Selective early-acquired fear memories undergo temporary suppression during adolescence

Siobhan S. Pattwell[a,b], Kevin G. Bath[a,c,d], B. J. Casey[a,c], Ipe Ninan[e,1], and Francis S. Lee[a,b,f,1]

[a]Department of Psychiatry, [b]Department of Neurology and Neuroscience, [c]Sackler Institute for Developmental Psychobiology, and [f]Department of Pharmacology, Weill Cornell Medical College, Cornell University, New York, NY 10065; [d]Department of Neuroscience, Brown University, Providence, RI 02912; and [e]Department of Psychiatry, New York University School of Medicine, New York, NY 10016

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Highly conserved neural circuitry between rodents and humans has allowed for in-depth characterization of behavioral and molecular processes associated with emotional learning and memory. Despite increased prevalence of affective disorders in adolescent humans, few studies have characterized how associative-emotional learning changes during the transition through adolescence or identified mechanisms underlying such changes. By examining fear conditioning in mice, as they transitioned into and out of adolescence, we found that a suppression of contextual fear occurs during adolescence. Although contextual fear memories were not expressed during early adolescence, they could be retrieved and expressed as the mice transitioned out of adolescence. This temporary suppression of contextual fear was associated with blunted synaptic activity in the basal amygdala and decreased PI3K and MAPK signaling in the hippocampus. These findings reveal a unique form of brain plasticity in fear learning during early adolescence and may prove informative for understanding endogenous mechanisms to suppress unwanted fear memories.

developmental period | synaptic plasticity | contextual conditioning

Early-life emotional experiences can profoundly impact future behavior and alter the risk for developing psychiatric disorders later in life (1, 2). The emergence of anxiety and other affective disorders peaks during adolescence (3, 4), a developmental period associated with continued organization of neural circuitry regulating emotional behaviors (5–8). In addition to the deleterious effects that psychiatric disorders can have on social and academic development, many of these disorders have the potential to persist into adulthood (4, 9).

Animal models have helped to bridge behavioral, molecular, genetic, pharmacological, and neuroanatomical gaps to provide insights into dysregulation of emotional circuitry that lies at the core of many anxiety disorders. Recent insights have been gained by examining emotional processes implicated in clinical disorders using Pavlovian-based fear-conditioning paradigms that can be applied across species (10–13). Conserved neural circuitry between rodents and humans has allowed for in-depth characterization of the behavioral and molecular processes associated with fear learning and memory (14–17).

Whereas many studies have expanded upon knowledge from adult fear-conditioning experiments to examine the ontogeny of a learned fear response in infant models (18–21), very few studies (22–24) examine intermediate age groups, rendering the developmental trajectory of fear-related behaviors nearly absent from existing fear-conditioning literature. Given the heightened prevalence of emotional disorders in adolescent humans (3), gaining a better understanding of developmental differences in fear-related learning and memory is translationally relevant. Here, using a mouse model, we examine amygdala-dependent cued and hippocampal-dependent contextual fear learning and memory during the transition into and out of adolescence. By examining behavioral, molecular, and electrophysiological responses, we delineate unexpected developmental differences in the acquisition, retrieval, and expression of fear memories, processes common to nearly all forms of learning and memory across species (14).

Results

Behavioral Results. ***Fear conditioning across adolescence.*** To explore developmental distinctions in fear learning and memory, we used three separate cohorts of mice [postnatal day (P) 29, 39, and 49]. These ages approximate early, mid, and late adolescence, respectively (7, 22, 25). Ten-week-old adult mice (P70) were used as a basis for comparison with existing adult fear-conditioning literature. Mice of all ages were fear-conditioned with three tone-shock pairings in the conditioning context, context A (grid floor, peppermint odor). The age groups under study did not have differences in foot-shock pain sensitivity (22), showed similar baseline locomotor activity (Fig. S1*A*), and did not differ in freezing behavior during fear acquisition (Fig. S1*B*). We assessed hippocampal-dependent contextual fear by returning the mice to context A 24 h postconditioning and examining the levels of freezing behavior (as previously described in ref. 26) (Fig. 1*A*). P29 mice froze significantly less than older mice [P39, P49, and adult mice, ANOVA, $F_{(3,\ 32)} = 15.073$, $P < 0.001$] (Fig. 1*B* and Fig. S2*A*), indicating the absence of a contextual fear response. In an attempt to enhance contextual fear memory expression, we fear-conditioned a separate cohort of P29 mice using a longer preshock acclimation period of 5 min, as this leads to increased levels of contextual fear in adult mice (Fig. S2*D*) (20), and also conditioned a separate cohort of mice to the context only, without any tone cues, but the apparent absence of a contextual fear response persisted in both cases (Fig. S2 *B* and *C*). To assess amygdala-dependent cued fear, we presented the mice with three tones in a novel context, context B (green cylinder, lemon odor), 48 h postconditioning. In contrast to low levels of contextual fear, P29 mice exhibited high levels of freezing to tone cues (Fig. S1*C*). This finding is not surprising, as the somatosensory and auditory processing associated with amygdala-dependent cued conditioning lack the hippocampally mediated spatial complexity associated with contextual fear conditioning (16). The amygdala-dependent freezing observed in younger mice was not attributable to inherent differences in auditory stimuli sensitivity (Fig. S1*B*) and confirms that the physiological response systems required for freezing behavior are intact.

Spatial memory. The lack of evidence for hippocampal-dependent contextual fear in early-adolescent (P29) mice could have been

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[1]To whom correspondence may be addressed. E-mail: ninani01@med.nyu.edu or fslee@med.cornell.edu.

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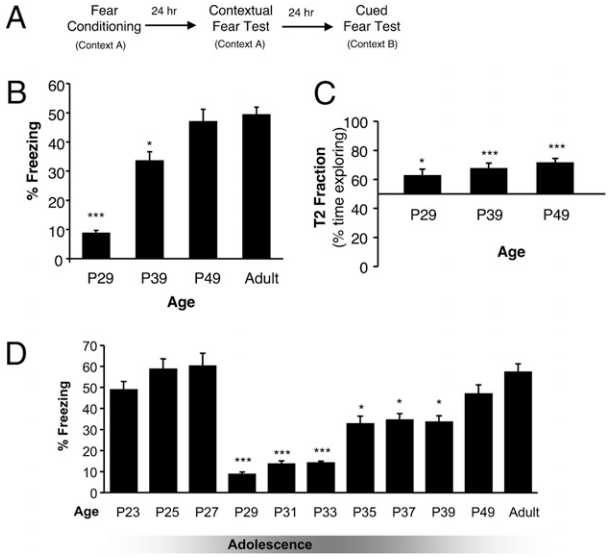

Fig. 1. Hippocampal-dependent memory across adolescent development. (*A*) Mice of all ages were fear-conditioned with three tone-shock pairings. Twenty-four hours later, they were returned to the conditioning context (context A) and freezing behavior was scored. Twenty-four hours after the contextual fear test, mice were placed in a novel context (context B) and scored for freezing behavior during five tone presentations. (*B*) P29 mice froze significantly less than older mice when tested for hippocampal-dependent, contextual fear. (*C*) In a hippocampal-dependent, spatial, novel object placement task, all age groups spent significantly more time than chance exploring objects in a novel location. (*D*) Adolescent mice (P29–P39) froze significantly less than both younger (P23–P27) and older (P49–P70) mice. All results are presented as a mean ± SEM determined from analysis of 7–10 mice per group (fear conditioning) and 28 mice per group (novel object placement) (*$P < 0.05$, ***$P < 0.001$). See also Figs. S1 and S2.

due to either hippocampal immaturity or immature connectivity in the medial prefrontal (mPFC)-amygdala-hippocampal fear circuit required for this task. To test these two possibilities, we used a hippocampal-dependent, spatial task optimized for the narrow developmental time period of interest and for which there was no underlying fear component: novel object placement (as previously described in refs. 27–29) (Fig. 1*C*). Although the novel object placement task assesses a short-term form of spatial memory, we were able to rely on it for a basic readout of hippocampal-dependent spatial learning. As P49 mice displayed hippocampal-dependent contextual fear indistinguishable from that of P70 mice, mice were tested at three time points (P29, P39, and P49) for preference of a novel object location. Acclimation trials confirmed that there were no differences in locomotor activity or exploratory behavior between age groups, and all age groups explored objects placed in novel locations significantly more than objects placed in familiar locations [P29: 55.65 ± 7.89 s novel to 22.90 ± 4.29 s familiar, trial 2 (T2) fraction of 62.40% ± 4.67; P39: 52.01 ± 5.02 s novel to 26.64 ± 3.99 s familiar, T2 fraction of 67.33% ± 3.86; P49: 51.38 ± 2.65 s novel to 22.92 ± 3.32 s familiar, T2 fraction of 71.22% ± 3.17; two-tailed *t* tests; P29: $t_{(27)} = 2.657$, $P < 0.05$; P39: $t_{(27)} = 4.491$, $P < 0.001$; P49: $t_{(27)} = 6.692$, $P < 0.001$], indicating intact hippocampal-dependent spatial abilities. Thus, in contrast to the contextual fear test results, P29 mice do not differ from older mice in their ability to successfully perform a basic, hippocampal-dependent spatial task [ANOVA, $F_{(1, 27)} = 1.1470$, $P = 0.248$]. Lack of contextual fear, but spared performance on the novel object placement task, indicates a selective lack of expression for hippocampal-dependent emotional processes in early-adolescent mice and potential alterations in integration of the mPFC-amygdala-hippocampal fear circuitry.

Preadolescent fear conditioning. Because previous groups have shown that aversive contextual learning is present in rats at ages younger than P29 (19, 20, 30), we wanted to further understand why contextual fear expression was absent or suppressed in the early-adolescent mice. To compare the observed contextual fear responses with previous studies, we tested separate cohorts of mice immediately before (P23–P27) and after (P31–P49) the transition into adolescence. Interestingly, mice fear-conditioned at early preadolescent ages displayed intact contextual fear expression that was indistinguishable from adult levels (Fig. 1*D*). As the mice transitioned into adolescence at P29, the expression of contextual fear was suppressed. Then, as the mice transitioned out of adolescence (P39), the expression of contextual fear reemerged (Fig. 1*D*). To assess whether a consolidated memory was suppressed during this transition into adolescence, separate cohorts of P27 mice were fear-conditioned and tested at P30 for contextual fear suppression. Whereas mice fear-conditioned at P27 and tested at P28 exhibit high levels of freezing behavior, mice conditioned at P27 and tested at P30 exhibit low levels of freezing behavior when tested for contextual fear (Fig. S3*A*). These results are consistent with the behavioral results observed in Fig. 1 and demonstrate that even previously consolidated fear memories are suppressed as the mice enter the adolescent transitional period (Fig. S3*A*).

Postadolescent retrieval of earlier acquired fear memories. In subsequent experiments, we targeted the potential physiological underpinnings of the suppressed contextual fear behavior at P29. Because preadolescent mice are capable of expressing a contextual fear response before P29, the lack of fear expression would likely not be explained by simple structural maturation in the fear-conditioning circuitry. Rather, a more complex series of events may be occurring during this sensitive developmental window. For successful expression of contextual fear, the conditioned stimulus (CS) and unconditioned stimulus (US) must first be successfully paired (acquisition). Subsequent to this pairing, the association must be consolidated such that it can be accessed later. Finally, when the CS is encountered again, the animal must be able to retrieve the associated memory in order for the appropriate response to be made (31). Because the lack of contextual fear expression at P29 could be due to selective failures in any of the aforementioned learning processes, we conditioned a separate cohort of P29 mice and tested them for contextual fear at both early (24 h) and late (between 3 and 14 d) time points, without the presence of reminder cues or additional US presentations (Fig. 2*A*). Because stable adult-like levels of contextual fear are not reached until between P39 and P49 (Fig. 1*B*), 14 d was chosen as the initial delay interval. Despite poor performance when tested 24 h postconditioning, mice that underwent fear conditioning at P29 demonstrated significantly higher levels of contextual fear expression 14 d postconditioning, as evidenced by increased freezing in context A [ANOVA, $F_{(1, 15)} = 15.541$, $P = 0.001$] (Fig. 2*B*). This increase in freezing response was also context-specific, as control and fear-conditioned mice had equivalently low levels of freezing when tested in context B 15 d postconditioning [ANOVA, $F_{(1, 15)} = 0.545$, $P = 0.472$] (Fig. S3*B*). To eliminate the possibility that the initial 24-h context test might be serving as a reminder and thus promoting an unmasking of the subsequent freezing after the 14-d delay, we fear-conditioned separate cohorts of P29 mice and tested them for contextual fear at various time points without administering the initial 24-h contextual fear test (Fig. S2 *E* and *F*). In addition, to confirm that this delayed expression of contextual fear memory occurs near the end of the adolescent period, separate cohorts of mice conditioned at P29 were tested throughout the 14-d interval (tested at P30–P43). Increased expression of contextual fear was not observed until 13 d postconditioning

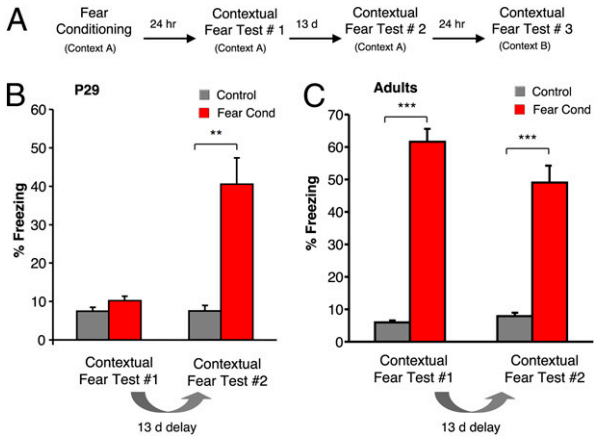

Fig. 2. Early-adolescent mice display impaired contextual fear at early but not late time points. (*A*) Mice were fear-conditioned (Fear Cond) with three tone-shock pairings (P29; n = 7 control, 10 fear-conditioned; adult, n = 10 control, 10 fear-conditioned). Twenty-four hours later, freezing behavior was scored in the conditioning context, context A. Fourteen days later, mice were retested for contextual fear in context A. Twenty-four hours after the second contextual fear test, mice were tested for cued fear in a novel context, context B. (*B*) Mice fear-conditioned at P29 did not display significant freezing to context A when tested 24 h postconditioning, but showed significantly enhanced freezing levels when tested 14 d postconditioning. (*C*) Mice conditioned as adults displayed significant freezing to context A when tested both 24 h and 14 d postconditioning. All results are presented as a mean ± SEM determined from analysis of 7–10 mice per group (**$P < 0.01$, ***$P < 0.001$). See also Figs. S2 and S3.

(Fig. S3*C*). This enhanced freezing response was specific to contextual fear conditioning, as control mice that had been exposed to the context and tone, but not the shock, did not show similarly elevated freezing levels when reexposed to context A 14 d later (Fig. 2*B*). Mice conditioned as adults froze significantly more than control mice at both test points, but did not show a similar augmentation of the fear response after the 14-d interval [ANOVA, 24 h: $F_{(1, 18)} = 190.38$, $P < 0.001$; 14 d: $F_{(1, 18)} = 53.672$, $P < 0.001$] (Fig. 2*B*). Adult fear did not generalize to the novel context after the delay interval [$F_{(1, 18)} = 3.48$, $P = 0.065$] (Fig. S3*B*), eliminating the possibility of a generalized, context-independent fear incubation artifact (32). Combined with the lack of contextual fear during early-adolescent time points, the delayed onset of contextual fear shows that the early-adolescent mice are indeed capable of contextual fear learning but that the fear undergoes a latent period in which the expression of the prototypical fear response associated with reexposure to the conditioning context is suppressed until early adulthood.

Physiological Correlates. ***Basal amygdala electrophysiology.*** To explore the potential physiological mechanism underlying the observed delay in contextual fear memory expression, we examined potential developmentally regulated changes in amygdala synaptic transmission during the periods in which we observed changes in behavioral performance. As the function of the hippocampus differs in recent versus remote memories (33) and because lesions to the basolateral amygdala complex have been shown to specifically impair long-term contextual fear memory (34), the amygdala was chosen as a stable indicator of long-term contextual fear-associated physiology. Based on previous characterization of amygdala subnuclei in fear learning (35–37) and intact cued fear observed in all age groups (Fig. S1*C*), we did not anticipate developmental differences in the lateral nucleus of the amygdala (LA). Given the specific lack of a contextual fear response at P29, followed by a recovery in contextual fear response after the 14-d delay interval, we hypothesized that there may be developmentally regulated functional differences in the basal nucleus (BA) of the amygdala, as this region has been strongly implicated in contextual fear (35–37). In vitro electrophysiological studies have demonstrated behaviorally induced modification of synaptic strength (38–40). Together with this finding and the known involvement of the BA in contextual fear conditioning, we tested whether contextual fear learning induces synaptic potentiation in the BA (36). We examined the field excitatory postsynaptic potential (fEPSP) slope recorded from the BA by stimulating the perirhinal cortex in brain slices (Fig. S4*A*) (41, 42). Consistent with intact fear memory in adult mice (Fig. S4*D*), we found an enhancement of fEPSP slope in the BA of fear-conditioned adult mice compared with the control mice, suggesting that conditioned fear led to long-term potentiation–like phenomena in BA synapses (Fig. 3*A*) [two-way ANOVA, $F_{(1, 32)} = 17.45$, $P < 0.001$]. This enhancement of BA fEPSP was also seen in P27 mice after fear conditioning (Fig. S4 *B* and *C*). Consistent with the observed suppression of contextual fear in P29 mice, fEPSP slopes from P29 fear-conditioned and control mice were not significantly different, suggesting the absence of synaptic potentiation in the BA of P29 animals after fear conditioning (Fig. 3*B* and Fig. S4*E*) [two-way ANOVA, $P > 0.05$]. This blunted response of the BA in P29 mice is in contrast to enhanced potentiation that is observed in the LA when P29 mice are tested for cued fear, confirming the specificity of the basal nucleus in the contextual fear memory (Fig. S5). Because the mice that underwent fear conditioning at P29 demonstrated significant improvements in contextual fear expression 14 d postconditioning (Fig. S4*F*), we hypothesized that the mice that underwent fear conditioning at P29 might exhibit synaptic po-

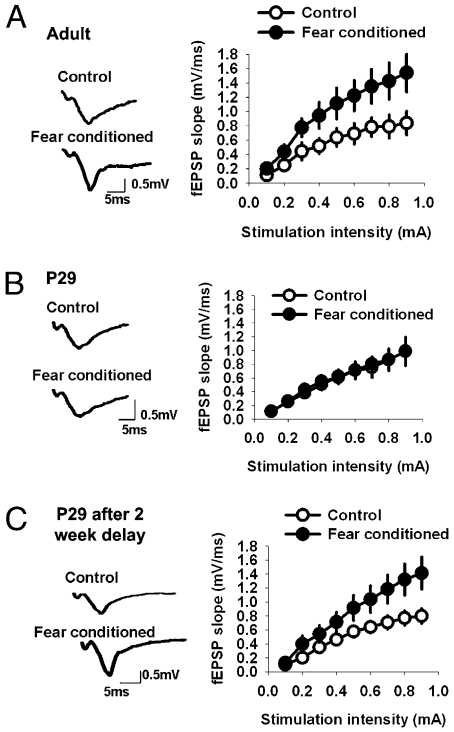

Fig. 3. Contextual fear conditioning–induced synaptic potentiation is present in adult mice but not in early-adolescent mice. Input–output curve of slopes of fEPSPs recorded from BA in adult mice (*A*) (n = 17 slices in each group, n = 5 mice in each group), early-adolescent mice (*B*) (n = 14 slices in each group, n = 5 mice in each group), and P43 mice fear-conditioned at P29 (*C*) (n = 15 slices in each group, n = 5 mice in each group). Examples of fEPSPs are shown on the left. All results are presented as a mean ± SEM. See also Figs. S4 and S5.

tentiation in the BA 14 d postconditioning. Indeed, consistent with behavior, we observed a significantly higher fEPSP slope in the BA 14 d postconditioning in mice that underwent fear conditioning at P29, suggesting that improvements in contextual fear expression observed 14 d postconditioning involved a delayed synaptic potentiation in the BA (Fig. 3*C*) [two-way ANOVA, $F_{(1,\ 28)} = 21.1$, $P < 0.001$]. Therefore, the suppression of fear expression at P29 is associated with an absence of immediate synaptic potentiation in the basal amygdala which is restored after the 14-d delay interval.

Although early-adolescent mice show no evidence of learning and have an initially blunted BA synaptic response at the 24-h contextual fear test, intact fear expression and enhanced BA reactivity at later time points suggest that the contextual fear memory was acquired. Because the 14-d interval falls beyond the limited time window of protein synthesis–independent short-term memory (31), memory consolidation was also not likely disrupted. Furthermore, as the mice were returned to context A for a 24-h postconditioning test, reconsolidation does not appear to be disrupted either, leaving retrieval as a remaining memory process in question.

Retrieval-associated signaling in the hippocampus. To examine the role of memory retrieval in the suppression of P29 contextual fear, we examined intracellular signaling within the hippocampus of P29 and adult mice, as contextual fear is mediated by both the amygdala and hippocampus. Unlike the previous experiments, which detailed electrophysiological recordings in the amygdala for a long-term memory of up to 2 wk, the hippocampus was chosen as the site to examine molecular signaling in the retrieval of 24-h contextual fear memory. Whereas it is well-established that the ERK/MAP kinase (ERK/MAPK) signaling cascade is involved in associative learning and the formation of contextual fear memories (43, 44), it has recently been shown that PI3K activity in the hippocampus is necessary and sufficient for the activation of p42 MAPK (ERK2) during contextual fear memory retrieval (45). To test the involvement of hippocampal PI3K and ERK/MAPK molecular cascades in contextual fear memory retrieval, and the potential lack thereof in early-adolescent mice, we fear-conditioned separate cohorts of adult and P29 mice and then divided them into two groups: a retrieval group that was tested for contextual fear 24 h postconditioning and a control group that was sacrificed 24 h postconditioning without experiencing a retrieval session. Fifteen minutes after contextual fear memory retrieval, mice in the experimental group were sacrificed for Western blot analysis. Consistent with previous results (45), phosphorylation levels of PI3K and MAPK downstream targets Akt and ERK2 were significantly increased in the hippocampi of adult mice after contextual fear memory retrieval [ANOVA, phosphorylated Akt (pAkt): $F_{(1,\ 17)} = 8.993$, $P < 0.01$; phosphorylated ERK2 (pERK2): $F_{(1,\ 17)} = 10.595$, $P < 0.01$] (Fig. 4 *A* and *B*). In contrast, pAkt and pERK2 levels remained unchanged in the hippocampi of P29 mice regardless of whether the mice underwent a retrieval session [ANOVA, pAkt: $F_{(1,\ 12)} = 0.278$, $P = 0.607$; pERK2: $F_{(1,\ 12)} = 0.026$, $P = 0.876$] (Fig. 4 *A* and *B*). The absence of a contextual fear response at the 24-h test combined with the blunted response of retrieval-associated signaling suggests that the suppression of contextual fear in early-adolescent mice is associated not only with a lack of BA activity but also with a lack of memory retrieval-associated signaling in the hippocampus.

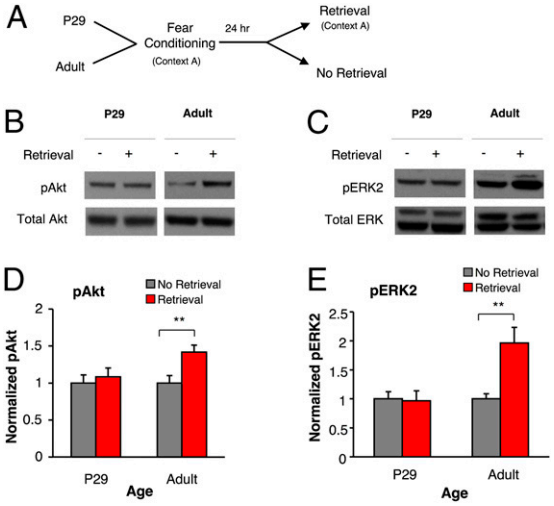

Fig. 4. PI3K and ERK/MAPK activation occurs in the hippocampi of adult but not early-adolescent mice during contextual fear memory retrieval. (*A*) Mice were fear-conditioned with three tone-shock pairings in context A (P29, $n = 14$; adult, $n = 19$). Twenty-four hours postconditioning, mice in the retrieval group were returned to context A for a contextual fear retrieval session and killed 15 min postretrieval, whereas control mice were killed without being given a retrieval session. (*B* and *C*) Representative Western blots showing activation of PI3K (*B*) and ERK/MAPK (*C*) signaling upon contextual fear memory retrieval in adult but not P29 mice. (*D* and *E*) Quantification of pAkt (*D*) and pERK2 (*E*) normalized to total Akt and ERK protein levels, respectively ($n = 6$–10 mice per group; $^{**}P < 0.01$).

Discussion

Our converging results suggest that differences in region-specific hippocampal-amygdala functioning underlie the temporary suppression of contextual fear in early-adolescent mice. Specifically, observed behavioral phenomena and electrophysiological responses (as shown in Figs. 2 and 3) are consistent with our notion of suppression of contextual fear retrieval at P29, rather than a failure to learn the association. The lack of contextual fear expression at P29 (Fig. 1*B*) combined with the blunted electrophysiological response in the BA confirms that suppression of contextual fear and associated synaptic activity occurs as the mice transition into adolescence. According to Fanselow's theory of contextual conditioning, the contextual representation of the conditioning environment must be relayed to the affective system as a fear-signifying cue after, and only after, such a representation has been constructed by the cognitive system (46). Our results suggest that whereas the contextual representation of the conditioning environment may have been encoded at P29, the context was temporarily incapable of eliciting a fear response until the mice began transitioning out of adolescence and into adulthood. Our results reveal a period of contextual fear suppression during the transition into adolescence, whereas our contextual fear results in preadolescent mice and adult mice remain consistent with existing literature (45, 47). From an evolutionary perspective, a temporary suppression of contextual fear during adolescence may prove highly adaptive, as it occurs just as the mouse transitions into higher exploratory behaviors away from the nest (48). For the early-adolescent rodent to exhibit high levels of contextual fear would likely prove maladaptive for exploratory behavior required for leaving the nest, exploring the surrounding environment, and becoming independent of the colony. Interestingly, contextual representations of particularly threatening environments that might be formed during this temporary window of contextual fear suppression remain intact and are capable of being retrieved as a fear-signifying context at later, postadolescent time points. Further characterization of developmentally regulated events within the neural circuitry underlying fear expression may prove useful for uncovering more about how the brain functions to inherently suppress unwanted fearful memories. Such ends have been the goal of many treatment regimens for a variety of anxiety disorders such as posttraumatic stress disorder, phobias, and social anxiety.

Methods

Animals. Male C57BL6/J mice were used for all experiments. To eliminate potential developmentally sensitive, shipping-induced stress effects, breeding pairs of C57BL6/J wild-type mice from Charles River were set up in the colony and monitored daily. Litters were weaned at P21 and males from various litters were randomly combined to eliminate any litter-driven effects on behavior. Mice were housed five per cage in a temperature- and humidity-controlled vivarium maintained on a 12-h light/dark cycle. Mice had ad libitum access to food and water. Separate cohorts of mice (age P23–P70) were used for all fear-conditioning, retrieval, and object placement tasks. All procedures regarding animal care and treatment were in compliance with guidelines established by Weill Cornell Medical College's Institutional Animal Care and Use Committee and the National Institutes of Health.

Fear Conditioning. Mice were fear-conditioned in a mouse test cage (Coulbourn Instruments) inside a sound-attenuated box. The chamber (context A) was cleaned in-between each mouse and scented by peppermint-scented (0.1%) ethanol (70%). On conditioning day, following a 2-min acclimation period (43), mice were conditioned with three trials consisting of a 30-s tone (5 kHz, 70 dB) that coterminated with a 1-s, 0.7-mA foot shock delivered through the electrified floor grid. Each trial was separated by a 30-s intertrial interval. After the final tone-shock pairing, mice remained in the conditioning chamber for 1 min before being returned to their home cages. Trials were videotaped, and freezing during the initial 2-min acclimation/exploration period was scored as a measure of baseline freezing to the conditioning context. Memory for the context was assessed after specified time intervals by returning the mice to context A, where freezing behavior was scored during the last 3.5 min of the total 5.5 min spent in the chamber. After the contextual fear test, mice were returned to their home cages. Memory for the cue was assessed by placing the mice in a novel context [green cylinder, cleaned with lemon-scented (0.1%) ethanol (70%); context B]. After a 2-min acclimation period in context B, mice were presented with three 30-s tones (5 kHz, 70 dB) separated by an intertrial interval of 30 s. Freezing behavior was scored during each of the 30-s tone presentations. After the last tone presentation, mice remained in the novel context for 1 min before being returned to their home cages.

Novel Object Placement. The novel object placement apparatus consisted of an open field made of a white Plexiglas arena (40 × 40 × 49 cm). The white floor was divided into 12 equal quadrants. The corners nearest the center of the northeast, northwest, southeast, and southwest quadrants served as placement locations for the objects, which were toy trees made of LEGO blocks (4.8 × 4.8 × 7 cm). A red "X" on the northern arena wall and a red square on the southern arena wall served as orienting cues. An overhead digital camera was connected to a video recorder and computer with an EthoVisionXT tracking system (Noldus). On day 1, a single mouse was placed in the center of the open field and behavior was recorded for 5 min and analyzed to confirm there were no differences in anxiety-related behavior (as measured by avoidance of center quadrants) or preference for one region of the arena over another. The arena was cleaned with 70% ethanol and dried in-between subjects to eliminate any potential odor cues left by previous subjects. On day 2, two identical objects were placed at adjacent northern locations within the arena and a single mouse was placed in the center of the arena. Behavior was recorded for 5 min and the time spent exploring each object was analyzed. After the initial 5-min exploration period, trial 1 (T1), subjects were removed and placed singly in a holding cage for 5 min while both the arena and objects were cleaned with 70% ethanol. One object was returned to its former northern location, while the other object was placed in the southern end of the box diagonally opposite the northern object (such that objects were either in northeast and southwest locations or northwest and southeast locations). Placement of the objects was counterbalanced across both trials and subjects. After the 5-min delay, the mouse was returned to the arena and the time spent exploring objects was recorded and analyzed for the second trial, trial 2 (T2). A T2 fraction was calculated by dividing the amount of time spent exploring the object in the novel location divided by the amount of time spent exploring both objects [T2 = ([time novel]/[time familiar + time novel]) × 100], such that mice were successful at the task if they spent significantly more time than chance (50%) exploring the object in the novel location.

Electrophysiology. Mice were fear-conditioned as described in *Fear Conditioning*, with three tone-shock pairings in context A. Twenty-four hours postconditioning, mice were returned to context A for a contextual retrieval test and decapitated under pentobarbital anesthesia 2 h later. Brains were quickly removed and placed in ice-cold artificial cerebrospinal fluid (ACSF) of the following composition: 118 mM NaCl, 2.5 mM KCl, 10 mM glucose, 1 mM NaH_2PO_4, 3 mM $CaCl_2$, 2 mM $MgCl_2$, and 25 mM $NaHCO_3$ bubbled with 95% O_2/5% CO_2 (pH 7.4). Coronal slices (400 μm) containing amygdala were cut with a vibratome and maintained at room temperature for 90 min in a brain-slice keeper before being transfered to an interface recording chamber exposed to humidified gas at 32 °C and perfused with ACSF (Scientific Systems Design). fEPSPs were recorded using an IE-210 amplifier (Warner Instruments) from basal nucleus with a glass electrode filled with 2 M NaCl and by stimulating perirhinal cortex with a concentric bipolar electrode (FHC). Bicuculline (10 μM) was included in the ACSF for recording fEPSPs. Synaptic strength was studied by plotting the stimulus current against slopes of fEPSPs to generate input–output relations.

Western Blot Analysis. Mice were fear-conditioned as described in *Fear Conditioning*, with three tone-shock pairings in context A. Twenty-four hours postconditioning, mice were returned to context A for a contextual retrieval test and hippocampi were dissected out 15 min later in 6% glucose and lysed on ice in extraction buffer (10 mM Tris, 150 mM NaCl, 1 mM EDTA, 1% NP-40, 1 μg/mL leupeptin, 1 μg/mL pepstatin, 2 μg/mL aprotinin, 0.5 mM phenylmethylsulfonyl fluoride, 10 mM NaF, 1 mM Na_3VO_4, pH 7.4). Extracts were clarified by centrifugation (14,000 rpm for 10 min). Proteins were resolved by SDS/PAGE (NuPAGE 10% bis-Tris gel; Invitrogen) and probed with specified antibodies obtained from Cell Signaling Technology. For densitometric analyses, immunoreactive bands were scanned and quantitated using National Institutes of Health ImageJ (Scion). All experiments were carried out at least in triplicate.

Statistics. When two means were compared, statistical significance was calculated using Student's *t* test or one-way ANOVA. For multiple comparisons, a general linear model, repeated-measures ANOVA, and Bonferroni post hoc correction were used to determine statistical differences.

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