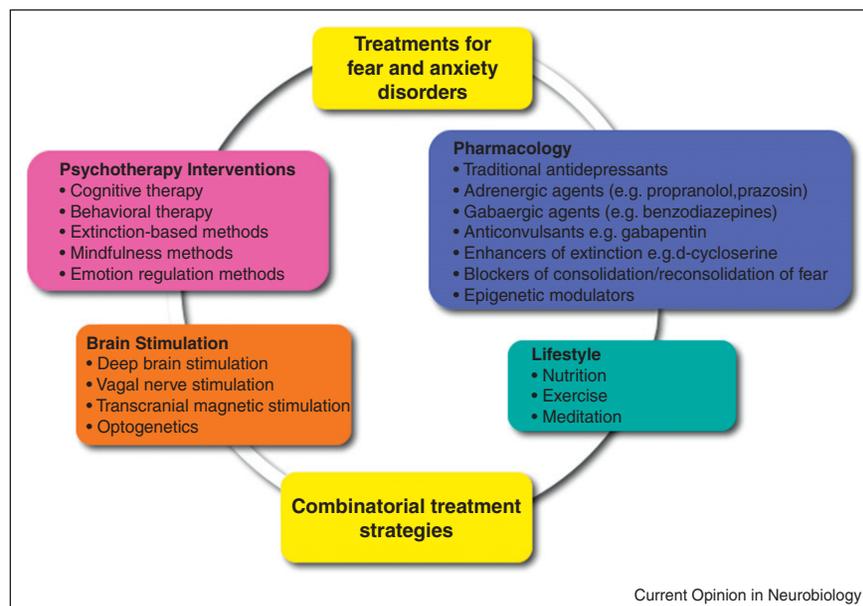
Therapeutic interventions for anxiety-related and fear-related disorders

Brain stimulation and optogenetics

There are many approaches ranging from current therapy and psychopharmacological treatments to future combination and brain-stimulation approaches that hold promise in the treatment of anxiety (Figure 3). Imaging studies have provided evidence to suggest that alteration of brain activity and structure within and between neural circuits plays a critical role in the manifestation of neuropsychiatric disorders [51–53]. Manipulating this altered brain activity using diverse techniques is proving to be a useful therapeutic strategy. Electroconvulsive therapy (ECT), vagal nerve stimulation (VNS), transcranial magnetic stimulation (TMS), and deep brain stimulation (DBS) are some of the strategies used to manipulate neural circuit function [54^{••}]. While they have historically been used in neurological disorders and to ameliorate depressive-like symptoms in Major Depressive Disorder (MDD), more recent studies emphasize their role in treating anxiety and fear (reviewed by [55^{••}]). However,

4 Social & emotional neuroscience

Figure 3



Current and promising new approaches to treatments for anxiety and fear-related disorders. Currently utilized treatments include antidepressants (targeting serotonin, norepinephrine, and dopamine monoaminergic pathways), GABA-acting benzodiazepines, and beta-adrenergic receptor blockers, as well as cognitive-behavioral therapies. However, all of these treatments have limited efficacy, and new direct and combined treatments specifically targeting known neural pathways underlying fear and anxiety are on the horizon.

the main efficacy of these treatments in most of these studies lies in their reversing of co-morbid depression. Most recently, more is becoming known about their reversal of the specific symptoms such as hyper-vigilance and arousal associated with anxiety, and cue-specific fear as in PTSD using rodent models [56]. Our current understanding of the mechanisms of action of these treatments speaks of their effect on the firing patterns of neuromodulatory systems in the brain such as the serotonergic and noradrenergic pathways [57]. While more challenging to specifically manipulate, future use of stimulation techniques would do well to affect the balance between excitatory (e.g. glutamatergic) and inhibitory (e.g. GABAergic) drive within specific regions of the brain, which some think is the root cause of anxiety and fear [58].

The recent use of controlling the firing of specific cell populations using light-based manipulation of ion channels termed 'optogenetics' provides a glimpse into what the future manipulation and understanding of neural activity in neuropsychiatric illness might look like [59]. From a basic science perspective, such approaches will give us a firmer appreciation for the specific molecular and neural circuitry involved in anxiety and fear. Further, it may allow us to refine brain coordinates that ought to be stimulated using the more traditionally used stimulation techniques. Treatments using an optogenetic strategy would first necessitate some manner by which to insert into specific brain regions ion channels that could eventually be

manipulated by light. In this direction, new advances in bio-safety and bio-engineering ought to make this a reality in the future. For example, manipulation of neural firing using optogenetic methods has already shown to be effective in rodent and non-human primate models of anxiety, fear-learning and depression [60,61–63]. More specifically, stimulation of Basolateral Amygdala fibers that project to the Central Amygdala reduced anxiety-like behavior in mice [61]. Such optogenetic approaches allow for more targeted manipulation of neural activity and could potentially reduce side-effects that might arise from the general non-targeted manipulation of activity that is a concern of the currently used stimulation strategies.

Pharmacological intervention

The symptoms of anxiety and fear can be treated in animals and humans to varying degrees of success via administration of benzodiazepines, common antidepressant treatments, and a suite of molecules like buspirone and propranolol that affect serotonergic, dopaminergic and noradrenergic neuromodulation (e.g. [26,64,65]). In several instances, chronic treatment is required for their therapeutic efficacy to come to the fore. With respect to reducing fear toward specific stimuli, recent success in both rodent and human models was achieved after the administration of a partial NMDA-receptor agonist, D-cycloserine (DCS) but only after the subjects had also been exposed to an extinction training regimen [27,66,67] (see combinatorial treatment regimens below). Looking to the future, the use of small molecules that cross the blood-brain-barrier and

target end-points such as BDNF signaling hold promise [27,28,41,68]. In addition, manipulating the epigenetic landscape in anxious and fearful states using drugs that interfere with histone modifications or DNA methylation (e.g. HAT inhibitors, HDAC inhibitors, DNMT inhibitors) may provide a fruitful therapeutic strategy [69,70]. For example, in mice, overexpression of Histone DeAcetylase2 (HDAC2) has been shown to impair memory processes, with these impairments being reversed after administration of HDAC inhibitors [71].

Cognitive Behavioral Therapy (CBT) and extinction-based intervention

Most of the success in treating anxiety-related and fear-related disorders comes from behavioral therapies that typically involve some form of extinction-based methodology [72]. This could take the form of exposing the subject to the stimulus that triggers fear in the absence of the negative outcome, or talking about the general state of anxiousness. Another behavioral approach taps into the reconsolidation theory of memory that posits that every time a memory or trigger is recalled, it becomes labile and reconsolidated. Behavioral or pharmacological interventions at this crucial timepoint have been shown to interfere with this reconsolidation [73,74,75,76]. In this direction, virtual reality environments have been especially useful in exposing subjects to environmental triggers [77]. While their mechanisms of action are unknown, the efficacy of lifestyle interventions like diet, exercise, and meditation will undoubtedly come to bear as efficient therapeutic strategies in the treatment of anxiety and fear [78,79].

Combinatorial treatment regimens

Given the complexity that underlies disorders like anxiety and fear, it is unlikely that one single form of intervention will be completely successful. What will more likely be needed are treatment regimens that act across several levels. Support for this comes from studies like the one wherein subjects with an intense fear of heights reported less fear after being subjected to a combinatorial treatment regimen that was comprised of virtual-reality extinction-based therapy and DCS administration [27,28,67], as well as the potential mechanisms of combined reconsolidation and extinction [74,75]. With the plethora of potential treatments listed above, the permutations and possibilities for combinatorial treatment are many.

In summary, while a lot has been understood about anxiety and fear from animal models and human case studies, we are only beginning to peel away the layers of complexity associated with these disorders. As this happens, we will not only understand new neural mechanisms that go awry in these disorders, but also gain new molecular, anatomical, and behavioral foci that can be targeted with advances in drug design, and technology. Until then a multi-pronged approach that combines basic research in animals, with

early diagnosis and intervention in humans will enable these disorders to be treated in a more efficient manner. We believe that due to the translational understanding of fear, which is the basis of anxiety-related disorders, this set of psychiatric syndromes will be the earliest understood at a mechanistic level among common psychiatric illnesses. With current rapid progress in understanding of neural mechanisms of fear and emotion regulation, the future of new and promising approaches is indeed bright.

Disclosures

Dr. Ressler is a founding member of Extinction Pharmaceuticals/Therapade Technologies to develop D-cycloserine, a generically available compound, for use to augment the effectiveness of psychotherapy. He has received no equity or income from this relationship within the last three years.

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