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Divergence Between Thalamic and Cortical Inputs to Lateral Amygdala During Juvenile–Adult Transition in Mice

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Abstract

Background—Adolescence is considered a critical time of life for emotional development in humans. During this period the amygdala, which regulates emotions, undergoes structural reorganization. Auditory fear conditioning, a form of amygdala-dependent emotional learning, occurs differently in juvenile and adult rodents. Because this learning is mediated by plastic changes in the thalamic and cortical inputs to lateral amygdala (LA), we investigated changes in synaptic properties of these inputs during juvenile-to-adult transition.

Methods—Whole-cell patch clamp recording in amygdala slices from juvenile and young adult mice was conducted to investigate long-term potentiation and basal synaptic transmission in the thalamic and cortical inputs to LA.

Results—We show that physiological differences develop between thalamic and cortical afferents to LA during the juvenile-to-adult transition. Although in juvenile mice the two pathways have similar properties, in young adult mice the thalamic pathway has reduced plasticity, increased number of quanta released by a single action potential, and decreased proportion of silent synapses.

Conclusions—Changes in thalamic but not cortical inputs to amygdala take place during late development and might contribute to differences in auditory fear conditioning between juveniles and adults.

Keywords

Amygdala; development; fear; juvenile; LTP; synaptic transmission

Adolescent development in humans involves changes in emotional behaviors and reorganization of the brain regions that control them (1,2). One of these regions is the amygdala, which is critical for emotional processing of sensory information, emotional memory, and expression of fear and anxiety (3–6). Maturation of the amygdala continues during juvenile–adult transition, as evidenced by increase of amygdala volume in humans (7,8) and continuing expansion of amygdala projections into the prefrontal cortex in rodents (9,10). Along with these anatomical changes, differences in fear learning between juvenile and adult mice (11, 12) suggest a possibility of physiological changes in the amygdala.

Synaptic inputs to lateral amygdala (LA) from the thalamus and cortex are believed to underlie emotional memories formed through fear conditioning (13–17), and the emergence of synaptic

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plasticity in these inputs coincides with the emergence of fear learning during early postnatal development (18). Yet, physiological analysis of these inputs has been performed mainly in juvenile (3–5 weeks old) rodents (19–22) whose developmental state is considered a match to that of adolescent humans (11), and no comparisons have been done between juveniles and adults. Thus, a question remains as to whether synaptic transmission in the amygdala inputs is altered during late development.

Here we compared synaptic properties of the two amygdala inputs in juvenile and young adult mice and found that thalamo-amygdala but not cortico-amygdala transmission undergoes substantial changes during the juvenile–adult transition. Such changes include an emergence of multiquantal release, a reduction in the proportion of silent synapses, and a decrease in spike-timing–dependent long-term potentiation (LTP). These findings support the notion that, during fear learning, contributions of the cortical and subcortical inputs to LA might differ between juveniles and adults.

Methods and Materials

Animals

All experiments were performed on male mice of a B6/129 hybrid background and approved by the National Institute of Mental Health Animal Care and Use Committee.

Electrophysiology

General—Amygdala slices were prepared from either 4–5-week-old or 8–10-week-old mice as described (17,23) with minor modifications. Mice were killed by decapitation, and brains were quickly removed to ice-cold oxygenated (95% oxygen/5% carbon dioxide) artificial cerebrospinal fluid (aCSF) containing (in mmol/L): 119 sodium chloride (NaCl), 2.5 potassium chloride, 1 magnesium sulfate, 2.5 calcium chloride, 10 glucose, and 26 sodium bicarbonate (pH 7.3). Slices containing LA were cut with a vibratome, incubated at 35°C for approximately 40 min, and maintained at room temperature for at least 1 hour before recording. Slices were then transferred to a recording chamber continuously superfused with aCSF at a constant rate of approximately 60 mL/hour. Temperature was held at 30°C ± 1°C unless indicated otherwise. Whole cell recordings of compound excitatory postsynaptic potentials (EPSPs)/excitatory postsynaptic currents (EPSCs) were obtained from principal cells in LA under visual guidance (differential interference contrast [DIC]/infrared optics) with an EPC-10 amplifier and Pulse v8.76 software (HEKA Elektronik, Lambrecht/Pfalz, Germany). Signals were filtered at 5 kHz with clamp amplifier circuitry. During “minimal stimulation” experiments, signals were filtered at 1 kHz. Compound synaptic responses were evoked by field stimulation of the fibers either in the external capsule (cortical input) or internal capsule (thalamic input) by a 1–3-M Ω glass stimulation electrode (6–8 M Ω for the “minimal stimulation”) filled with aCSF. In most experiments patch pipets were filled with (in mmol/L): 120 K-gluconate, 5 NaCl, 1 magnesium chloride, 10 HEPES, .2 ethylene glycol bis-(β -aminoethyl ether)-*N,N'*-tetraacetic acid (EGTA), 2 ATP-Mg, and .1 GTP-Na. The pH was adjusted to 7.3 with potassium hydroxide and osmolality to 285 Osm with sucrose. Cesium was used instead of potassium (K⁺) (the pH adjusted to 7.3 by cesium hydroxide) in experiments investigating the decay of *N*-methyl-D-aspartate receptor (NMDAR) currents in the presence of dizocilpine (MK-801) and in all “silent synapse” experiments. The patching pipet resistance was 5 M Ω –7 M Ω when filled with the K⁺-containing solution and 3 M Ω –5 M Ω when filled with the cesium ion–containing solution. One hundred micromolar picrotoxin was routinely added to the bath solution to block γ -aminobutyric acid (GABA)_A receptors. All membrane potentials were corrected by a junction potential of 12 mV. Series resistance (Rs) was in the range of 10 M Ω –20 M Ω and monitored throughout experiments. If Rs changed more than 20% during recording, the data were not included in analysis. To measure membrane time constant, we injected a

square current pulse (10 msec, 200 pA) into a cell held at -70 mV. The time constant for membrane potential decay was determined offline with Origin software (OriginLab Corporation, Northampton, Massachusetts). To compare rise time of evoked EPSCs, we adjusted stimulus intensity to obtain responses at 30% of the maximum. The 20%–80% rise time was determined offline with Clampfit software (Molecular Devices, Sunnyvale, California). Data were expressed as mean $\pm$ SEM. For visualization of the patched neurons, .2% biocytin was included in intracellular solution; slices were postfixed in 4% paraformaldehyde overnight, stained with avidin Alexa Fluor 488 conjugate (Invitrogen Corporation, Carlsbad, California), cryoprotected in 30% sucrose, and resliced at $70\text{ }\mu\text{mol/L}$ for imaging.

LTP—In LTP experiments, stimulus intensity was adjusted to produce potency approximately 20%–30% of the maximum synaptic response. For a spike-timing dependent plasticity (STDP) protocol, membrane potential was held at -80 mV for basal state recording, and LTP was induced with trains of 10-Hz monosynaptic EPSPs with each EPSP followed by individual action potentials (elicited by 1 nA, 5-msec current steps: EPSP onset to action potential peak delay was no more than 10 msec). This pattern was repeated 15 times at .1 Hz. The LTP was quantified by normalizing data collected in the last 5 min of recordings to the mean value of baseline EPSP, which was recorded at .05 Hz for at least 6 min before the LTP induction.

Minimal Stimulation Experiments for Quantal Analysis—For minimal stimulation (24), stimulus intensity was adjusted to just above a threshold: 10% reduction in the stimulus intensity resulted in total failures in responses, whereas 20% increase above the threshold did not alter the average potency of responses. The latencies of responses were invariable throughout the repeated stimuli. Stimuli were given in pairs (50-msec interval). Only cells with potencies of responses to the first and second stimuli showing no significant difference were included in the analysis. This criterion was not applied to the thalamic pathway in the adult mice, because of multiquantal release (Figure S1 in Supplement 1).

MK-801 Block—For assessing the rate of NMDAR current blockade by MK-801, neurons were clamped at $+30$ mV, and synaptic responses to presynaptic stimulation at .1 Hz—alternating between the thalamic and cortical pathways—were recorded for 5 min as a baseline to insure stability of responses. It was followed by bath application of MK-801 ($20\text{ }\mu\text{mol/L}$) without stimulation (25). Ten minutes later, the stimulation at .1 Hz was resumed for 20 min. The EPSC amplitudes were normalized by that of the EPSC elicited by the first stimulus during the MK-801 application.

Silent Synapses—We used two approaches to detect silent synapses in the cortical and thalamic inputs to amygdala. First, we performed coefficient of variation (CV) analysis of evoked α -amino-3-hydroxy-5-methylisoxazole propionate receptor (AMPA) and NMDAR currents as previously described (26). In brief, AMPAR currents were evoked when cell was held at -70 mV, whereas for NMDAR currents the cell was held at $+40$ mV. The AMPAR current was measured at the peak response at -70 mV, whereas NMDAR current was determined at 50 msec after the peak. In one-half of experiments cells were first held at -70 mV and then at $+40$ mV, and in the other one-half the order was reversed. The CVs were estimated for epochs of 30 consecutive trials separated by intervals of 10 sec. Sample variances (SVs) were calculated for the amplitudes of EPSC or noise with sweeps obtained with or without stimulation, respectively. The CVs were then taken as the square root of the differences of the sample variances ($\text{SV}[\text{EPSC}] - \text{SV}[\text{noise}]$), divided by EPSC mean. As shown before (26), the CV of NMDAR EPSCs was higher than previously reported (27), because NMDAR EPSCs were measured at 50 msec after the peak of response in the absence of CNQX, thus bringing more variance to the measurement.

Second, we detected silent synapses by analyzing the success rate of EPSC evoked by low-intensity stimulation. Because silent synapses contain NMDARs but not AMPARs, those synapses only contribute to EPSCs registered at +40 mV. When a small number of synapses are activated, the proportion of successful EPSCs sampled at +40 mV should be higher than that recorded at -70 mV (26). For each cell, we first recorded at -70 mV and adjusted stimulation intensity to obtain a success rate for AMPAR EPSCs of approximately 50% and recorded responses to 100 stimuli (interstimulus interval 15 sec). After this, we switched membrane potential to +40 mV and recorded AMPAR/NMDAR mixed responses to another set of 100 stimuli. To ensure stability of recording, we compared the success rate of both AMPAR and AMPAR/NMDAR mixed currents between the first and last 50 traces of each of the 100-trace series, and only recordings with changes in success rates of <20% were included in the analysis. Note that this stimulation protocol is different from the one used for the minimal stimulation in the quantal analysis, where the threshold criteria (not the success rate criterion) was used for choosing stimulus intensity.

Statistical Methods

Unless indicated otherwise, unpaired *t* test was used for the comparisons of LTP, minimal response amplitude, quantal size, CV, and EPSC amplitude during low-intensity stimulation and intrinsic properties of LA neurons; paired *t* test was used for comparison of paired-pulse ratio (PPR) and the success rate of EPSC at +40 mV and -70 mV; decay curves during MK-801 application were compared with two-way repeated measures analysis of variance (ANOVA).

Results

Loss of Spike-Timing–Dependent Plasticity During the Juvenile–Adult Transition in Thalamic But Not Cortical Inputs to the Amygdala

To investigate possible changes in synaptic plasticity within the thalamic and cortical inputs to LA during late postnatal development, we recorded excitatory postsynaptic potentials (EPSPs) from LA principal neurons of p28–35 (juvenile) and p56–70 (young adult) mice (Figure 1A). The EPSPs were evoked by stimulating either the internal capsule that contains afferents from the auditory thalamus or the external capsule that contains afferents from the cortex (Figure 1B). The LTP was induced with a STDP protocol (Figure 1B), which is a physiological model of synaptic plasticity during learning (28). We first replicated our previous finding that this protocol evokes LTP in the cortical but not thalamic input of young-adult mice (23) (cortical: $154.7 \pm 10.1\%$, $n = 7$; thalamic: $108.8 \pm 6.4\%$, $n = 10$, $p = .003$) (Figures 1D and 1E). In contrast to the young adults, in juvenile mice LTP was robust in both inputs to LA (cortical: $166 \pm 13.7\%$, $n = 9$; thalamic: $159.8 \pm 16.8\%$, $n = 9$, $p = .757$) (Figures 1C and 1E). Thus, during the juvenile–adult transition, a significant decline of plasticity occurs in the thalamic but not cortical pathway.

Thalamic and Cortical Inputs to the Amygdala Diverge in Presynaptic Properties During the Juvenile–Adult Transition

Alteration of synaptic plasticity during postnatal development often correlates with changes in the properties of basal synaptic transmission, such as neurotransmitter release probability (Pr) (29), quantal size (30), and number of quanta released by a single action potential (quantal content) (31). Accordingly, we next tested whether these parameters change during the juvenile–adulthood transition.

To directly compare Pr between inputs at different ages, we recorded gradual decreases of NMDAR EPSCs evoked in the cortical and thalamic inputs in the presence of a noncompetitive NMDAR antagonist MK-801 (Figure S2A in Supplement 1). The rate of blockade of NMDAR current is directly related to Pr (25,32). Despite a tendency toward a faster rate in the thalamic

pathway of young adults, no significant difference was detected in the blocking rates between thalamic and cortical pathways in either age group [juveniles: $F(1,15) = .19$, $n = 8$, $p = .67$, young adults: $F(1,15) = 1.61$, $n = 8$, $p = .23$; repeated measure ANOVA] or in individual pathways across ages [thalamic: $F(1,15) = .72$, $p = .41$, cortical: $F(1,15) = .08$, $p = .78$], suggesting that Pr did not differ between the inputs or across ages.

To further investigate presynaptic properties of inputs to LA, we tested paired pulse facilitation (PPF) while altering calcium ion (Ca^{2+})/magnesium (Mg^{2+}) ratio in the external solution. The PPRs were similar between thalamic and cortical inputs in either juvenile or young adult mice (juvenile: $n = 8$; young adult: $n = 8$, $p > .3$ for all cortico-thalamic comparisons under different $\text{Ca}^{2+}/\text{Mg}^{2+}$ ratio in either juvenile or young adult mice) (Figures S2B and S2C in Supplement 1), indicating that amygdala inputs have similar presynaptic release in both ages.

To examine quantal properties of synaptic transmission, we recorded EPSCs evoked by minimal stimulation, which presumably recruits a single axonal fiber (33,34). The amplitude of such responses is proportional to the quantal content and quantal size. The mean amplitudes of currents evoked by minimal stimulation of the cortical inputs did not differ between ages (juvenile: $15.1 \text{ pA} \pm 1.3 \text{ pA}$, $n = 11$; young adults: $14.5 \text{ pA} \pm 1.6 \text{ pA}$, $n = 10$; $p = .822$). In contrast, the current amplitudes in the thalamic input of young adult mice were almost twice that of juvenile animals (juvenile: $15.3 \text{ pA} \pm 2.9 \text{ pA}$, $n = 16$; young adult: $28.9 \text{ pA} \pm 4.6 \text{ pA}$, $n = 11$, $p = .019$) (Figures 2A and 2B).

To estimate quantal size, we recorded asynchronous quantal responses induced by stimulating either pathway in the presence of strontium ion (Sr^{2+}) substituting for extracellular Ca^{2+} (35). The mean amplitudes of these responses (juvenile-cortical: $12.7 \text{ pA} \pm .6 \text{ pA}$, $n = 13$; juvenile-thalamic: $12.5 \text{ pA} \pm .5 \text{ pA}$, $n = 18$, $p = .813$; young adult-cortical: $12.2 \text{ pA} \pm 1.0 \text{ pA}$, $n = 13$; young adult-thalamic: $13.0 \text{ pA} \pm .4 \text{ pA}$, $n = 14$, $p = .434$) did not differ between pathways and across ages (Figures 2C and 2D).

Given the similar quantal sizes in both inputs and across ages, the quantal content in the young adult thalamic pathway must be two times higher than in the juvenile thalamic pathway (juvenile: $1.2 \pm .1$, $n = 16$; young adult: $2.2 \pm .4$, $n = 11$, $p = .025$). In contrast, the quantal content in the cortical pathway did not differ across ages (juvenile: $1.2 \pm .1$, $n = 11$; young adult: $1.2 \pm .1$, $n = 10$, $p = .966$).

The Thalamic Input Has Fewer Silent Synapses in Young Adult Mice

Besides the multiquantal glutamate release from presynaptic sites, a change in the proportion of silent synapses, which lack AMPAR current but exhibit NMDAR current, can contribute to loss of plasticity during development (36,37). To determine whether the thalamic loss of LTP during the juvenile–adult transition was accompanied by loss of silent synapses, we employed two approaches (26,27). First, we compared the CVs of the AMPAR EPSCs and NMDAR EPSCs (Figures 3A–3E). The larger the number of synapses that contribute to responses, the lower the CV of EPSC becomes. Therefore, the ratio between CV for NMDAR and AMPAR EPSCs ($\text{CV}_{\text{NMDAR}}/\text{CV}_{\text{AMPAR}}$) inversely correlates with the proportion of silent synapses (27). For the thalamic pathway, this ratio was higher in young adult mice (juvenile: $.60 \pm .10$, $n = 9$; young adult: $1.27 \pm .14$, $n = 9$, $p < .001$) (Figure 3E), which indicates a decrease in silent synapses with aging. A comparison of the raw CV measurements between the two age groups indicated that this change is due to difference in CV_{NMDAR} between juvenile and young adults but not in CV_{AMPAR} (CV_{NMDAR} ; juvenile: $.07 \pm .01$; young adult: $.14 \pm .01$; $p = .005$; CV_{AMPAR} ; juvenile: $.13 \pm .01$; young adult: $.11 \pm .01$, $p = .634$) (Figure 3D). For the cortical pathway, the $\text{CV}_{\text{NMDAR}}/\text{CV}_{\text{AMPAR}}$ ratio was independent of age (juvenile: $.74 \pm .12$, $n = 9$; young adult: $.82 \pm .06$, $n = 9$, $p = .531$) (Figures 3C and 3E).

Second, we employed low-intensity stimulation (as described in Methods and Materials) to compare success rates of synaptic responses mediated by either AMPAR EPSCs or mixed AMPAR and NMDAR EPSCs recorded at -70 or $+40$ mV, respectively (Figures 3F and 3G). Because silent synapses can only contribute to NMDAR EPSCs registered at $+40$ mV, the presence of silent synapses results in a success rate of AMPAR EPSCs at -70 mV that is lower than that of mixed EPSCs at $+40$ mV (26,27). Consistent with the CV analysis, in the thalamic pathway, the success rate of mixed EPSCs exceeded that of AMPAR EPSCs in juvenile but not young adult mice (juvenile: $.67 \pm .04$ vs. $.46 \pm .03$, $n = 8$, $p < .001$; young adult: $.52 \pm .03$ vs. $.53 \pm .04$, $n = 8$, $p = .785$) (Figures 3H and 3I), whereas in the cortical pathway the success rate of mixed EPSCs exceeded that of AMPAR EPSCs in both ages (juvenile: $.53 \pm .08$ vs. $.41 \pm .05$, $n = 7$, $p = .032$; young adult: $.67 \pm .06$ vs. $.47 \pm .04$, $n = 10$, $p = .005$).

The differences in the success rate of mixed EPSCs and AMPA EPSCs together with the CV analysis suggest loss of silent synapses in the thalamic input with aging. However, this conclusion might be compromised by the fact that the higher success rate of mixed versus AMPAR-mediated EPSCs can result not only from silent synapses but also from the spillover of glutamate from neighboring synapses (38). Actually, the effect of glutamate spillover was found to be prominent in the thalamic input to LA of juvenile rats (39). To determine whether the spillover was also responsible for the higher success rate of mixed EPSCs at $+40$ mV in slices from juvenile mice, we repeated the comparisons at $36^\circ\text{C} \pm 1^\circ\text{C}$, when the spillover effects are minimal because of higher efficiency of glutamate transporter (38). Under these conditions, the difference in the success rate between the two types of EPSCs was much smaller than that obtained at $30^\circ\text{C} \pm 1^\circ\text{C}$ (36°C : $.06 \pm .02$, $n = 10$; 30°C : $.21 \pm .03$, $n = 8$, $p = .002$, unpaired t test) (Figures 4A and 4C), indicating a significant involvement of glutamate spillover. Despite this, the success rate of the mixed EPSCs was still higher than that of AMPAR EPSCs (mixed: $.57 \pm .06$; AMPAR: $.50 \pm .04$, $n = 10$, $p = .032$) (Figures 4A and 4B), pointing to the presence of “silent synapses” in thalamic inputs in juvenile mice. By contrast, no such difference was found in young adult mice (mixed: $.49 \pm .07$; AMPAR: $.52 \pm .06$, $n = 8$, $p = .316$) (Figures 4A and 4B). Interestingly, young adult mice did not show such difference even at the low temperature (Figure 4C), which might suggest that glutamate reuptake is less sensitive to temperature in older animals. These data confirm the loss of silent synapses in the thalamic pathway during the juvenile–adult transition but do not replicate observations in slices from 3–5-week-old rats, in which failure rates of synaptic responses at 36°C did not differ at -70 and $+40$ mV (39). The exact reason for this discrepancy is unclear, but one possible explanation could be a difference in the proportion of silent synapses between mouse and rat at this developmental stage.

Intrinsic Membrane and Electrotonic Properties of LA Neurons

Patch recording from cell body reflects not only synaptic events but also electrical properties of the plasma membrane and dendrites (40), which might change during development. To examine properties of plasma membrane and dendritic transmission, we determined input resistance, membrane time constants, and latency and rise time of compound EPSCs. The input resistance ($p = .821$), membrane time constants ($p = .907$), and EPSC latency (cortical: $p = .675$; thalamic: $p = .117$) did not differ between amygdala neurons in juvenile and young adult mice. The EPSC rise time (20%–80%) had a tendency to be shorter for cortical inputs in both ages (juvenile: $p = .065$; young adult: $p = .067$, paired t test) and for young adult mice in both pathways (cortical: $p = .187$, thalamic: $p = .285$, unpaired t test) (Table 1).

Discussion

Earlier studies have identified several differences between the thalamic and cortical inputs to LA in juvenile 3–5-week-old rodents. These include a higher AMPAR/NMDAR current ratio

in the cortical pathway (19), an L-type Ca^{2+} channel dependent form of LTP specific to the thalamic pathway (41), a greater size of dendritic spines receiving thalamic projections (20), and a selective involvement of glutamate receptor 3 (GluR3) AMPAR subunits in LTP within the cortical but not thalamic pathway (21). The present study documents that a physiological divergence between the two amygdala-based fear pathways becomes greater during the juvenile–adult transition.

In juvenile mice, the two pathways had indistinguishable quantal contents and glutamate release probabilities. They equally expressed LTP induced by STDP protocol, which was consistent with a previous study comparing glutamatergic transmission in the two pathways in juvenile rats (22). It is interesting, however, that STDP protocol with fewer pairings (45 pairings instead of 150 in this study) failed to induce LTP in the cortical input of 3–4-week-old mice (20). In addition, we found that silent synapses were present in both pathways in juveniles, as evidenced by: 1) the relatively higher success rate of mixed AMPAR/NMDAR EPSCs evoked by low-intensity stimulation, and 2) the lower variation of NMDAR than AMPAR EPSCs. Yet, the proportion of silent synapses seemed to be low, because the difference in the success rates of the mixed EPSCs was only slightly higher than that of the AMPAR EPSCs.

We found differences in synaptic properties between the two pathways in young adult mice, unlike in juveniles, which included a larger quantal content and a lower proportion of postsynaptically silent synapses in the thalamic than in the cortical input. The Pr did not seem to differ between the two pathways, when estimated by measurements of the PPR and the decay of NMDAR current in the presence of MK-801. Because synaptic unsilencing in the somatosensory cortex during early postnatal development has been implicated in the developmental loss of LTP (31,36), we speculate that the similar changes in the thalamic input account for the reduced plasticity during adulthood. A report of less plasticity in the thalamic than in the cortical pathway in 6–12-week-old mice seems consistent with our findings, assuming most animals in this study were older than 8 weeks (42).

Given the strong correlation between LTP in amygdala inputs and fear learning (14,15,17, 43), the occurrence of late developmental changes in plasticity of the thalamic but not cortical pathway raises the question of how these changes might affect the functional role of each pathway and whether the contribution of the thalamic input in fear learning and other emotional behaviors is altered during the juvenile–adult transition. That thalamic pathway loses STDP-LTP but not the pairing protocol-induced LTP, which is robust in both young adult (23) and juvenile mice (44), indicates that only some forms of plasticity in this pathway disappear, whereas others remain intact. The LTP in the thalamic pathway can consistently be induced in vivo (45). A plausible parallel interpretation of these findings is that some functions of the thalamic pathway might be lost, whereas others remain intact during late development.

Previous studies have suggested that the cortical pathway plays a predominant role in fear conditioning in adult animals, because its temporary inactivation by muscimol during training or testing interfered with fear learning or fear retrieval, respectively (46), and post-training lesions in the cortical pathway also eliminated fear memory (47,48). Yet, thalamic pathway can support fear learning when cortical input is destroyed (13). If the cortical pathway dominates fear learning during adulthood due to its greater plasticity, the thalamic pathway would be expected to contribute more in fear learning in juveniles, which exhibit similar plasticity between the two pathways. Consistently, a recent study reported stronger fear learning in juvenile than in adult mice of C57 background (11). In addition, the higher plasticity in the thalamic pathway might contribute to enhanced fear generalization in juvenile mice (12).

The late developmental alterations in thalamo-amygdala synapses raise an intriguing possibility that the relative contributions of subcortical and cortical fear pathways to emotional behavior in humans might differ between adolescents and adults.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Loss of synaptic plasticity in thalamic (Th) but not cortical (Cx) input during the juvenile–adult transition. **(A)** Examples of biocytin-filled neurons from juvenile (left) and young adult (right) mice. **(B)** Stimulation scheme and spike-timing dependent plasticity (STDP) protocol: 15 trains of 10 presynaptic stimuli (10 Hz), each followed (10-msec delay) by a 5-msec 1-nA current injection into the lateral amygdala (LA) neuron, applied at .1 Hz. **(C,D)** STDP in Cx and Th inputs to LA in juvenile **(C)** and young adult **(D)** mice. Arrow shows induction of STDP. Insets show examples of excitatory postsynaptic potentials (EPSPs) recorded before [1] and after the induction [2]. **(E)** Summary of long-term potentiation (LTP) expression in

both inputs across ages. Data shown as means $\pm$ SEM during the last 5 min of baseline before LTP induction [1]. $**p < .01$ (unpaired t test).

Figure 2.

Multiquantal glutamate release emerged in Th but not Cx inputs to LA during juvenile–adult transition. **(A)** Examples of minimal responses from juvenile (left) and young adult (right) mice. For Cx input, the success rate of EPSCs is $.26 \pm .04$ in juvenile and $.27 \pm .06$ in young adult mice. For Th input, these values are $.30 \pm .04$ and $.35 \pm .05$, respectively. **(B)** Comparison of minimal responses between the Cx and Th inputs at different ages. **(C)** Representative traces of asynchronized quantal responses from juvenile (Left) and young adult (Right) mice in the presence of Sr^{2+} . **(D)** Mean EPSC amplitudes of responses after stimulation of either the Th or Cx inputs in juvenile and young adult mice in the presence of Sr^{2+} . * $p < .05$; ** $p < .01$. Abbreviations as in Figure 1.

Figure 3.

Proportion of silent synapses decreases in Th but not Cx input during the juvenile–adult transition. (**A,B**) Plots of the amplitudes of compound EPSCs recorded from representative neurons held at +40 mV and –70 mV during repetitive stimulation of the Th and Cx inputs in juvenile (**A**) and young adult mice (**B**). (**C,D**) Coefficient of variation (CV) value for α -amino-3-hydroxy-5-methylisoxazole propionate receptor (AMPA) and *N*-methyl-D-aspartate receptor (NMDAR) currents in Cx (**C**) and Th (**D**) inputs across ages. (**E**) Ratios between CV_{NMDAR} and CV_{AMPA} in the Cx and Th inputs across ages. (**F,G**) AMPAR and AMPAR/NMDAR mixed responses to the low-intensity stimulation of the Th and Cx pathways in juvenile (**F**) and young adult mice (**G**). Examples of traces recorded from individual neurons

held at -70 mV (upper traces) or at $+40$ mV (lower traces). **(H,I)** Comparisons of success rates between AMPAR (AMPA) and AMPAR/NMDAR-mixed responses (mixed) in juvenile **(H)** and young adult **(I)** mice. **(H)** The success rates of AMPAR EPSCs are $.41 \pm .05$ in Cx inputs and $.46 \pm .03$ in Th inputs. **(I)** The success rates of AMPAR EPSCs are $.47 \pm .04$ in Cx inputs and $.52 \pm .04$ in Th inputs. $*p < .05$; $**p < .01$; and $***p < .001$. Other abbreviations as in Figure 1.

Figure 4.

Silent synapses in the Th input of juvenile but not young adult mice are detected at 36°C.

(A) Examples of traces recorded from individual neurons held at -70 mV (upper traces) or at $+40$ mV (lower traces). (B) Comparisons of success rates between AMPAR and AMPAR/NMDAR-mixed responses (mixed) in juvenile and young adult mice. The success rates of AMPAR EPSCs are $.50 \pm .05$ in juvenile and $.52 \pm .06$ in young adult mice. (C) Difference in the success rate of mixed and AMPAR responses in slices from juvenile and young adult mice for recordings at 30°C and 36°C . $*p < .05$; $**p < .01$. Abbreviations as in Figure 1.

Table 1
Comparisons of Intrinsic Properties of LA PNs Between Juvenile and Young Adult Mice

Cortical	EPSCs Latency (ms)		20%-80% Rise Time (ms)		Membrane Constant (ms)	
	Thalamic	Cortical	Thalamic	Rin (MΩ)		
Juvenile	2.2 ± .1 (8)	2.3 ± .1 (8)	2.5 ± .4 (8)	2.8 ± .3 (8)	105.8 ± 2.9 (24)	57.0 ± 3.7 (10)
Young Adult	2.1 ± .1 (8)	2.2 ± .1 (8)	1.8 ± .3 (8)	2.3 ± .2 (8)	107.0 ± 4.4 (25)	57.7 ± 4.6 (9)

The number in the parentheses represents the number of tested neurons.
LA, lateral amygdala; EPSCs, excitatory postsynaptic currents; PN, principal neurons; Rin, input resistance.