*Biological Psychiatry:* Celebrating 50 Years

# Archival Report

# Diminished Fear Extinction in Adolescents Is Associated With an Altered Somatostatin Interneuron–Mediated Inhibition in the Infralimbic Cortex

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## ABSTRACT

**BACKGROUND:** Rodents and humans show an attenuation of fear extinction during adolescence, which coincides with the onset of several psychiatric disorders. Although the ethological relevance and the underlying mechanism are largely unknown, the suppression of fear extinction during adolescence is associated with a diminished plasticity in the glutamatergic neurons of the infralimbic medial prefrontal cortex, a brain region critical for fear extinction. Given the putative effect of synaptic inhibition on glutamatergic neuron activity, we studied whether gamma-aminobutyric acidergic neurons in the infralimbic medial prefrontal cortex are involved in the suppression of fear extinction during adolescence.

**METHODS:** We assessed membrane and synaptic properties in parvalbumin-positive interneurons (PVINs) and somatostatin-positive interneurons (SSTINs) in male preadolescent, adolescent, and adult mice. The effect of fear conditioning and extinction on PVIN-pyramidal neuron and SSTIN-pyramidal neuron synapses in male preadolescent, adolescent, and adult mice was evaluated using an optogenetic approach.

**RESULTS:** The development of the membrane excitability of PVINs is delayed and reaches maturity only by adulthood, while the SSTIN membrane properties are developed early and remain stable during development from preadolescence to adulthood. Although the synaptic inhibition mediated by PVINs undergoes a protracted development, it does not exhibit a fear behavior–specific plasticity. However, the synaptic inhibition mediated by SSTINs undergoes an adolescence-specific enhancement, and this increased inhibition is suppressed by fear learning but is not restored by extinction training. This altered plasticity during adolescence overlapped with a reduction in calcium-permeable glutamate receptors in SSTINs.

**CONCLUSIONS:** The adolescence-specific plasticity in the SSTINs might play a role in fear extinction suppression during adolescence in mice.

*Keywords:* Adolescence, Fear extinction, GABA, Infralimbic medial prefrontal cortex, Parvalbumin-positive neurons, Somatostatin-positive neurons, Synaptic plasticity

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A prior knowledge of potential threats allows an organism to detect future dangers and take the appropriate actions for its safety. However, a suppression of threat memory in the absence of danger is equally important to permitting other survival functions. An impairment of such coping mechanisms to attenuate threat memory might result in maladaptive behaviors. A diminished ability to extinguish fear memory has been implicated in anxiety- and trauma-related disorders (1–3). Consistently, behavioral therapy for anxiety- and trauma-related disorders relies on extinction learning. Given the high incidence of psychiatric disorders during adolescence (4,5), it is important to understand whether fear regulation is affected during the transition into and out of adolescence. Past studies on fear extinction (FE) in rodents and humans have made a remarkable observation, i.e., a lack of a prototypical FE during adolescence (6,7). The diminished FE during adolescence might be advantageous to exert a constraint on risky behaviors during the transition from dependence on a caregiver to being an independent adult. However, an interaction of this reduced FE with potential environmental and genetic risk factors could exacerbate anxiety and trauma-like behaviors in adolescents.

A significant number of adults who are diagnosed with anxiety- and fear-related disorders exhibited symptoms during their childhood and adolescent years (8,9). Understanding the mechanism involved in the onset of anxiety- and fear-related

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disorders might be critical for the prevention and management of these disorders across the lifespan. It is possible that the brain regions involved in the regulation of fear behavior exhibit a development-dependent plasticity, which might be responsible for the suppression of FE during adolescence (6,7,10). Consistently, we observed a lack of FE-dependent synaptic and intrinsic plasticity in the layer 5 pyramidal neurons of the adolescent infralimbic medial prefrontal cortex (IL-mPFC), a brain region critical for FE (6,11,12). However, the mechanism underlying the lack of IL-mPFC plasticity during adolescence is unclear. As synaptic inhibition plays important roles in sculpting cortical circuitry, pyramidal neuron activity and the generation of cortical rhythms (13,14), gamma-aminobutyric acidergic (GABAergic) neuron development could play a role in the attenuation of IL-mPFC plasticity and FE during adolescence. Consistent with the notion that the phylogenetically newer brain areas undergo a protracted development (15), the mPFC is believed to undergo a prolonged rearrangement (16,17). Earlier studies have demonstrated a protracted synapse development in the GABAergic neurons of the mPFC (18–21). However, an in-depth understanding of synapse development in IL-mPFC GABAergic neurons from preadolescence to adulthood has been lacking. Therefore, we undertook a systematic analysis of the membrane and synaptic properties of the IL-mPFC parvalbumin-positive interneurons (PVINs) and somatostatin-positive interneurons (SSTINs), which primarily innervate the somatic and proximal dendritic areas and the distal dendrites, respectively, of the pyramidal neurons, in preadolescent, adolescent, and adult mice (22).

## METHODS AND MATERIALS

### Animals

The following mouse lines were purchased from Jackson Laboratory (Bar Harbor, ME) and subsequently bred in the Skirball division of New York University Medical School animal facility: B6 $PV^{cre}$ (017320, C57BL/6J), sttm2.1(cre)Zjh/J (013044, C57BL/6/129S4SvJae/C57BL/6J), Ai32(RCL-ChR2(H134R)/EYFP) (024109, C57BL/6J), B6.Cg-Gt(ROSA)26Sortm14(CAG-tdTomato)Hze/J (007914, C57BL/6J), and GAD67/GAD67-EGFP (G42, 007677, BALBc/C57BL/6J) mice. PV-channelrhodopsin-2 (PV-ChR2) mice were generated from homozygous B6 $PV^{cre}$ and homozygous Ai32(RCL-ChR2(H134R)/EYFP) mice. SST-ChR2 mice were generated from homozygous sttm2.1(cre)Zjh/J and homozygous Ai32(RCL-ChR2(H134R)/EYFP) mice. SST-tdTomato mice were generated from homozygous sttm2.1(cre)Zjh/J and homozygous B6.Cg-Gt(ROSA)26Sortm14(CAG-tdTomato)Hze/J mice. We have used the same line for specific tests across 3 developmental stages. Naïve mice were used for studying the development-dependent changes in membrane and synaptic properties. Mice were maintained on a 12-hour light-dark cycle at 23°C with access to food and water ad libitum. All experiments were carried out in male mice. The Institutional Animal Care and Use Committee of the New York University School of Medicine approved all the procedures.

### Behavior

Mice were categorized as preadolescent (postnatal day 24 [P24]), adolescent (P29), and adult (>P60), as described before (11,23). Fear conditioning (FC) or tone alone (TA) exposure was performed on P22 for preadolescent, P27 for adolescent, or ≥P60 for adult mice. For conditioning, a mouse was placed in the conditioning chamber (context A) within a soundproof box (Coulbourn Instruments, Whitehall, PA). After a 2-minute exploration, mice received 2 habituation tones (5 kHz, 30 dB, 30-second duration) followed by 3 tones (30-second duration) that co-terminated with foot shocks (2-second duration, 0.5 mA, 30-second interval). Mice were returned to the home cages 30 seconds after the final tone-shock pairing. The TA group received the same tone presentations as the fear conditioned group but without foot shocks. FE training was performed 24 hours after conditioning and consisted of 30 tone presentations (30-second duration) at 30-second intervals (context B). For all groups, fear memory was tested 48 hours after the initial conditioning by exposing the animal to 3 tones (30-second duration) at 30-second intervals (context B). Freezing was measured using FreezeFrame3 software (Coulbourn Instruments). Mice were anesthetized for electrophysiology studies 1 hour after fear memory test on day 3.

### Electrophysiology

Mice were anesthetized by an intraperitoneal injection of pentobarbital (120 mg/kg). A transcardial perfusion with ice-cold and oxygenated artificial cerebrospinal fluid containing NaCl (118 mM), glucose (10 mM), KCl (2.5 mM), $NaH_2PO_4$ (1 mM), $CaCl_2$ (1 mM), and $MgSO_4$ (1.5 mM) (325 mOsm, pH 7.4) was performed for approximately 45 seconds. Immediately following the perfusion, the brain was isolated and 300-μm brain slices were prepared on a vibratome (Campden Instruments, Loughborough, United Kingdom). To allow for recovery, slices were maintained for at least 1 hour at room temperature. Following recovery, one slice was transferred to the recording chamber and superfused with the aforementioned artificial cerebrospinal fluid containing 2.5 mM $CaCl_2$ at 32°C at 2 to 3 mL/min. The IL-mPFC was located using a 4× objective. The layer 5 pyramidal neurons were visualized using a 40× water immersion objective and video-enhanced differential interference contrast microscopy and were confirmed by their morphology and accommodating action potential firing characteristics. PVINs positive for enhanced green fluorescent protein (EGFP)/enhanced yellow fluorescent protein (EYFP) and EYFP/tdTomato-positive SSTINs were identified under fluorescence microscopy. Recording pipettes of 3 to 5 MΩ resistance were filled with internal solution containing K-gluconate (130 mM), KCl (10 mM), $MgCl_2$ (5 mM), MgATP (5 mM), NaGTP (0.2 mM), EGTA (0.5 mM), and HEPES (5 mM), pH adjusted to 7.4 with KOH. Electrophysiological recordings were performed with a Multiclamp 700B amplifier connected to a Digidata 1550A (Molecular Devices, San Jose, CA). Signals were sampled at 20 to 100 kHz and filtered at 2 kHz. Neuronal excitability was measured in current clamp mode by injecting currents from −50 to 200 pA (10-pA increments). Spontaneous excitatory postsynaptic currents (sEPSCs) were recorded at −60 mV in the presence of bicuculline (20 μM). In experiments involving electrical stimulation, a concentric bipolar stimulating electrode was placed in layer 2/3. *N*-methyl-D-aspartate (NMDA) receptor currents were measured at +40 mV in the presence of bicuculline (20 μM) and DNQX (10 μM) using an electrode solution containing CsCl (130 mM), HEPES (10 mM), EGTA (0.5 mM), MgATP (5 mM), sucrose (10 mM), and QX314 (5 mM), pH adjusted to 7.4 with CsOH. The same CsCl internal solution containing 10 mM spermine was used for rectification index experiments. Currents were evoked at different holding potentials from −60 to +60 mV (20-mV increments) in the presence of bicuculline (20 μM) and APV (50 μM), and rectification index was calculated as the ratio of the slopes of the linear current/voltage relationship at positive (0 to 60 mV) and negative (−60 to 0 mV) holding potentials (24). For light stimulation of GABAergic terminals in PV-ChR2 and SST-ChR2 mice, blue light (470 nm) was emitted from a Lumen 1600-LED (Prior Scientific, Cambridge, United Kingdom) at increasing durations (0.05 to 0.5 ms, 0.1 Hz). Electrophysiological recordings were rejected when series resistance or holding current changed by 10% or more.

## Data Analysis

Membrane properties and evoked currents were analyzed using Clampfit 10.5 (Molecular Devices, San Jose, CA). Passive membrane properties were measured from the membrane voltage response to hyperpolarizing current injections of −50 to −10 pA. Input resistance ($R_{in}$) was calculated as the slope of the current-voltage relationship from −50 to 0 pA and membrane time constant tau was calculated by fitting the initial change in membrane voltage in response to a −50-pA step to a single exponential function. Membrane capacitance ($C_m$) was calculated from tau and $R_{in}$. Statistical analyses were performed using GraphPad Prism 7 (GraphPad Software, San Diego, CA) or SPSS statistics software, version 25 (IBM Corp., Armonk, NY). One-way analysis of variance with Tukey's post hoc test or Kruskal-Wallis with Dunn's post hoc test were used to compare passive membrane properties, spontaneous neurotransmission, and FE memory. Two-way analysis of variance with Tukey's post hoc test was used to analyze neuronal excitability.

## RESULTS

### Suppression of Fear Extinction in Adolescent Mice

To compare FE in preadolescent, adolescent, and adult mice, we have pooled together behavioral data from PV-ChR2 (Supplemental Figure S1) and SST-ChR2 mice (Supplemental Figure S2). There were 3 treatment groups: TA, FC, and FE. In preadolescent mice, FC resulted in a robust freezing behavior compared with the TA group (Figure 1A). Furthermore, FE caused a significant reduction in freezing compared with the FC group (Figure 1A). Similar to the preadolescent group, adolescent mice exhibited a significant increase in freezing following FC (Figure 1B). However, FE training did not result in the reduction of freezing 24 hours later in adolescent mice (Figure 1B). Although the magnitude of FC was lower in adult mice compared with the younger mice, they exhibited a strong fear memory (Figure 1C). Furthermore, FE resulted in a significant decrease in freezing (Figure 1C). These behavioral data further confirm the suppression of FE during adolescence (6,7).

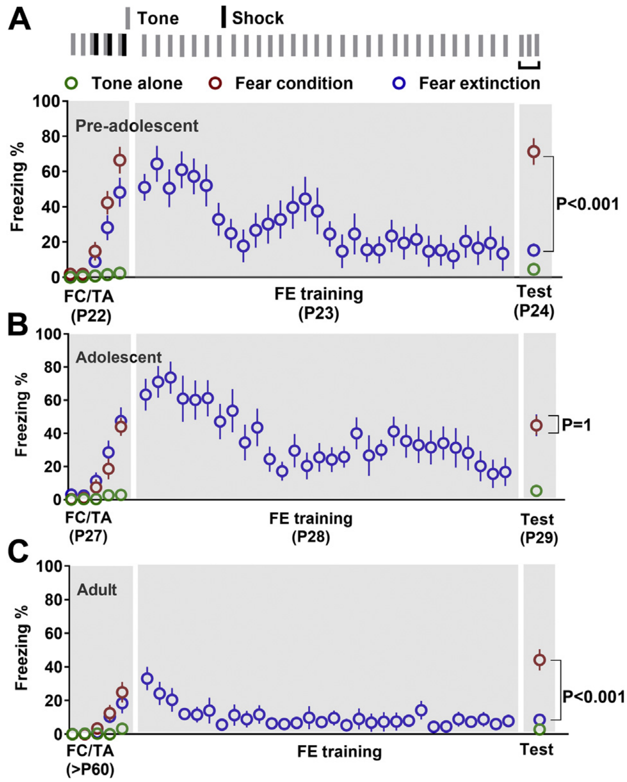


**Figure 1.** Suppression of fear extinction (FE) during adolescence. Average freezing in tone alone (TA), fear conditioning (FC), and FE groups on days 1 (TA or FC), 2 (extinction training), and 3 (memory test). **(A)** Preadolescent: TA (10 mice), FC (10 mice), and FE (10 mice), comparison of freezing on day 3 ($F_{2,27} = 50.6$, $p < .001$; TA vs. FC: $p < .001$; TA vs. FE: $p = .4$; FC vs. FE: $p < .001$); **(B)** adolescent: TA (11 mice), FC (10 mice), and FE (10 mice), comparison of freezing on day 3 ($F_{2,28} = 17.2$, $p < .001$; TA vs. FC: $p < .001$; TA vs. FE: $p < .001$; FC vs. FE: $p = 1$); and **(C)** adult: TA (10 mice), FC (9 mice), and FE (9 mice), comparison of freezing on day 3 ($F_{2,25} = 26.7$, $p < .001$; TA vs. FC: $p < .001$; TA vs. FE: $p = 1$; FC vs. FE: $p < .001$). A part of this behavioral data appeared in an earlier publication (11). We also observed a development-dependent decrease in fear acquisition ($F_{2,55} = 12.9$, $p < .001$; preadolescent vs. adolescent: $p = .25$; preadolescent vs. adult: $p < .001$; adolescent vs. adult: $p = .005$) and fear memory ($F_{2,26} = 4.7$, $p = .014$; preadolescent vs. adolescent: $p = .017$; preadolescent vs. adult: $p < .016$; adolescent vs. adult: $p = .93$) in preadolescent, adolescent, and adult mice. P, postnatal day.

### Protracted Development of Intrinsic Excitability in IL-mPFC PVINs

As membrane excitability plays an important role in neuronal output, an adolescence-specific modulation of the membrane properties of IL-mPFC PVINs could influence FE. To determine whether the IL-mPFC PVINs show an adolescence-specific change in membrane excitability, we compared the number of action potentials in response to current injection in EYFP-positive PVINs in the IL-mPFC of preadolescent, adolescent, and adult PV-ChR2 mice. We observed a development-dependent increase in the number of action potentials from preadolescence to adulthood (Figure 2B). The

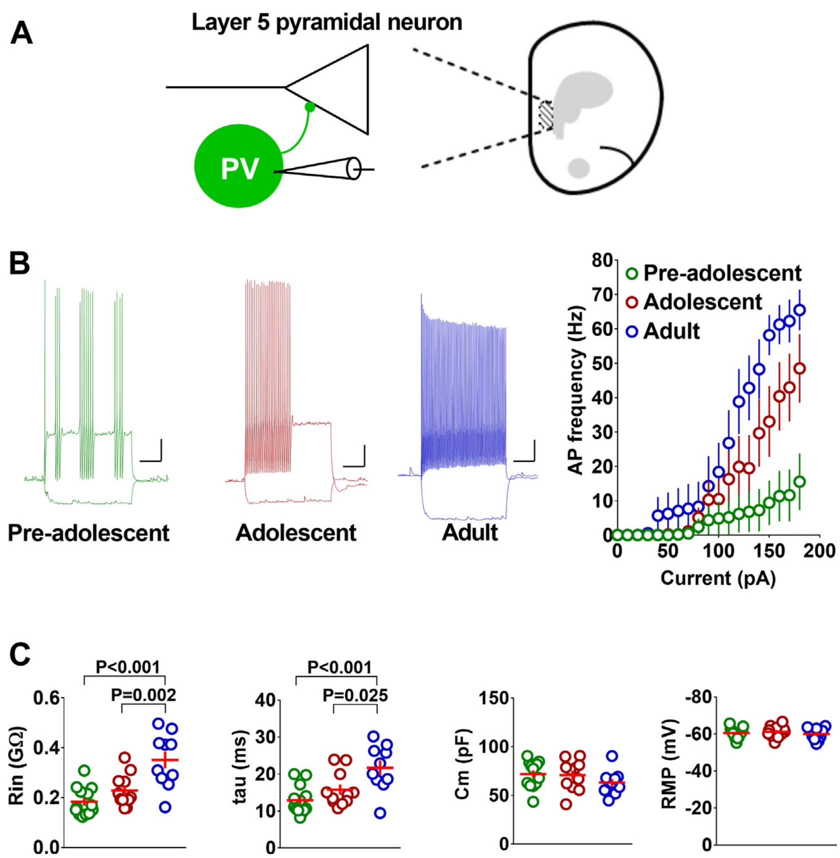


**Figure 2.** Protracted development of membrane excitability in the infralimbic medial prefrontal cortex parvalbumin-positive interneurons (PVINs). **(A)** Schematic presentation of whole-cell recording in PVINs. **(B)** Mean action potential (AP) frequency in response to current injection in PVINs from preadolescent (13 neurons/5 mice), adolescent (11 neurons/4 mice), and adult (9 neurons/4 mice) mice ($F_{2,30}$ = 5.7, $p$ = .008; preadolescent vs. adolescent: $p$ = .12; adolescent vs. adult: $p$ = .243; preadolescent vs. adult: $p$ = .006). Left panel shows example traces of voltage responses to hyperpolarizing (−50 pA) and depolarizing (+180 pA) current steps in PVINs. Scale = 250 ms/10 mV. **(C)** Passive membrane properties, input resistance (Rin) ($F_{2,31}$ = 13.76, $p <$ .001), membrane time constant (tau) ($F_{2,31}$ = 9.334, $p$ = .0007), membrane capacitance (Cm), and resting membrane potential (RMP), in the PVINs of preadolescent (13 neurons/5 mice), adolescent (11 neurons/4 mice), and adult (10 neurons/4 mice) mice. Horizontal line in each group represents mean and vertical line represents SEM.

development-dependent increase in membrane excitability was accompanied by a progressive increase in $R_{in}$ and membrane time constant (tau) without any significant change in membrane capacitance or resting membrane potential (Figure 2C). These results strongly suggest that PVINs in the IL-mPFC undergo a protracted development and reach maturity only by adulthood.

## Early Maturation of Intrinsic Excitability in IL-mPFC SSTINs

Given the protracted development of PVIN membrane properties, we examined whether the active or passive membrane properties of tdTomato-positive SSTINs in the IL-mPFC change during development. A comparison of the number of evoked action potentials in response to current injection in preadolescent, adolescent, and adult mice did not reveal a statistically significant effect (Figure 3B, C). Consistent with the lack of change in membrane excitability, we did not observe any change in $R_{in}$, membrane time constant, membrane capacitance, or resting membrane potential in the SSTINs of the preadolescent, adolescent, and adult groups (Figure 3D–G). Therefore, unlike the PVINs, the SSTINs in the IL-mPFC undergo an early maturation and exhibit stable membrane properties during development from preadolescence to adulthood.

## Comparison of Spontaneous Glutamatergic Transmission in the IL-mPFC PVINs and SSTINs of Preadolescent, Adolescent, and Adult Mice

In addition to intrinsic membrane excitability, the excitatory synaptic drive could play an important role in the GABAergic output of PVINs and SSTINs. To test whether the glutamatergic input to these GABAergic neurons exhibits any adolescence-specific changes, we measured the frequency, amplitude, rise time, and decay time of sEPSCs in the EYFP-positive PVINs and tdTomato-positive SSTINs of preadolescent, adolescent, and adult mice. Although the adult group exhibited a decrease in sEPSC frequency and amplitude in PVINs compared with the preadolescent and adolescent groups, only the change in amplitude reached statistical significance (Figure 4A). Both the rise time and decay time of sEPSCs in PVINs remained unchanged during development from preadolescence to adulthood (Figure 4A). Unlike the PVINs, SSTINs did not show any change in sEPSC frequency or amplitude in the preadolescent, adolescent, or adult groups (Figure 4B). Interestingly, we observed an increase in both the rise time and decay time in adult mice compared with the younger groups (Figure 4B). This modification of sEPSC kinetics might be due to a change in alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor subunit composition during development.

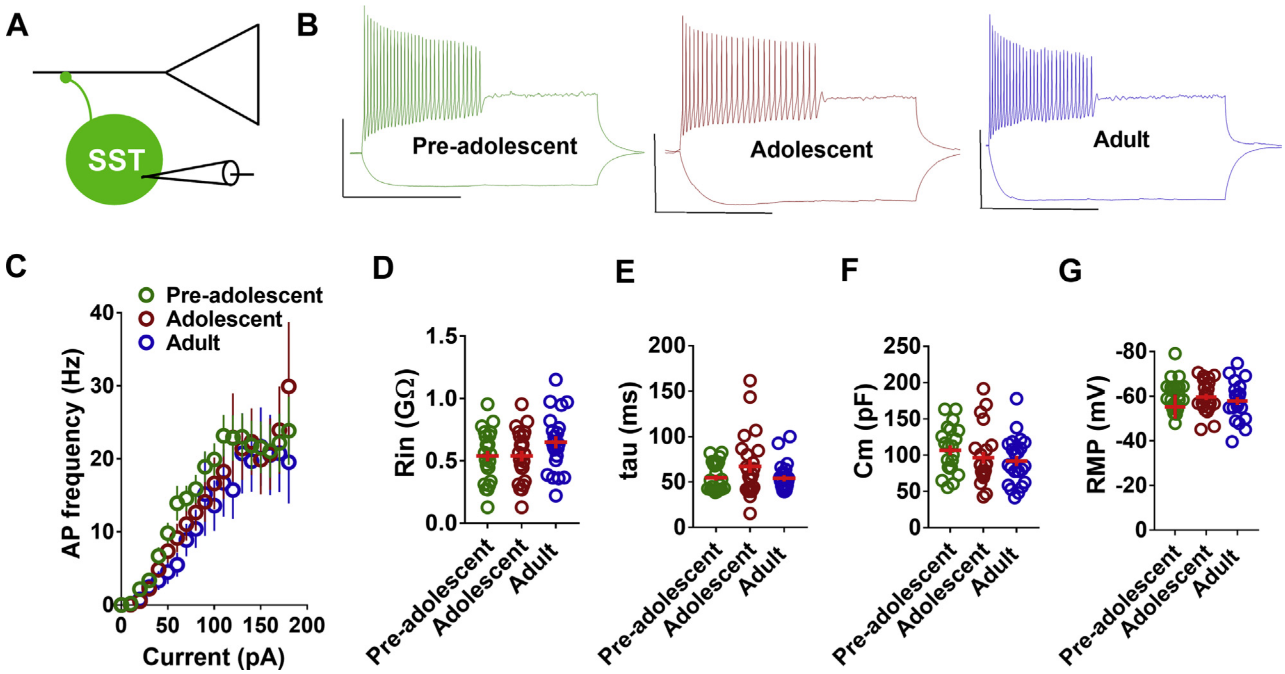


**Figure 3.** Early maturation of membrane excitability in the infralimbic medial prefrontal cortex somatostatin-positive interneurons (SSTINs). **(A)** Schematic presentation of whole-cell recording in SSTINs. **(B)** Example traces of voltage responses to hyperpolarizing (−50 pA) and depolarizing (+180 pA) current steps in SSTINs. Scale bar = 500 ms/50 mV. **(C)** Mean action potential (AP) frequency in response to current injection in SSTINs from preadolescent (21 neurons/7 mice), adolescent (21 neurons/7 mice), and adult (21 neurons/7 mice) mice ($F_{2,60} = 0.944$, $p = .395$). **(D–G)** Passive membrane properties, input resistance (Rin), membrane time constant (tau), membrane capacitance (Cm), and resting membrane potential (RMP), in the IL-mPFC SSTINs of preadolescent (21 neurons/7 mice), adolescent (21 neurons/7 mice), and adult (21 neurons/7 mice) mice. Horizontal line in each group represents mean and vertical line represents SEM.

## Synaptic Calcium Permeable AMPA Receptors in the IL-mPFC PVINs and SSTINs of Preadolescent, Adolescent, and Adult Mice

Changes in receptor subunit composition could modify the kinetics of AMPA receptor currents and glutamatergic transmission (25). Therefore, we examined whether the AMPA receptor subunit composition, particularly the presence of GluA2 subunit lacking synaptic calcium-permeable AMPA receptors (CPARs), a key mediator of plasticity in GABAergic neurons (26), changes during development from preadolescence to adulthood. Based on the inward rectification property of CPARs, we compared the inward rectification of electrically evoked AMPA currents in the EGFP-positive PVINs of preadolescent, adolescent, and adult G42 mice (24,27). A comparison of rectification index in the IL-mPFC PVINs of the preadolescent, adolescent, and adult groups did not show a statistically significant difference (Figure 5A). However, a similar analysis in the tdTomato-positive SSTINs showed a higher rectification index in the adolescent and adult groups compared with the preadolescent group, indicating a development-dependent decrease in synaptic CPARs (Figure 5B). These results suggest a development-dependent switch in the subunit composition of AMPA receptors, leading to an increase in synaptic calcium impermeable AMPA receptors in SSTINs, while PVINs exhibited a stable presence of synaptic CPARs during development from preadolescence to adulthood.

## NMDA Receptor Transmission in the IL-mPFC PVINs and SSTINs of Preadolescent, Adolescent, and Adult Mice

Similar to CPARs, NMDA receptors are a major source of calcium signaling at glutamatergic synapses and play an important role in synaptic plasticity. Therefore, we examined whether NMDA receptor transmission is diminished in PVINs or SSTINs during adolescence. A comparison of the amplitude of electrically evoked NMDA EPSCs in EGFP-positive PVINs of G42 mice revealed a development-dependent decrease in NMDA receptor transmission. The adult group showed a significantly lower NMDA receptor transmission compared with the preadolescent mice (Figure 6A). Although the NMDA receptor transmission in the adolescent group was lower than the preadolescent group, this effect did not reach statistical significance. A similar examination of NMDA EPSC amplitude in the tdTomato-positive SSTINs of preadolescent, adolescent, and adult mice showed an increase in NMDA EPSC amplitude in adult mice compared with preadolescent and adolescent mice (Figure 6B). Therefore, PVINs and SSTINs in the IL-mPFC exhibit an opposite effect on synaptic NMDA receptors during the transition from preadolescence to adulthood.

## Experience-Dependent Plasticity at PVIN-Pyramidal Neuron Synapses of the Preadolescent, Adolescent, and Adult IL-mPFC

Given the development-dependent changes in membrane excitability and glutamatergic transmission in PVINs, it is

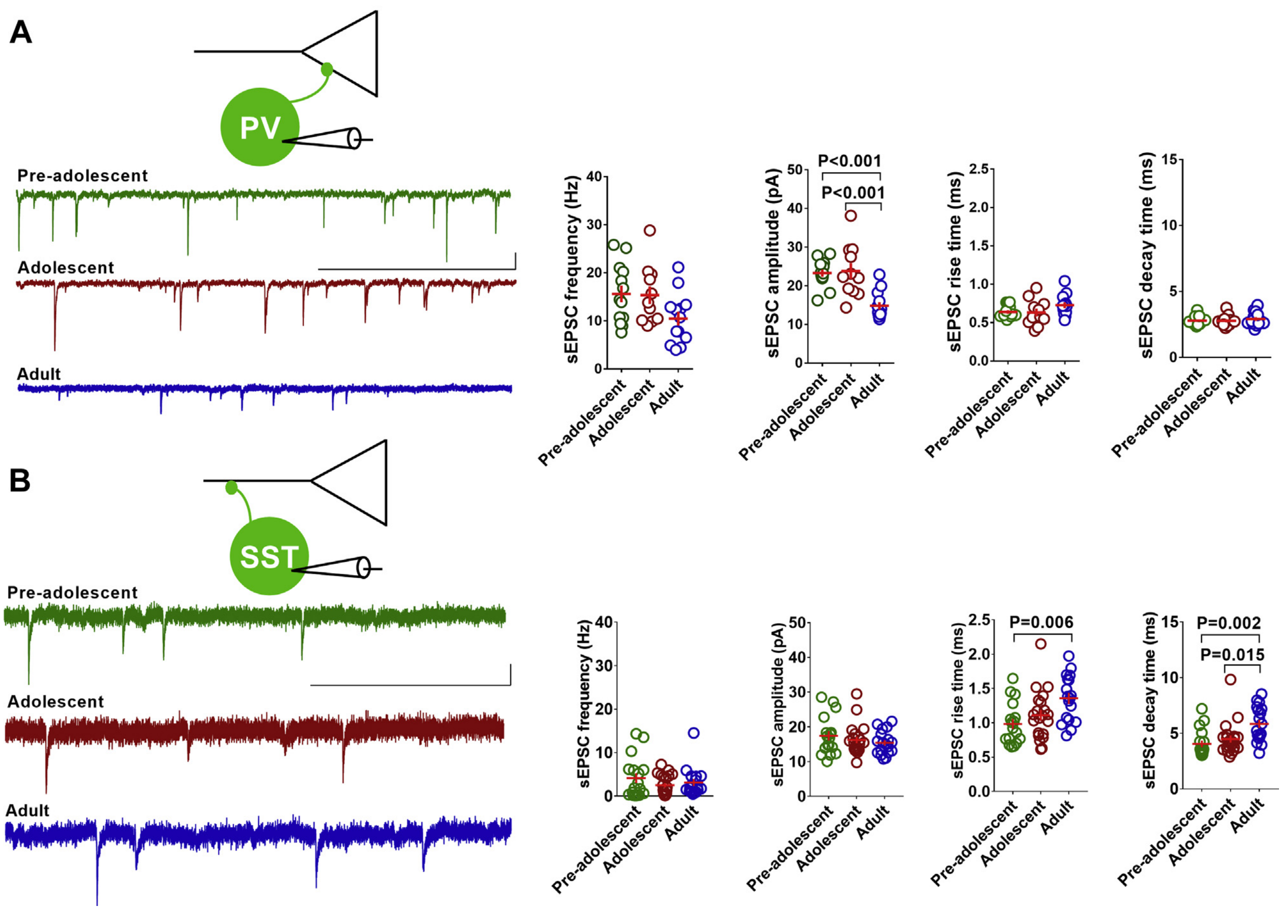


**Figure 4.** Development-dependent modulation of spontaneous glutamatergic transmission on to parvalbumin-positive interneurons (PVINs) and somatostatin-positive interneurons (SSTINs) in the infralimbic medial prefrontal cortex. **(A)** Mean spontaneous excitatory postsynaptic current (sEPSC) frequency, amplitude ($F_{2,34} = 14.6$, $p < .001$; preadolescent vs. adolescent: $p = 1$; preadolescent vs. adult: $p < .001$; adolescent vs. adult: $p < .001$), rise time, and decay time in the infralimbic medial prefrontal cortex PVINs of preadolescent (13 neurons/4 mice), adolescent (11 neurons/4 mice) and adult (13 neurons/4 mice) mice. Left panel shows example traces. Scale bar = 400 ms/10 pA. **(B)** Mean sEPSC frequency, amplitude, rise time ($F_{2,53} = 5.6$, $p = .006$; preadolescent vs. adolescent: $p = .77$; preadolescent vs. adult: $p = .006$; adolescent vs. adult: $p = .079$), and decay time ($F_{2,53} = 7.5$, $p = .001$; preadolescent vs. adolescent: $p = 1$; preadolescent vs. adult: $p = .002$; adolescent vs. adult: $p = .015$) in the infralimbic medial prefrontal cortex SSTINs of preadolescent (17 neurons/8 mice), adolescent (21 neurons/8 mice), and adult (18 neurons/6 mice) mice. Left panel shows example traces. Scale bar = 400 ms/10 pA. Horizontal line in each group represents mean and vertical line represents SEM.

interesting to know whether PVIN-pyramidal neuron GABAergic synapses undergo an experience (FC and FE) and/or development-dependent plasticity. Therefore, we measured the amplitude of light-evoked inhibitory postsynaptic currents (IPSCs) in the IL-mPFC layer 5 pyramidal neurons of TA, FC, and FE PV-ChR2 mice. In the preadolescent mice, the FE group but not the FC group showed an increase in IPSC amplitude (Figure 7B). Although there was a similar trend in adolescent mice, the FE group did not show a statistically significant increase in IPSC amplitude compared with the TA or FC groups (Figure 7C). A similar analysis in the adult mice showed a lack of effect of FC and FE on PVIN-pyramidal neuron GABAergic transmission (Figure 7D). Therefore, a successful extinction in preadolescent mice, but not adult mice, was associated with a potentiation of PVIN-pyramidal neuron transmission.

To determine whether PVIN-pyramidal neuron GABAergic synapses exhibit a development-dependent plasticity, we compared IPSC amplitude in control TA group from preadolescent, adolescent, and adult mice. We observed an increase in PVIN-pyramidal neuron GABAergic transmission with development (Figure 7B–D), suggesting that PVIN-pyramidal neuron GABAergic transmission undergoes a protracted development and reaches maturity only by adulthood. These results are similar to the protracted development of the membrane properties of PVINs (Figure 2).

## Experience-Dependent Plasticity at SSTIN-Pyramidal Neuron Synapses of the Preadolescent, Adolescent, and Adult IL-mPFC

Next, we studied the effect of FC and FE on light-evoked IPSCs in the IL-mPFC layer 5 pyramidal neurons of preadolescent, adolescent, and adult SST-ChR2 mice. Neither FC nor FE affected IPSC amplitude in preadolescent mice (Figure 8B). However, in the adolescent mice, we observed a robust

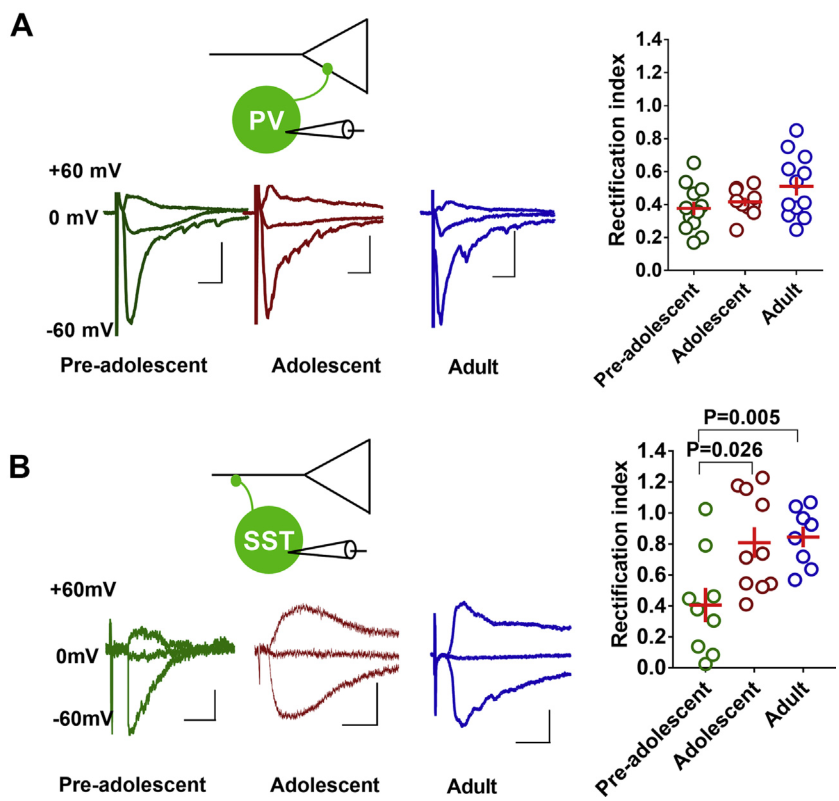


**Figure 5.** Development-dependent changes in synaptic calcium-permeable alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (CPARs) in parvalbumin-positive interneurons (PVINs) and somatostatin-positive interneurons (SSTINs) in the infralimbic medial prefrontal cortex. **(A)** Rectification index in the PVINs of preadolescent (12 neurons/3 mice), adolescent (10 neurons/3 mice), and adult (12 neurons/3 mice) mice ($F_{2,31}$ = 2.577, $p$ = .0922). Left panel shows example traces. Scale bar = 10 ms/20 pA. **(B)** Rectification index in the SSTINs of preadolescent (9 neurons/5 mice), adolescent (10 neurons/5 mice), and adult (9 neurons/5 mice) mice ($F_{2,25}$ = 6.9, $p$ = .004; preadolescent vs. adolescent: $p$ = .026; preadolescent vs. adult: $p$ = .005; adolescent vs. adult: $p$ = 1). Left panel shows example traces. Scale bar = 10 ms/20 pA.

suppression of IPSC amplitude in the FC group compared with the TA group. Furthermore, FE failed to modify FC-induced suppression of IPSC amplitude (Figure 8C). The effect of FC and FE on SSTIN-pyramidal neuron GABAergic transmission in adult mice was similar to that of preadolescent mice, as neither FC nor FE affected IPSC amplitude (Figure 8D).

To test whether SSTIN-pyramidal neuron GABAergic synapses exhibit a development-dependent plasticity, we compared IPSC amplitude in the control TA group from preadolescent, adolescent, and adult mice. Interestingly, we observed a potentiation of SSTIN-pyramidal neuron GABAergic transmission during adolescence (Figure 8B–D). Therefore, the SSTIN-mediated inhibition is enhanced during adolescence, and these potentiated synapses undergo a depression in response to FC. Furthermore, FE failed to restore FC-induced suppression of SSTIN-pyramidal neuron synapses in adolescent mice.

## DISCUSSION

Our current study reports the changes in the membrane properties, glutamatergic input, and GABAergic output of PVINs and SSTINs in the IL-mPFC during the transition from preadolescence to adulthood in mice. While PVINs undergo a protracted development and reach maturity only by adulthood, SSTINs are developed early but exhibit an adolescence-specific GABAergic plasticity. The surge in SSTIN-mediated inhibition of pyramidal neurons during adolescence and an irreversible suppression of this GABAergic transmission after FC might play a role in diminished FE in adolescents. Although a recent study has demonstrated the effect of SSTINs on glutamatergic transmission (28), future studies will be necessary to understand how SSTIN activity affects plasticity in the IL-mPFC pyramidal neurons, which regulate the amygdala in a top-down fashion to mediate FE (29–34).

The prolonged development of PVINs described in the current study is consistent with the notion that the mPFC undergoes a protracted development (15,17,20,35,36). Congruent with the earlier suggestion that mPFC development during adolescence is characterized by a slowly increasing inhibitory synaptic transmission and a diminishing glutamatergic transmission (17), we observed a progressive increase in PVIN-pyramidal neuron GABAergic transmission and a simultaneous decrease in glutamatergic transmission in PVINs, particularly those mediated by NMDA receptors, during the transition from preadolescence to adulthood. A previous study also reported a similar suppression of NMDA receptor transmission in older mice compared with juvenile mice (19). These results are particularly relevant given the purported role of a NMDA receptor hypofunction in the mPFC fast-spiking GABAergic neurons in schizophrenia, a mental disorder with an adolescent onset (17,37,38). However, the presence of CPARs, a key mediator of synaptic plasticity in PVINs (26), did not change during development. It is possible that CPARs are sufficient to mediate synaptic calcium signaling in PVINs and, therefore, a decrease in NMDA receptors in older mice might

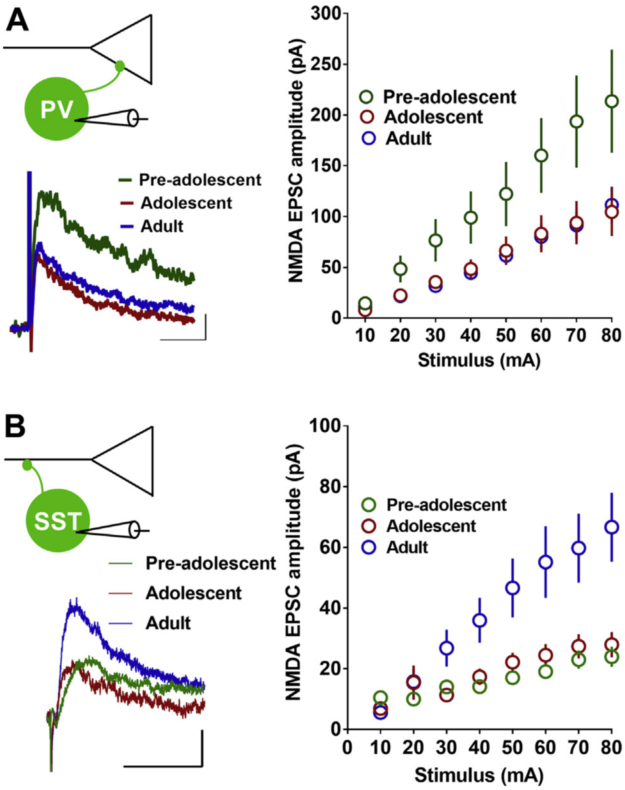


**Figure 6.** Development-dependent changes in *N*-methyl-D-aspartate (NMDA) receptor transmission in parvalbumin-positive interneurons (PVINs) and somatostatin-positive interneurons (SSTINs) in the infralimbic medial prefrontal cortex. **(A)** NMDA receptor-mediated currents in the PVINs of preadolescent (11 neurons/3 mice), adolescent (10 neurons/3 mice), and adult (14 neurons/3 mice) mice ($F_{2,32}$ = 3.8, $p$ = .033; preadolescent vs. adolescent: $p$ = .075; preadolescent vs. adult: $p$ = .043; adolescent vs. adult: $p$ = .99). Left panel shows example traces. Scale bar = 50 ms/20 pA. **(B)** NMDA receptor–mediated currents in the SSTINs of preadolescent (10 neurons/5 mice), adolescent (10 neurons/5 mice), and adult (13 neurons/6 mice) mice ($F_{2,30}$ = 5.8, $p$ = .007; preadolescent vs. adolescent: $p$ = 1; preadolescent vs. adult: $p$ = .013; adolescent vs. adult: $p$ = .033). Left panel shows example traces. Scale bar = 50 ms/20 pA. EPSC, excitatory postsynaptic current.

not affect plasticity in PVINs. The successful FE was associated with a potentiation of PVIN-pyramidal neuron synapses in preadolescent but not in adult mice, suggesting that the PVIN-pyramidal neuron synaptic plasticity might play a selective role in FE in preadolescents but not in adults. Our earlier study suggested the possibility of distinct FE mechanisms in preadolescents and adults, as only adults, and not preadolescents, show fear relapse following extinction (6).

Unlike in the PVINs, the membrane properties of SSTINs did not change during development from preadolescence to adulthood. Consistently, a recent study in the anterior cingulate cortex SST-positive neurons showed stable membrane characteristics in preadolescent and adolescent stages (18). However, the SSTIN-pyramidal neuron GABAergic transmission underwent a potentiation during adolescence. Consistently, somatostatin expression in the mPFC shows an increase during the fourth postnatal week (36). It is possible that an adolescence-specific neuromodulation is responsible for the increase in SSTIN-pyramidal neuron GABAergic transmission during adolescence. A recent study suggested that dendritic brain-derived neurotrophic factor in the excitatory neurons regulates their SSTIN input (39). These potentiated GABAergic synapses were depotentiated by FC. Consistent with the lack of FE in adolescent mice, FE training failed to restore this FC-induced suppression of SSTIN-pyramidal neurons GABAergic transmission. As SSTIN activity could modulate glutamatergic synapses (28), this adolescence-specific plasticity in the SSTINs could play an important role in the lack of intrinsic and synaptic plasticity in the layer 5 pyramidal neurons (6,11). Therefore, FC-induced suppression of SSTIN-pyramidal neuron GABAergic transmission and its irreversible nature might be a key mechanism in the suppression of FE during adolescence. We also observed a switch in synaptic calcium signaling in SSTINs during development. The CPAR-predominant glutamatergic synapses in preadolescent mice become calcium impermeable AMPA receptor-predominant glutamatergic synapses in adolescent and adult mice. Consistently, the rise and decay times of sEPSCs in older animals were slower compared with preadolescent mice (25). Although this development-dependent decrease in synaptic calcium signaling was compensated by an increase in synaptic NMDA receptors, this effect occurred only by adulthood. Therefore, the glutamatergic synapses in adolescent SSTINs lacked a robust calcium signaling owing to low levels of synaptic CPARs and NMDA receptors. Future studies are necessary to understand whether a lack of synaptic calcium signaling is responsible for the aforementioned development- and experience-dependent plasticity in SSTINs. Furthermore, it will be very interesting to examine the mechanism underlying the development-dependent regulation of synaptic CPARs and NMDA receptors. Although we observed a development-dependent decrease in FC, this effect was not correlated to either FE or experience-dependent plasticity.

We have previously shown that both synaptic and intrinsic plasticity in IL-mPFC pyramidal neurons are involved in FE in preadolescents and adults but not adolescents (6,11). Specifically, FE involves a potentiation of glutamatergic transmission in IL-mPFC layer 5 pyramidal neurons in preadolescents and adults but not in adolescents (6). Meanwhile, preadolescent and adult mice exhibited a bidirectional modulation of the excitability of IL-mPFC layer 5 pyramidal neurons following FC and FE, i.e., FC reduced membrane excitability, whereas FE reversed this effect (11). However, FE training failed to reverse FC-induced suppression of membrane excitability in adolescent mice (11). An mPFC-mediated top-down regulation of the amygdala is believed to be involved in FE (29,30,32–34,40–47). Therefore, the altered SSTIN-pyramidal neuron transmission during adolescence could affect plasticity in the IL-mPFC pyramidal neurons and, hence, FE. Our current findings shine a light on potential opportunities to enhance IL-mPFC plasticity and, hence, FE during adolescence. A recent study showed that a pharmacological enhancement of $GABA_A$ receptor transmission restores the mPFC gamma oscillation and cognitive flexibility in Dlx5/6$^{+/-}$ mice with impaired GABAergic neuron development (48). Given the delayed development of PVINs, an enhancement of PVIN-mediated GABAergic transmission during adolescence might enhance IL-mPFC plasticity and FE.

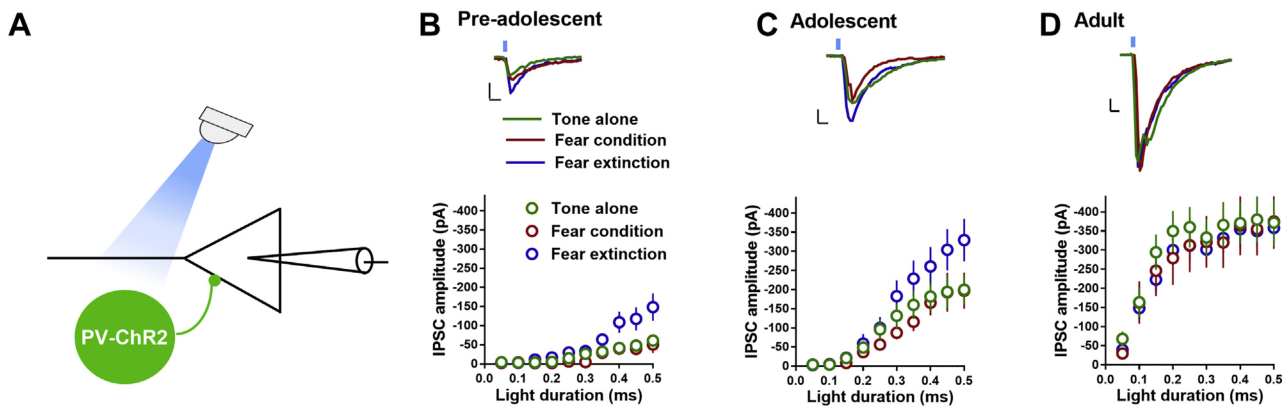


**Figure 7.** Experience-dependent modulation of parvalbumin-positive interneuron (PVIN)–mediated inhibition of infralimbic medial prefrontal cortex (IL-mPFC) layer 5 pyramidal neurons. **(A)** Schematic presentation of light-evoked inhibitory postsynaptic current (IPSC) recording in the IL-mPFC layer 5 pyramidal neurons of PV-ChR2 mice. **(B)** Mean IPSC amplitude in the IL-mPFC pyramidal neurons of preadolescent tone alone (TA) (15 neurons/5 mice), fear conditioning (FC) (15 neurons/5 mice), and fear extinction (FE) (17 neurons/5 mice) groups ($F_{2,44}$ = 4.9, $p$ = .011; TA vs. FC: $p$ = .88; TA vs. FE: $p$ = .051; FC vs. FE: $p$ = .015). **(C)** Mean IPSC amplitude in the IL-mPFC pyramidal neurons of adolescent TA (17 neurons/5 mice), FC (19 neurons/5 mice), and FE (14 neurons/5 mice) groups ($F_{2,47}$ = 2.12, $p$ = .13; TA vs. FC: $p$ = .82; TA vs. FE: $p$ = .33; FC vs. FE: $p$ = .11). **(D)** Mean IPSC amplitude in the IL-mPFC pyramidal neurons of adult TA (12 neurons/4 mice), FC (12 neurons/4 mice), and FE (14 neurons/4 mice) groups ($F_{2,35}$ = 0.15, $p$ = .85; TA vs. FC: $p$ = .9; TA vs. FE: $p$ = .86; FC vs. FE: $p$ = .99). Comparison of IPSC amplitude in preadolescent, adolescent, and adult TA groups: ($F_{2,41}$ = 26.6, $p$ = .007; preadolescent vs. adolescent: $p$ = .07; preadolescent vs. adult: $p < .001$; adolescent vs. adult: $p < .001$). Upper panels show example traces. Scale bar = 5 ms/50 pA. ChR2, channelrhodopsin-2.

Also, an enhancement of NMDA receptor transmission might benefit both IL-mPFC plasticity and FE in adolescents, as they show a diminished synaptic calcium signaling in SSTINs (7,49). Finally, understanding whether and how neuromodulators contribute to the development of the IL-mPFC might benefit efforts to enhance FE during adolescence.

Although our study is limited to the examination of 2 classes of GABAergic neurons, PVINs and SSTINs, and the current study does not demonstrate the causal role of these neurons in the adolescence-specific suppression of FE, we have demonstrated a protracted development of both the membrane and synaptic properties of PVINs and an adolescence-specific plasticity in SSTIN-pyramidal synapses in the IL-mPFC. These findings might facilitate a further understanding of how this differential development of PVIN- and SSTIN-mediated inhibition shapes pyramidal neuron activity, and hence regulates FE during development. There is a higher incidence of affective and anxiety disorders in women

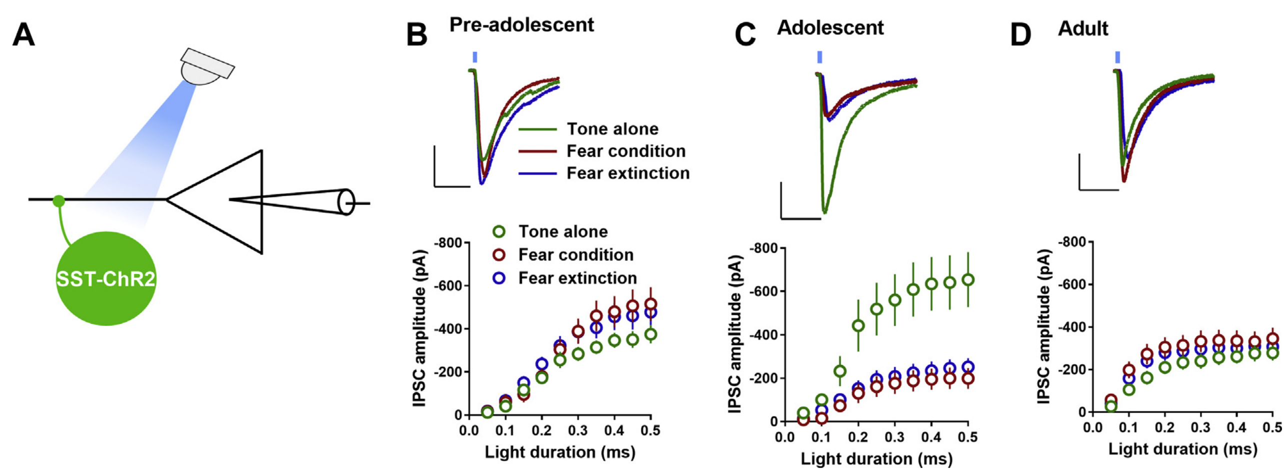


**Figure 8.** Experience-dependent modulation of somatostatin-positive interneuron (SSTIN)–mediated inhibition of infralimbic medial prefrontal cortex (IL-mPFC) layer 5 pyramidal neurons. **(A)** Schematic presentation of light-evoked inhibitory postsynaptic currents (IPSC) recording in the IL-mPFC layer 5 pyramidal neurons of SST-ChR2 mice. **(B)** Mean IPSC amplitude in the IL-mPFC pyramidal neurons of preadolescent tone alone (TA) (13 neurons/5 mice), fear conditioning (FC) (14 neurons/5 mice), and fear extinction (FE) (15 neurons/5 mice) groups ($F_{2,39}$ = 1.14, $p$ = .33; TA vs. FC: $p$ = .57; TA vs. FE: $p$ = .6; FC vs. FE: $p$ = 1). **(C)** Mean IPSC amplitude in the IL-mPFC pyramidal neurons of adolescent TA (18 neurons/6 mice), FC (15 neurons/5 mice), and FE (18 neurons/5 mice) groups ($F_{2,48}$ = 7.8, $p$ = .001; TA vs. FC: $p$ = .003; TA vs. FE: $p$ = .006; FC vs. FE: $p$ = 1). **(D)** Mean IPSC amplitude in the IL-mPFC pyramidal neurons of adult TA (15 neurons/6 mice), FC (15 neurons/5 mice), and FE (18 neurons/5 mice) groups ($F_{2,45}$ = 1.05, $p$ = .36; TA vs. FC: $p$ = .49; TA vs. FE: $p$ = 1; FC vs. FE: $p$ = 1). Comparison of IPSC amplitude in preadolescent, adolescent, and adult TA groups: ($F_{2,43}$ = 4.45; $p$ = .017; preadolescent vs. adolescent: $p$ = .071; preadolescent vs. adult: $p$ = 1; adolescent vs. adult: $p < .031$). Upper panels show example traces. Scale bar = 20 ms/200 pA. ChR2, channelrhodopsin-2.

compared with men, and this sex difference is believed to emerge after midpuberty (50–54). The dynamic changes in GABAergic neuron synapses, described in the current study, along with the known effects of sex hormones on both pre- and postsynaptic plasticity (55–57), warrant future studies to understand sex differences and the role of sex hormones in the developmental regulation of FE.

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## ARTICLE INFORMATION

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