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Development of anxiety: the role of threat appraisal and fear learning

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Abstract

Anxious individuals exhibit threat biases at multiple levels of information processing. From a developmental perspective, abnormal safety learning in childhood may establish threat-related appraisal biases early, which may contribute to chronic disorders in adulthood. The current review illustrates how the interface among attention, threat appraisal, and fear learning can generate novel insights for outcome prediction. This review summarizes data on amygdala function, as it relates to learning and attention, highlights the importance of examining threat appraisal, and introduces a novel imaging paradigm to investigate the neural correlates of threat appraisal and threat-sensitivity during extinction recall. This novel paradigm can be used to investigate key questions relevant to prognosis and treatment.

Keywords

fear conditioning; generalization; attention; amygdala; ventromedial prefrontal cortex

INTRODUCTION

Childhood anxiety disorders can be viewed as “gateway” conditions because they signal increased risk for various mental illnesses. Indeed, childhood anxiety disorders predict a two-to-three fold increased risk for adult disorders, particularly anxiety disorders and major depressive disorder (MDD) [1–4]. Nevertheless, many anxious children mature to become healthy adults, free of psychopathology [2]. As a result, there is a need to understand the factors that distinguish between the subgroups of anxious children that have relatively high and low risk for adverse outcomes.

Long-term adverse outcomes may vary based on patterns of information processing and associated neural responses engaged when confronting threats that signal impending danger. Specifically, anxious individuals exhibit threat biases at multiple levels of information processing, including aspects of attention orienting, cognitive appraisal, and learning [5,6]. In addition, neuroimaging studies conducted in separate samples of children, adolescents, and adults implicate similar brain regions in tasks measuring these anxiety-related information-processing biases [7–10]. This work raises questions on the degree to which neural responding to threats at one point in life predicts outcome at later points in life. As such, neuroimaging may eventually be used to identify subgroups of anxious children most likely to develop chronic conditions.

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Two prior reviews set the stage for the current review. One review focused on integrating clinical and basic perspectives on anxiety [5]; the other focused on using neuroscience to inform therapeutics [6]. The current review focuses more narrowly on neurocognitive influences on fear learning, a topic not addressed in these past reviews. The goal here is to illustrate how a narrow focus on the interface among attention, threat appraisal, and fear learning can generate novel insights for outcome prediction. This review proceeds in three stages. The first section broadly summarizes data on amygdala function, as it relates to learning and attention; the second section then focuses more narrowly on one specific information-processing function, threat appraisal. The final section introduces a novel imaging paradigm to investigate the neural correlates of threat appraisal during extinction recall.

LEARNING, ATTENTION, AND AMYGDALA PLASTICITY

Conditioning and Extinction

Considerable research on learning examines the functioning of the amygdala, a brain region that plays a key role in stimulus-reinforcement learning. The amygdala is often examined through classical fear conditioning and extinction experiments, both of which can be considered instances of “fear learning”. In conditioning experiments, a neutral conditioned stimulus (CS) acquires the ability to provoke fear through stimulus-reinforcement learning when the CS is paired with an aversive unconditioned stimulus (UCS). In animals, experimental manipulations show that intact amygdala function is required for the acquisition and expression of fear conditioning [11,12]. Many such studies use simple fear conditioning paradigms, where a single CS is paired with the UCS; whereas, translational work in humans typically employs differential conditioning paradigms, involving two CSs. The CS+ is paired with the UCS, and the CS- is never paired with the UCS. Following conditioning, fear responses can be attenuated through the process of extinction, a second form of stimulus-reinforcement learning. In this learning process, the CS+ is repeatedly presented in the absence of the UCS, which leads to reduced fear responding. While some initial research attributed extinction to forgetting, more recent findings suggest that extinction involves active learning of the CS+-no threat association [13,14]. After extinction, the organism learns to re-classify a CS+ that was previously viewed as threatening. Using such paradigms with shock as the UCS, functional magnetic resonance imaging studies (fMRI) in adults implicate the amygdala in fear conditioning and extinction [15,16], which is consistent with animal work.

Research on fear conditioning and extinction has long been considered relevant to anxiety disorders. Anxiety disorder patients have exaggerated fear responses to simple cue conditioning [17,18]. Nevertheless, psychophysiological research in both pediatric and adult anxiety disorder patients demonstrates relatively subtle perturbations in differential cue conditioning [19,20]. Moreover, contrary to initial predictions that anxious individuals condition to a greater extent [21], most research has failed to find enhanced levels of differential conditioning; anxious individuals have enhanced responding to conditioned safety cues, possibly due to deficits in stimulus classification [19]. The inconsistent findings from physiology research could reflect the failure to account for possible information-processing perturbations occurring when patients learn about danger and safety. These perturbations could prevent patients from recognizing safety signals or inhibiting fear responses when safety cues are present [22].

While these physiological data provide modest evidence for an association between fear learning and clinical anxiety, much stronger evidence emerges from research on exposure therapy, which relies on principles of extinction. Patients treated with exposure therapy are taught to acquire stimulus-safety learning through threat exposure, where the patient learns

to reduce fear reactions over time. In the case of anxiety disorders, excessive fears manifest in safe contexts, and exposure therapy teaches patients to react appropriately in these contexts. When recalled in the extinction context, the amount of fear elicited by exposure is expected to reflect the competition between the original fear memory and the extinction memory. If the extinction memory is successfully recalled as part of therapy, then the fear reaction should diminish. Fully effective treatment allows these instances of extinction learning to produce clinical benefit when skills learned during exposure generalize to other situations. Thus, fear reactions also should diminish to a range of other real-world stimuli resembling the original feared object. Moreover, fear learning interacts with other psychological processes and associated brain regions. Through these interactions, attention control and orienting, appraisal of fear states, conceptualized by elaborations of classifications, and the ability to discriminate threat/safety in situations when this discrimination is difficult shapes fear learning. Figure 1 presents a schematic of these processes and associated neural architecture.

Attention and the Amygdala

Attention is the process whereby capacity-limited neurocognitive resources are allocated based on the relative salience of environmental cues [23,24]. This process of attention allocation may influence stimulus response learning, partially through effects on the amygdala. Amygdala-attention interactions involve both bottom-up and top-down mechanisms [12,24,25], and both mechanisms may influence fear learning. For bottom-up processes, the amygdala can respond very rapidly to threat-related stimuli, thereby changing the focus of attention and facilitating stimulus-response learning about a salient stimulus and other stimuli that predict its occurrence [12,26]. For top-down processes instantiated in the prefrontal cortex (PFC), the representation of task-related goals influences amygdala engagement, which, in turn, influences stimulus-response learning. For example, when attention is allocated to a demanding cognitive task, task-related goals can reduce the amygdala response to task-irrelevant emotionally-salient cues [27]. Such an effect of attention on the amygdala also would be expected to account for well-known effects of top-down attention on stimulus-response learning.

Clinical expressions of anxiety are known to involve perturbed attention allocation in potentially dangerous situations, where attentional capture in response to threat may be strengthened and regulation may be diminished. Threatening displays from conspecifics, such as an angry facial display from a stranger, or other innately dangerous stimuli, such as snakes or cues of suffocation, evoke threat responses, even in the absence of prior exposure or learning with these stimuli [28,29]. Threat-related attention biases are seen in the anxiety disorders, even when threats (e.g., angry faces) are presented too rapidly to be labeled [30]. These perturbations in attention are thought to influence risk for anxiety by shaping physiologic and neural responding during learning. While, based on available data, it is reasonable to suggest that attention shapes risk for anxiety through effects on the amygdala and fear learning, few neuroimaging studies examine the physiology of fear learning in anxiety disorders. As a result, virtually no data directly consider the impact of attention on between-group differences in fear-learning through effects on brain circuitry. Some research does investigate the interactions between attention and fear learning in healthy adults [31–34], setting the stage for future work in patients.

As with behavioral research on attention, most imaging research on amygdala function in anxiety disorders relies on paradigms that expose participants to various facial expressions in the absence of any learning-related manipulation. This research consistently finds enhanced amygdala responding to threatening faces in a range of adult and pediatric anxiety disorders [7–9,26]. For example, anxious children exhibited greater amygdala activation to overt fearful faces, and amygdala activation correlated positively with trait anxiety measures

[35]. While the exaggerated responses to emotional stimuli in anxiety often are viewed as reflecting perturbed conditioning-related processes, few research studies directly evaluate this possibility [36–38]. Nevertheless, other imaging work does focus on the relationships among clinical anxiety, attention, and amygdala function. Two studies compared anxiety disorder patients and healthy subjects exposed to rapidly-presented, difficult-to-detect, threat-cues. Both studies found evidence of amygdala hyper-activation in anxiety disorder patients [8,26]. Other studies compared amygdala responding when patients and healthy subjects are required to view threat cues in a series of alternating attention states, such as during passive viewing, incidental threat processing, and cognitive tasks that directly focus attention on threat content [7]. In healthy individuals, amygdala activation is suppressed under cognitive tasks, which require high levels of effort [24,27,39], and this attention effect moderates between-group differences in amygdala activation [7,40]. To fully integrate basic and clinical approaches of attention and amygdala function, work is needed to extend such findings to research on conditioning and extinction.

Amygdala Plasticity

Research on amygdala plasticity also informs brain imaging. Molecular research on amygdala function delineates factors that support stimulus-reinforcement learning, as reflected in conditioning and extinction. Such learning requires engagement of particular molecular signaling cascades previously shown to support cellular plasticity [41,42]. High levels of plasticity exhibited by the amygdala are thought to enable stimulus-reinforcement learning [43]. While such plasticity often is quantified through invasive techniques, it also may manifest in the temporal dynamics of amygdala functioning, as assessed through neuroimaging. For example, amygdala activation rapidly habituates during fear-conditioning [44,45] and face-viewing [46,47], possibly reflecting cellular aspects of amygdala plasticity. Moreover, the amygdala response also resets eventually [48,49], which may represent an adaptive, plasticity-related aspect of amygdala function that maintains vigilance for subsequent threats or other salient cues. Thus, habituation failures or, alternatively, sensitization in the amygdala response may contribute to heightened anxiety [50,51].

As with other work linking anxiety to amygdala responding, research on novelty demonstrates different amygdala response patterns in behaviorally-inhibited and non-inhibited individuals [52,53]. These temperamental differences may reflect difficulties in resolving ambiguity in novel stimuli. As such, increased vigilance to novelty may reflect a failure to adapt, which, in turn, may arise from perturbed amygdala plasticity. Evidence of these between-group differences in amygdala plasticity demonstrates the importance of using brain imaging to examine the interface between clinical anxiety and either conditioning or extinction.

THREAT APPRAISAL

Defining Threat Appraisal

The term “appraisal” refers to the process of stimulus classification, based on goal relevance for the organism [5,54]. The term “threat appraisal” refers to such classification when it is based on danger or a stimulus’s capacity for harming the organism. To create such classifications, stimuli must be evaluated based both on their emotional valence and their goal relevance.

Research on threat appraisal is complex, due to the fact that appraisal is indexed by multiple measures. Research indexing appraisal in rodents and non-human primates typically relies on motor and associated physiological responses [55,56]. Such work finds threat-related responding to exhibit complex associations with threat intensity. In some situations, a group of measures each show coherent, linear relationships to threat intensity, but in other

situations, they show discordant, non-linear relationships. For example, high levels of arousal are associated with both approach-attack and freezing behaviors, representing high and low levels of motor activity, respectively [57]. Though arousal levels may be high in response to threat, the behavioral reaction may depend on additional factors. For example, imminent and direct threats can elicit approach-related attack [58]; whereas, anticipated or ambiguous threats can elicit freezing as opposed to behavioral avoidance (i.e., flight). Thus, different threats produce distinct forms of motor-physiology output, complicating attempts to precisely quantify threat appraisals. Given the complex nature of appraisal, multiple measures are needed to precisely quantify learning-related mammalian appraisal biases.

In humans, as in rodents and non-human primates, threat appraisal can be indexed by physiologic arousal and defensive/avoidance behaviors, though humans also use language to classify stimuli. In fact, anxiety disorders are defined by subjective reports of inappropriately-experienced fear and avoidance, which can be considered one form of threat-appraisal bias. Patients with anxiety disorders and individuals scoring high on anxiety scales classify some stimuli as dangerous that healthy individuals classify as safe [59–62]. As such, verbal reports of an individual's internally experienced fear reflect aspects of threat appraisal that are particularly relevant to clinical work. For research with humans, this investigation involves asking research participants to rate their fear, which might engage different neurocognitive processes than when naturally encountering threats. As such, the act of appraisal itself might complicate research on fear by influencing the physiology of threat processing. In some threatening situations, verbal reports positively correlate with motor and physiologic response patterns [63,64], but in other situations they either do not correlate or even show opposite patterns of correlation [65,66]. This discordance further emphasizes the need in research on appraisal to acquire data using multiple measures and the need to understand how appraisal may change learning. Thus, biased threat appraisals in anxiety disorders can manifest as correlated patterns in verbal, motor, and physiological measures or as a particularly aberrant response pattern in any one of these measures.

Threat Appraisal in Clinical Anxiety

Anxiety disorder patients have biased appraisals of threats, as reflected in exaggerated physiological arousal, avoidance, and reports of fear. Such biases consistently manifest in patients, as discussed in a prior review [5]. This prior review focuses on biased appraisals of innately feared dangers, those dangers, in the absence of prior exposures, which are capable of evoking fear and associated threat responses. These dangers include cues of suffocation or threatening social displays, which evoke enhanced fear responses specifically in separation anxiety disorder and social anxiety disorder, respectively [5]. In fact, exaggerated fear of innately feared threats may represent the strongest correlate of anxiety disorders in laboratory-based research [67]. The current review does not focus on responses to innately feared threats but rather focuses on appraisal biases that manifest during fear learning, the process where a neutral stimulus acquires a threat value through its pairing with an aversive experience. Moreover, the current review focuses on the role of learning as a shared feature across the anxiety disorders and does not focus on individual anxiety disorders. This approach follows from the fact that studies of conditioning and exposure therapy implicate learning-related biases in many anxiety disorders [13,68].

Studies of fear conditioning and extinction learning draw parallels with studies of emotion regulation [31]. Emotion regulation work focuses on the process of reappraisal and its influence on the amygdala. In reappraisal, a subject first attempts to monitor or “appraise” their emotional reaction to a stimulus, and then they attempt to change or “reappraise” this reaction. For example, individuals might be asked to reinterpret or distance themselves from initial reactions to a threat, a process which dampens amygdala responding and negative

affect in healthy individuals [69]. Both appraisal and reappraisal shape perceptions of emotional significance through the medial PFC [69–71].

Not only do patients with anxiety disorders exhibit biased initial appraisals of threats, but they also show a reduced capacity to alter these initial appraisals [5,72,73], which may be reflected in aberrant PFC engagement [73,74], a key regulator of amygdala engagement [69,75–77]. Of note, as with other research on appraisal biases, work on reappraisal in anxiety primarily focuses on responding to intrinsic as opposed to learned, newly acquired fears. Nevertheless, the work on emotion regulation does inform research on fear learning. In fact, one study showed that both emotion regulation and extinction activated the medial PFC/subgenual ACC [31]. Studies of conditioning, like those of appraisal, might model neural and psychological factors related to amygdala engagement during the initial stages of threat encounters. Conversely, studies of extinction, like those of reappraisal, might model PFC regulation of this initial amygdala engagement, as it facilitates attempts to reclassify the stimulus as non-threatening.

Appraisal, Conditioning, & Classification

Prior work on conditioning supports the importance of studying how appraisal biases are learned. Previously, anxiety disorders had been thought to result from enhanced conditioning; however, a more recent perspective suggests that patients with anxiety disorders show difficulty distinguishing threat from safety when studied with conditioning and extinction paradigms [18,60]. The available data in this area also suggests that appraisal biases in anxiety disorders can manifest as perturbed fear-generalization gradients [19].

Fear generalization is a natural process where the “threat” value of a feared stimulus is transferred to stimuli resembling the feared stimulus [78]. To study fear generalization in more detail, Lissek and colleagues developed a paradigm that involved two procedures: fear conditioning and a generalization test [79]. During differential fear conditioning, a small and large circle served as the CS- and the CS+. During the generalization test, circles of varying size between the CS- and the CS+ were shown to the participant. Following conditioning, the CS+ was a clear, unambiguous threat cue, but the meaning of the each stimulus resembling the CS+ was ambiguous. As is important for studies of appraisal, data from multiple measures were recorded, including eye-blink startle, motor-response times, and perceived risk. In healthy subjects, each measure showed signs of varying in tandem with features of the CS, consistent with the presence of a generalization gradient. This gradient fell along the continuum between the CS- and CS+, the two extreme stimuli. For startle and risk-perception data, the greatest responses occurred to the CS+ and decreased along a curvilinear pattern as the CS became more like the CS-. Brain regions are also expected to show graded responses to stimuli that resemble each other [80,81].

Adults with panic disorder exhibited signs of an appraisal bias on this task, when compared to healthy subjects [82]. In healthy adults, subjective fear ratings and psychophysiology data indicated the ability to discriminate “safety” cues that were quite similar in appearance to the CS+. Interestingly, on multiple measures, the fear responses to a clear, unambiguous CS+ and a clear, unambiguous CS- differed from each other, both in patients and in healthy adults, with no evidence of between-group differences in conditioning. This result suggests that adults with panic disorder and healthy subjects appraise some forms of overt threat as similarly dangerous. However, for more ambiguous threats, a threat-appraisal bias did manifest in panic disorder patients, through the process of overgeneralization or deficient discrimination. While groups similarly appraised the unambiguous CS+ threat, patients appraised an *ambiguous* CS threat as more dangerous than did the healthy adults, with parallel between-group differences in physiology and subjective ratings. In essence, the “threat” value of the CS+, acquired through learning, had been more extensively transferred

to stimuli resembling the CS+ in patients than healthy subjects [82]. Thus, some appraisal biases in a range of anxiety disorders may reflect a compromised capacity for learning the boundaries separating safe and threatening stimuli and inhibiting fear responses in safe contexts.

Extinction and Classification

Extinction is another learning process where boundaries among threats are necessary. However, whereas generalization gradients reflect boundaries along a stimulus-feature gradient [82], extinction reflects boundaries along a temporal and contextual gradient. In extinction, a time-related re-classification must occur; stimuli that are currently dangerous must be distinguished from those that were previously dangerous [13]. This process requires reappraising the emotional value of a previously-feared stimulus. Thus, extinction, the process of learning a new stimulus-safety association, is linked to the process of emotion regulation of conditioned fear.

Considerable work in the rodent examines neural mediators of extinction learning, and this research provides a strong foundation for examining neural correlates of the anxiety disorders. Lasting extinction of conditioned fear in the rodent requires intact functioning of neurons connecting the infralimbic cortex to the intercalated cells of the amygdala [76,83,84]. Similar findings have been generated in humans. In an fMRI study, ventromedial PFC (vmPFC) and amygdala activation have been detected in extinction learning [16,85]. In addition, during a reversal learning task in healthy adults, the amygdala tracked the fear signal, while the vmPFC tracked the safety signal [86]. Finally, individual differences in extinction learning and the underlying neural circuitry influence the emergence of anxiety. The methionine (Met) allele variant of the brain-derived neurotrophic factor (BDNF) Val66Met single-nucleotide polymorphism is associated with anxiety and impairs extinction learning in both rodents and humans. Less vmPFC activation and greater amygdala activation during extinction were detected in human carriers of the Met allele [87], and this effect of the Met allele on fear-circuitry function may manifest uniquely in anxious and healthy individuals [88].

In both rodent and human studies, the infralimbic cortex (IL)/or vmPFC involvement appears particularly important for the process of extinction recall, which differs in subtle ways from extinction learning. In extinction learning, the organism demonstrates the capacity to acutely lower responses to the CS+, shortly after the CS+ has been presented multiple times in the absence of the UCS. Extinction recall refers to the process whereby the organism retains this ability over time. During extinction recall, the organism is re-exposed to the previously-extinguished CS+ after a considerable delay following extinction learning. Whereas IL lesions do not disrupt the learning of extinction contingencies, they do prevent consolidation of this learning. Rodents with IL lesions show normal conditioning and extinction, but they show an exaggerated return of fear on re-testing one day after extinction training [89]. Neuroimaging studies of extinction recall among adults demonstrate the clinical relevance of such findings. Here, vmPFC structure and function is linked to fear-related behavior during extinction recall, based on physiological [85,90] and clinical indices [36].

Learning and Development

Development constrains the neural pathways that support various types of learning, including fear learning [91]. In other words, the ability of an organism to learn about safety and danger varies across development, such that immature organisms rely on different brain structures and show unique learning-related changes, relative to mature organisms. While no neuroimaging study examines the interactions between human development and fear

learning, the early appearance of individual differences in fear responses suggest these interactions exist. In rodents, diverse experiences occurring at key stages in development can produce similar changes in underlying neural circuitry and associated behaviors engaged by threats. For example, pups either separated from their mothers during critical development periods or reared by mothers with impaired licking/grooming abilities have high stress reactivity, suggesting that developmental experiences can alter the threat response [92,93]. On the other hand, similar experiences occurring at different developmental stages can produce unique behaviors. For example, amygdala lesions in childhood monkeys increase fear responses to conspecifics; whereas, this fear is reduced with adult lesions [94]. Finally, behaviors acquired at different stages of development can be mediated by distinct circuits. Studies of language and motor learning suggest that different neural pathways support skill acquisition at different stages in life [95]. In humans, developmental trajectories of facial expression recognition suggest that sensitivity to discrimination is refined with age [96,97]. At least in some contexts, children may exhibit greater amygdala activation to neutral faces, relative to fearful faces [35]. These data may suggest that neutral faces are deemed more ambiguous until discrimination and appraisal processing mature. In fact, amygdala activation to fearful faces is greater in adolescents compared to adults [98,99]. In addition, at least in rodents, hippocampal contributions to fear learning appear to mature later than amygdala contributions [100]. As such, the ability to discriminate among a group of complex threat-related stimuli occurs later, in tandem with maturation in the hippocampus, than the ability to discriminate from overtly safe and dangerous stimuli [101]. Therefore, it is likely that the developmental stage influences the capacity for fear learning, and these interactions are mediated by neural circuitry changes. This developmental work on fear learning can inform therapeutics, since different strategies may be most efficient when attempting to alter behaviors in two individuals that are mediated by unique ontogeny.

Fear and safety learning may interact with development in several important ways, and this interaction, in turn, may predict the outcome of pediatric anxiety. Although the ability of the amygdala to generate conditioned fear responses likely emerges early [102,103], cortical regions reach maturity later in development [104]. Moreover, the neural circuitry underlying fear conditioning and extinction may change with age as the vmPFC and the connectivity among the amygdala, hippocampus, and vmPFC matures [98,105,106]. In addition, developmental changes in brain structure and function may enable increased cognitive appraisals and classification of complex threats [107–110]. Within the context of conditioning experiments, the capacity to discriminate “threat” from “safety” also may mature with development, such that failures to increase this discrimination capacity in childhood may contribute to persistent anxiety disorders.

Epidemiological data suggests that data on the physiological correlates of extinction are needed in pediatric anxiety. The prevalence of anxiety is high in childhood and adolescence [111]; however, most of these disorders remit by adulthood [2]. Interestingly, during this same developmental transition, newly-onset anxiety disorders also become increasingly rare [1,2]. This trajectory suggests that the failure to overcome pediatric anxiety accounts for a significant proportion of anxiety in adults. Adult anxiety may reflect a failure to extinguish childhood fear reactions, expressed in inappropriate contexts. As such, deficient extinction may predict persistence of anxiety disorders into adulthood.

DEVELOPING A NOVEL IMAGING PARADIGM

This final section reviews factors that inform the development of imaging paradigms for assessing neural correlates of extinction recall. Such efforts face technical hurdles, including complications related to UCS selection, methods for quantifying generalization gradients,

and for constraining the effects of attention on neural circuitry function. This section delineates a range of approaches for addressing these hurdles and then presents one illustrative paradigm. The proposed paradigm is provided as only one option because additional paradigms will possess other advantages [112]. However, the delineation of specific procedures and their associated justification provides a useful guide for considering alternative approaches. Finally, research questions are posed to illustrate how novel imaging paradigms might extend our basic understanding of conditioning and extinction to generate relatively clear, specific hypotheses concerning extinction recall. This future work will shape research on pediatric anxiety disorder outcome and treatment.

UCS Selection

Attempts to examine fear learning are shaped by the nature of the UCS. Levels of conditioning are influenced by UCS potency, with a strong, novel and evolutionarily-relevant UCS generating strong conditioning [67,113]. This UCS selection also heavily shapes attempts to study extinction [114]. Extinction tends to occur quickly in studies of physiological responding among humans [16]. As a result, studies that employ a relatively weak UCS will possess limited ability to examine individual differences in extinction. For these reasons, the use of a relatively potent UCS carries clear advantages.

In conditioning research on adult anxiety disorders, electric shock represents the UCS that generates the most consistent findings. For example, fear-learning paradigms employing a shock-UCS generate relatively robust increases in sustained fear, as reflected in physiological reactions to the experimental context [115]. Such measures of sustained fear correlate with clinical measures of anxiety and are reduced by treatment with clinically-effective medications [116]. Moreover, the over-generalization of conditioned fear in panic disorder patients emerged in a shock-UCS paradigm [60,82]. However, shock is not the only viable UCS. Lissek and colleagues compared the subjective response to various UCSs in research with adults and showed no differences in the anxiety provoked by white noise, tone, alarm and screams (Figure 2). In some circumstances, more robust between-group differences may emerge in research on anxiety disorders that relies on relatively mild as opposed to more aversive threat-related stimuli [117]. However, in other circumstances, studies using a mild UCS will be insensitive to relevant between-group differences. For example, subsequent work substituted air-puffs to the throat, a mildly aversive UCS, for shock [118,119]. This milder UCS failed to generate sustained elevations in fear in paradigms previously generating consistent clinically-relevant findings [115].

Obvious ethical questions emerge concerning the use of shock-UCS research in pediatric anxiety disorders. One could argue that shock-UCS represents a minimal risk procedure, given that shock yields less pain than that associated with venipuncture and the shock level is determined by the subject to be only mildly aversive, not painful. On the other hand, given the vulnerable state of anxious children, it is important to consider an alternative UCS. Aversive air-puffs, loud sounds, and aversive pictures have been used in fear-conditioning and fear-potentiated startle research as alternatives to shock [17,20,119–121]. Considerable research, including research on temperament, clinical anxiety, and amygdala response, demonstrates meaningful associations between pediatric anxiety and response to aversive air-puffs [122,123]. However, the magnitude of rated fear generated is low, raising questions on the suitability of this probe for conditioning research [122].

The suitability of a novel UCS involving an aversive photograph of a fearful woman, coupled with a loud shrieking scream, has been evaluated. This novel UCS extended other research on conditioning and imitation, showing that observing extreme fear in a conspecific serves as a potent UCS in both humans and other mammals [67]. Moreover, the subjective and physiologic response to this novel UCS fall between that associated with shock UCS

and milder UCSs, such as loud sounds or aversive pictures, presented in isolation [124]. Finally, this UCS was subsequently used successfully in a conditioning study of pediatric anxiety disorders [20]. As a result, this “screaming lady” UCS represents a reasonable alternative to an aversive shock UCS and mild auditory or tactile UCSs (e.g., loud sounds, airpuffs). In addition, this UCS is well-suited for an imaging study on extinction recall.

Generalization

The “screaming lady” paradigm possesses other advantages associated with its utility for testing fear-related generalization gradients. Following conditioning using circles of two different sizes (CS+ and CS-), Lissek and colleagues had presented circles with varying diameters to contrast generalization gradients in healthy adults and adults with panic disorder [82]. Using a similar approach, the screaming-lady paradigm allows the examination of such gradients in pediatric anxiety disorders. Specifically, in the screaming lady paradigm, photographs of two ladies are used in conditioning. One lady serves as the CS+ and the other lady as the CS- [20]. Through morphing software, a continuum of stimuli can be generated by mixing perceptual features of these CS+ and CS- stimuli to study generalization gradients in fear response to these stimuli. Figure 3 illustrates the two types of stimuli used to assess generalization gradients following conditioning, circles of two different sizes and two different women with neutral facial expressions. Figure 4 illustrates procedures for examining generalization gradients during threat appraisal in research on pediatric anxiety disorders. In this illustration, differential conditioning to the two neutral photographs occurs and is followed by extinction. At a later period, subjects are exposed to both the CS+ and CS-, during which time they attempt to recall various aspects of each stimulus. Subjects also are exposed to stimuli varying along a continuum of similarity between the features of the CS+ and CS-. Thus, the screaming-lady paradigm can be used to examine generalization gradients during extinction recall in pediatric anxiety disorders.

Attention & Appraisal

The “screaming-lady” paradigm generates reasonable, relatively stable between-group differences in reported fear to the conditioned faces [20]. Moreover, these between-group differences can be quantified using generalization gradients that have meaningfully informed research on adult anxiety disorders [82]. The culmination of ideas sets the stage for an imaging study of extinction recall in pediatric anxiety disorders using the “screaming-lady” paradigm. Work reviewed above suggests the feasibility of using such an approach to examine aspects of extinction recall. Research on extinction is clinically relevant, given that exposure therapy relies on procedures from extinction to generate clinically-meaningful benefits for both pediatric and adult patients. Moreover, prior basic science work, also reviewed above, generates relatively specific hypotheses concerning the role of a relatively specific neural circuit in individual differences. Finally, from the developmental perspective, extinction emerges as a particularly relevant process. Pediatric anxiety is extremely common, and the typical outcome of such anxiety is remission, possibly reflecting instances of successful extinction. As a result, adult anxiety disorders can be conceptualized as failures to extinguish pediatric fears, a view that directly informs outcome prediction.

From this perspective, we developed an appropriate imaging paradigm of extinction recall. This paradigm possesses three essential features: fear learning, attention modulation, and fear generalization. First, in terms of fear learning, fear conditioning and immediate extinction would be conducted in the psychophysiological laboratory, using similar procedures employed by Lau et al. (2008). Second, at a later date, research participants would undergo fMRI to study how the neural correlates of extinction recall are modulated by attention. During scanning, subjects would attempt to recall their experience with extinction under different attention states. This approach would effectively model brain

circuits engaged during extinction recall, a particularly important, clinically-relevant process. However, such an approach to imaging between-group differences also should incorporate understandings of attention as it influences amygdala engagement. As reviewed above, attention powerfully influences amygdala engagement in research among healthy and anxious children and adults. Most importantly, between-group differences in pediatric anxiety disorders are gated by attention. In particular, the most consistent between-group differences in the amygdala and ventral PFC emerge during fear-appraisals, when subjects focus attention on their internal reaction to a fearful stimulus [7,40]. Therefore, this extinction-recall imaging paradigm assessed amygdala and vPFC activity in an emotionally-relevant attention state, i.e., focusing on internal fear, as well as in a two additional attention states, where subjects are asked to recall the CS+-UCS association and to rate a physical feature of the stimulus.

Combining these two aspects of this novel approach, amygdala and prefrontal cortical function can be contrasted as part of a fear-learning process in anxious and healthy youth in varying attention states, and, in particular, during threat appraisals. This investigation can be accomplished through a paradigm where subjects view blocks of images and are instructed to make a yes/no judgments according to three instructions: 1) Are you afraid? (threat appraisal); 2) Did she scream? (explicit memory); and 3) Is her hair jet black? (perceptual discrimination). Finally, this paradigm generates data on fear generalization in the context of extinction recall. Specifically, in each block, morphed images that form a continuum of similarity between the CS+ and CS- are randomly presented. Morphed images are used in this paradigm to precisely characterize threat sensitivity, as reflected in levels of behavioral and neural discrimination among similar images.

Hypotheses

Several hypotheses emerge from this novel neuroimaging paradigm that incorporates fear learning, attention modulation, and fear generalization principles. First, as previously noted, anxiety disorders in childhood predict anxiety disorders and major depression in adulthood. However, not all children and adolescents with an anxiety disorder will have long-term adverse outcomes. Activation of the vmPFC, a region involved in emotion regulation and extinction processes, during threat appraisal may reflect the ability to discriminate threat and safety. Perturbations found in vmPFC activation and fear overgeneralization during threat appraisal may identify groups that are likely to develop chronic disorders. Reduced vmPFC activation is expected to be associated with poor long-term outcomes because perturbations in vmPFC activation are expected to lead anxious youth to appraise ambiguous stimuli as dangerous (i.e., extinction deficit and fear overgeneralization), and thereby, contribute to the development of anxiety disorders. In addition, from a developmental perspective, deviations from the normal maturation trajectory of vmPFC function and threat-safety classification ability may allow the identification of sensitive periods for clinical expression [125]. Secondly, perturbations in vmPFC activation and associated deficits in safety learning may predict clinical outcome. Exposure therapy draws on principles of extinction. With repeated exposures to a feared stimulus, the fear reaction is expected to decrease; however, threat-safety discrimination ability must be intact. Greater vmPFC activation during threat appraisal, indicative of better discrimination capability, may be associated with greater symptom improvement during exposure therapy. In addition, pharmacologic manipulations of glutamatergic system via the *N*-methyl-D-aspartic acid (NMDA) receptor (e.g., D-cycloserine, DCS) may facilitate extinction [126]. Giving DCS prior to exposure therapy enhances fear reduction in social phobia [127] and acrophobia [128]. These treatments may have the ability to alter fear learning by facilitating the ability to disambiguate threats. Assessing vmPFC activation during extinction recall may help identify individuals who would show greater benefits from pharmacological manipulation of extinction. For example,

pharmacological treatment may be best suited for individuals with less vmPFC activation. In summary, research that investigates the boundaries separating threat- and safety-related stimuli may carry major therapeutic implications.

CONCLUSIONS

The current review summarizes the manner in which attention impacts fear learning through appraisal biases in anxiety disorders. From a developmental perspective, abnormal fear-safety learning in childhood may establish threat-related appraisal biases early. These appraisal biases may, in turn, contribute to the development of chronic disorders in adulthood. Individuals with anxiety disorders tend to classify ambiguous stimuli as threatening more so than healthy individuals. Therefore, the boundary separating threat and safety may be blurred in patients with anxiety. Integrating principles from fear learning, threat appraisal, and fear generalization, a novel imaging paradigm was developed to investigate the neural correlates of threat appraisal and threat-sensitivity to ambiguous stimuli during extinction recall. This paradigm can be used to investigate key questions relevant to prognosis and treatment.

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Figure 1. Influences on Fear Learning

Figure 1 Legend: As illustrated in the highlighted box, fear learning primarily involves the amygdala and the ventromedial prefrontal cortex (vmPFC). Through repeated pairing of the conditioned stimulus (CS+) and unconditioned stimulus (UCS), the neural representation of the CS+ is activated in tandem with the amygdala response to the UCS. Following learning, the CS+ alone excites the amygdala and its projections to brain regions mediating the autonomic (e.g., heart-rate, respiration, and sweating response), endocrine (cortisol response), and behavioral (e.g., freezing, flight/fight response) constituents of the fear response. When the CS+ is presented in the absence of the UCS during extinction, the vmPFC is proposed to inhibit this amygdala activation. While fear learning principally involves the amygdala and vmPFC, attention, appraisal, and safety/threat discrimination processes and associated brain regions interact with fear learning to influence the fear response. The ventrolateral prefrontal cortex (vlPFC) and anterior cingulate cortex (ACC) influence the control of attention orienting and resource allocation. The dorsomedial prefrontal cortex (dmPFC) and dorsolateral prefrontal cortex (dlPFC) elaborate the learned classification via threat appraisal and emotion regulation processes. The hippocampus provides context and fear generalization information. Together, these processes and the underlying brain network influence the fear response.

Figure 2. Unconditioned Stimulus Selection

Figure 2 Legend: Thirty-five adults (mean age=27.9) rated similar anxiety levels following a 95dB, 3 second exposure to white noise, 2Hz tone, alarm and a scream (all $p>0.3$). Mean and standard deviations are displayed.

Figure 3. Morph Images used for Generalization

Figure 3 Legend: Several types of stimuli may be used to assess generalization gradients. A) Circles. The circles at the extremes are the images used in the fear conditioning procedure. Circles of varying size created a continuum of stimuli between these two extremes. [79]. B) Faces. The faces at the extremes are the pure images of the light-haired and dark-haired women with neutral facial expressions used in the fear conditioning procedure. The light-haired woman is morphed into the dark-haired woman using 10% increments, providing a continuum of similarity between the two women. The continuum of circles or faces can be used to assess fear generalization gradients following conditioning.

Figure 4. Threat Appraisal Paradigm

Figure 4 Legend: On Day 1, participants undergo fear acquisition and extinction procedures using the screaming lady paradigm in the psychophysiology laboratory using similar methods as Lau et al., 2008 [20]. Two women with neutral facial expressions are shown. One woman (CS+) is paired with a screaming lady (UCS); whereas, the other woman (CS-) never gets paired with the UCS. Approximately two weeks later, participants return and undergo extinction recall in the magnetic resonance imaging (MRI) scanner. Participants are shown a continuum of neutral face stimuli that vary between the CS+ and CS- while attending to their emotional state. A generalization gradient in brain activation in the ventromedial prefrontal cortex is expected. CS=conditioned stimulus, UCS=unconditioned stimulus, vmPFC=ventromedial prefrontal cortex.