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The Teenage Brain: Altered Fear in Humans and Mice

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Abstract
Fear learning is an adaptive, evolutionarily conserved process that allows people to respond appropriately to threats in the environment. These threats can vary across different contexts and across the life course. Taking into account the high degree of neural and behavioral conservation across species in fear regulation and its underlying neural circuitry, we examined how fear learning changes across contexts and over the course of development, focusing specifically on the environmentally changing and challenging period of adolescence. We show two surprising developmental findings specific to adolescents, relative to older and younger individuals: (a) diminished fear to previously aversive contexts and (b) heightened fear to previously aversive cues. These behavioral changes are paralleled by developmental changes in frontolimbic circuitry. We discuss how these evolutionarily conserved mechanisms may be essential to survival of the species, given the changing environmental demands (social, sexual, and physical) of adolescence. Our findings also have important implications for unremitting forms of fear at the core of anxiety-related disorders, which peak during adolescence, and for when during development specific treatments for these disorders may be most effective.

Keywords
adolescence, fear, mouse, human, amygdala, hippocampus, prefrontal cortex, development

Introduction

Throughout their lives, people experience fear. Children often fear the dark or imaginary monsters hiding under the bed. Teenagers may fear social rejection and humiliation. Adults may fear for their jobs or for their families' well-being. These examples suggest that fear persists across the life span but takes various forms as environmental demands change in each phase of life. This paper highlights how people's response to and regulation of fear varies with age, especially during adolescence. We discuss how these changes may be adaptive or maladaptive, depending on the changing demands of the environment.

By definition, adolescence poses new environmental challenges and potential threats, as a period during which individuals move from dependence on their parents to relative independence and must rapidly adapt to new social, sexual, and intellectual challenges. In parallel with these changes are reported increases in the prevalence of anxiety and stress-related disorders, which affect as many a 10% of youths (Kessler et al., 2005; Merikangas et al., 2010; Newman et al., 1996). These health statistics highlight the importance of understanding how fear-related behavior and the brain circuitry at the core of these disorders change during this period.

Fear Learning and Memory

Under normal circumstances, fear learning is a highly adaptive process that allows people to respond appropriately to threats in the environment. However, fear that persists long after the removal of any threat is often referred to as pathological. This unremitting form of fear is a core component of many anxiety and stress-related disorders, which affect nearly 40 million Americans, or 20% of the adult population, and contribute to the country's near $58 billion in annual mental health care expenses (Agency for Healthcare Research and Quality, 2006; Merikangas et al., 2011). Too often, scientists trying to discover novel diagnostics and treatments for

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psychiatric disorders quickly jump into examining model systems of aberrant behavior without a detailed understanding of the typical development of these disorders' core behaviors and underlying neural circuitry. The large portion of youths represented in U.S. mental health statistics underscores the need to understand anxiety from a neurodevelopmental perspective. Specifically, what are the core behaviors underlying anxiety? How do they develop? What are their neurobiological bases, and when does their development go awry?

In our work, we have attempted to address these questions by (a) examining periods of typical developmental transitions during which these disorders have been shown to peak, such as the transition into and out of puberty, and (b) using translational developmental neuroscience to understand how behavior is translated across species. These two approaches require behavioral paradigms that can be used across both developmental stages and species. Fear-learning paradigms are advantageous in this regard because they can be used equally well to assess fear learning in humans and in rodents and to assess fear learning across development. Moreover, because there is a high degree of neural and behavioral conservation across species in fear learning and fear circuitry (LeDoux, 2000), these paradigms have the added benefit of allowing us to delineate mechanisms of change in nonhuman species that would be more difficult to ascertain in humans.

In our experiments, we have used a Pavlovian fear-conditioning paradigm to directly examine how responses to threat change during adolescence (Pattwell et al., 2012). Specifically, we wanted to examine the development of the ability to regulate fear once the threat of fear is removed (i.e., *extinction learning*). Pavlovian conditioning involves pairing an inherently threatening or unpleasant stimulus (*unconditioned stimulus*), such as an electric shock or an aversive noise, with a neutral stimulus, such as a flicker of light or an auditory tone (*conditioned stimulus*). Through multiple pairings of the conditioned stimulus with the unconditioned stimulus, an association is formed, such that the former becomes predictive of the latter. Eventually, after the association between the two types of stimuli has been learned, presentations of the conditioned stimulus alone are capable of eliciting a fear response similar to that elicited by the unconditioned stimulus. In humans, this *conditioned response* is often characterized physiologically by changes in autonomic arousal and behavior, as indexed by changes in perspiration via skin conductance response; in rodents, this response is characterized by freezing behavior. For both humans and rodents, the acquisition and expression of a fear response involves the amygdala, a subcortical structure within the temporal lobe that is implicated in emotion processing (Milad & Quirk, 2012)

Once an association between the conditioned and unconditioned stimuli has been formed, the conditioned response can be extinguished through multiple presentations of the conditioned stimulus alone, in the absence of the unconditioned stimulus. The initial pairing of the stimuli is not forgotten during the extinction process: The fear almost always returns after standard extinction training via renewal (i.e., when fear returns after exposure to the fear-conditioning context), reinstatement (i.e., when fear returns after an unsignaled presentation of an aversive stimulus), or the passage of time (Bouton, Westbrook, Corcoran, & Maren, 2006). Extinction learning, which is not to be confused with the process of mere forgetting, is an inhibitory process during which the association between the conditioned and unconditioned stimuli and expectancies related to this pairing are modified (Sotres-Bayon & Quirk, 2010). By presenting the conditioned stimulus repeatedly, in the absence of any unconditioned stimulus, one can reevaluate the outcome it predicts and thus learn that this stimulus that was once associated with threat has become safe.

Extinction learning is mediated by the infralimbic cortex in rodents and by the ventromedial prefrontal cortex in humans (Milad & Quirk, 2012; Phelps, Delgado, Nearing, & LeDoux, 2004). Uncovering the mechanisms involved in the development of fear acquisition and of fear extinction in particular has wide clinical implications, given that the most common and validated treatment for anxiety disorders involves exposure-based cognitive behavioral therapy. This treatment consists of identification of what triggers the anxiety, followed by desensitization of the patient to it through repeated exposures, a process that builds directly on the principles of extinction (Rothbaum & Davis, 2003). Strong cross-species conservation of the neural circuitry implicated in fear-extinction learning has been supported by human and nonhuman-animal studies, further bolstering the translational credibility of rodent models for studying fear regulation and extinction (Gottfried & Dolan, 2004). We present evidence from studies involving two forms of fear conditioning: cued fear and contextual fear learning. These studies have highlighted changes in fear regulation and extinction and their neural correlates across development.

Cued-fear-extinction learning

In parallel behavioral experiments across development in human children, adolescents, adults, and mice on postnatal days 23, 29, and 70, we examined cued fear learning and extinction. Previous studies with rodents have shown that cued-fear acquisition is intact across all ages (McCallum, Kim, & Richardson, 2010). In research with humans, fear acquisition has been shown to be intact in adolescents and adults, but it is diminished in adolescents when social cues (e.g., facial expressions) serve as unconditioned stimuli (Lau et al., 2011). Our own work with both mice and humans, in which we have used behavioral paradigms with nonsocial cues (tones or colored squares) across childhood, adolescence, and adulthood, has shown that mice and humans

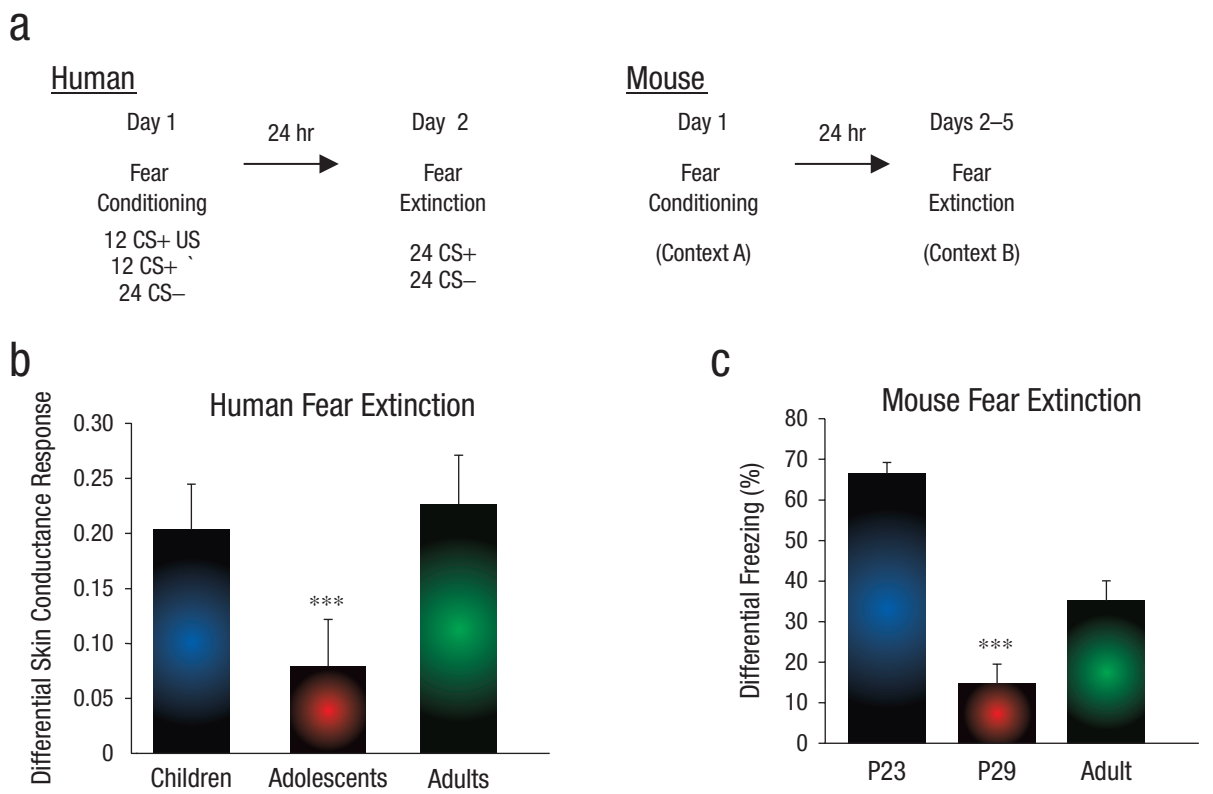

Fig. 1. Cued-fear extinction learning across development in mice and humans. Panel (a) shows behavioral paradigms for parallel fear-conditioning experiments in humans and mice. For humans, conditioned stimuli (CS) are visual cues, one of which (CS+) is initially paired with an unconditioned stimulus (US; an aversive noise) during acquisition and then presented without it during extinction. The graph in panel (b) shows results from an analysis of extinction indices (averaged values from first two extinction trials minus averaged values from last two extinction trials) revealing a main effect of age group for humans, such that adolescents displayed attenuated fear-extinction learning compared with children and adults, based on changes in skin conductance response (adolescents: 0.05916 ± 0.06904; children: 0.25435 ± 0.04839; adults: 0.22510 ± 0.05931). The graph in panel (c) shows results indicating a lack of extinction learning and retention of extinction memory in adolescent mice (29 days old; P29), compared with older (70 days old; adults) and younger mice (23 days old; P23), as revealed by significantly decreased differential extinction indices (calculated by comparing the percentage of freezing behavior in response to the first tone of the extinction trials with the percentage of freezing behavior in response to the last tone of extinction trials, P23: 66.5% ± 2.75; P29: 14.72% ± 4.79; P70: 35.17% ± 4.89). Adapted from "Altered Fear Learning Across Development in Both Mouse And Human," by S. S. Pattwell et al., 2012, *Proceedings of the National Academy of Sciences, USA, 109*, p. 16319. Adapted with permission.

successfully acquire conditioned cued-fear memory equally well across all ages (Pattwell et al., 2012). Surprisingly, after the initial cued-fear memory has been acquired, adolescents show attenuated fear-extinction behavior compared with younger and older subjects (see Fig. 1). This diminished extinction learning during adolescence has been observed in both humans and rodents (McCallum et al., 2010; Pattwell et al., 2012). Exploiting the mouse-model system to further characterize the neural circuitry via electrophysiology and immunohistochemistry revealed blunted responses in ventromedial prefrontal subregions during extinction learning in adolescent mice compared with younger and older mice. Together, these findings suggest that fear regulation does not increase with age, but rather reflects a deflection (nonlinear change) in both behavior and the underlying neural circuitry during adolescence.

Contextual fear learning

In addition to exploring the learning of fear responses to discrete stimuli using cued fear conditioning, our laboratories also have examined the learning of fear responses to discrete contexts using contextual fear conditioning (Pattwell, Bath, Casey, Ninan, & Lee, 2011). Unlike fear responses to discrete cues, which involve neural projections between the sensory thalamus, amygdala, and prefrontal cortex, fear responses to one's context integrate spatial aspects of the surrounding environment (Maren, 2011). The hippocampus, through its

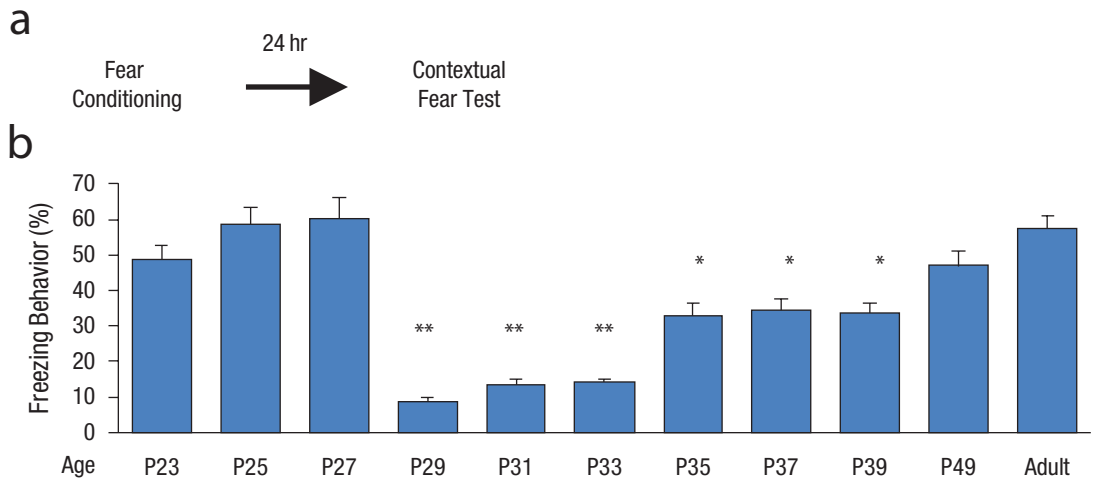

Fig. 2. Hippocampal-dependent contextual fear memory across development. In our paradigm, (a) preadolescent (< 27 days old), adolescent (approximately 29–39 days old), and adult (approximately 49–70 days old) mice were fear conditioned with three tone-shock pairings. Twenty-four hours later, they were returned to the conditioning context, and their freezing behavior was scored by dividing the total number of seconds spent freezing by the total number of seconds in a trial. As the graph (b) shows, adolescent mice froze significantly less than both younger and older mice. All results are presented as a means for each age group comprising 7 to 10 mice (*$p < .05$, **$p < .001$); error bars show standard errors of the means. P = postnatal day. Adapted from "Selective Early-Acquired Fear Memories Undergo Temporary Suppression During Adolescence," by S. S. Pattwell, K. G. Bath, B. J. Casey, I. Ninan, and F. S. Lee, 2011, *Proceedings of the National Academy of Sciences, USA*, *108*, p. 1183. Adapted with permission.

projections to the amygdala and prefrontal cortex, mediates fear responses on the basis of the level of safety or threat in the environment. When mice are fear conditioned in a given context and subsequently returned to it at later time points, they exhibit a fear response (freezing) to the conditioning context alone, in the absence of any auditory-tone cues.

To explore contextual fear across development, we fear conditioned preadolescent, adolescent, and adult mice and tested them the following day for contextual fear (see Fig. 2). As expected, adult mice showed an increased freezing response upon being returned to the context in which they had previously been shocked (Pattwell et al., 2011). Interestingly, preadolescent mice showed contextual fear responses that were indistinguishable from those of adult mice. Adolescent mice, however, showed a lack of fear when returned to the conditioned context (Pattwell et al., 2011). When the same adolescent mice were retested for contextual fear at later, postadolescent time points, contextual fear expression emerged, which suggests that the expression of contextual fear was merely temporarily suppressed during adolescence.

This suppression of contextual fear expression during adolescence is associated with altered molecular signaling in the hippocampus and blunted activity in the basal nucleus of the amygdala, which receives hippocampal inputs during contextual conditioning. It may not be surprising that this temporary suppression of contextual-fear expression coincides with the developmental period encompassing the transition into and out of adolescence, during which animals engage in heightened exploratory behavior required for sexual reproduction and survival (Spear & Brake, 1983). For adolescents, exhibiting heightened levels of contextual fear during this period of exploration may prove maladaptive. Specifically, adolescents may be unwilling to leave the safety of their parents' niche and explore new environments. It is important to note, however, that memories of threatening environments remain intact and are capable of being retrieved in adulthood when animals must find safe environments for themselves and their offspring.

Discussion

Together, the results from our studies suggest that the development of cued-fear extinction and contextual-fear expression occur in a nonlinear progression, such that adolescents show diminished abilities relative to preadolescents and adults (Casey, Duhoux, & Cohen, 2008; Casey, Getz, & Galvan, 2010). This pattern of behavior may be adaptive, in that the only way adolescents can transition from dependence on their parents to relative independence is to explore new habitats in which to find new sources of food and mates. If an animal is afraid to venture out of the home environment, then

it may exploit and deplete food in the home environment and fail to find a mate. However, if the animal ventures out and is attacked by a predator, it will be no more likely to procreate or survive than if it had stayed at home. Thus, the animal needs to be highly vigilant to cues of threat in new environments, which may explain the heightened fear response to cues of threat.

Fear learning and memory have garnered significant interest in recent years because of their potential role in anxiety and stress-related disorders (LeDoux, 2000). Regulating fear is a principal component of these disorders. By studying the neural circuitry of fear learning and memories, insight can be gained into not only how these systems function normally across development, but also how they may go awry in the case of adolescent psychiatric disorders. By taking into account various developmental, environmental, and genetic factors, the hope is that insights may be gained toward finding better treatments and preventative measures for specific vulnerable populations. Specifically, our studies suggest that extinction learning is attenuated during adolescence. Exposure therapy, which relies heavily on basic principles of extinction learning, may yield inadequate treatment responses if administered to adolescent patients. Likewise, if contextual fear expression is suppressed during adolescence, but contextual memories are encoded and expressed at later, postadolescent time points, then tapping into contextual elements of fears may be a useful treatment approach. By highlighting developmental changes in the brain and in behavior that underlie fear learning, we seek to gain a better understanding of both normative and aberrant behaviors, which would be masked by perspectives that conceptualize neural development as a unidirectional, rigid process.

Recommended Reading

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Declaration of Conflicting Interests

The authors declared that they had no conflicts of interest with respect to their authorship or the publication of this article.

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