Neuropsychopharmacology www.nature.com/npp

ARTICLE

Downregulation of parvalbumin expression in the prefrontal cortex during adolescence causes enduring prefrontal disinhibition in adulthood

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The expression of the calcium binding protein parvalbumin (PV) has been observed in several cortical regions during development in a temporal pattern consistent with increased afferent-dependent activity. In the prefrontal cortex (PFC), PV expression appears last and continues to substantially increase throughout adolescence, yet the significance of this increase remains unclear. Because of the expression of PV in fast-spiking GABAergic interneurons, we hypothesized that PV upregulation during adolescence is necessary to sustain the increase in GABAergic activity observed in the PFC during this period. To test this hypothesis, we utilized an RNAi strategy to directly downregulate PV levels in the PFC during adolescence and examined its impact on prefrontal GABAergic function, plasticity, and associated behaviors during adulthood. The data indicate that a mere 25% reduction of adult PV levels in the PFC was sufficient to reduce local GABAergic transmission onto pyramidal neurons, disrupt prefrontal excitatory–inhibitory balance, and alter processing of afferent information from the ventral hippocampus. Accordingly, these animals displayed an impairment in the level of extinction learning of a trace fear conditioning response, a behavioral paradigm that requires intact PFC-ventral hippocampus connectivity. These results indicate the PV upregulation observed in the PFC during adolescence is necessary for refinement of prefrontal GABAergic function, the absence of which results in immature afferent processing and a hypofunctional state. Importantly, these results suggest there is a critical window of plasticity during which PV upregulation supports the acquisition of mature GABAergic phenotype necessary to sustain adult PFC functions.

Neuropsychopharmacology (2020) 45:1527–1535; https://doi.org/10.1038/s41386-020-0709-9

INTRODUCTION

The elucidation of mechanisms mediating the transition from childhood to adulthood has received increasing attention under the premise that understanding cortical developmental trajectories will shed light onto disturbances leading to the onset of psychiatric disorders later in life [1]. As the cradle of cognitive functions, the prefrontal cortex (PFC) is one of the cortical regions whose development extends into adolescence [2, 3], a period during which neurotransmitter systems in the PFC undergo intense reconfiguration (reviewed in [4]). The PFC's involvement in developmental psychiatric disorders with cognition and affect dysregulation [5, 6] suggests its protracted maturation also extends its window of susceptibility until young adulthood. Nonetheless, the mechanisms conferring PFC vulnerability during adolescence remain largely undefined.

Parvalbumin (PV) is a member of the EF-hand family of calcium binding proteins with broad expression in the brain and muscle [7, 8]. Long considered a "slow calcium buffer", the presence of PV grants the cells the ability to regulate the decay phase of the calcium transient [9–11], especially upon high-frequency stimulation [12]. In general, PV levels are strongly regulated throughout development [13–21], with mRNA and protein expression displaying an exquisite activity-dependence in both neural and nonneural tissue [22–26]. In the cortex, PV has been typically considered a marker for a subset of local GABAergic interneurons which provide strong feedforward inhibition to pyramidal neurons [27]. In this role, PV-positive interneurons are considered major regulators of the excitatory–inhibitory balance in cortical circuits [28] and ultimately contribute to the organization and synchronization of afferent input [29]. Of relevance, the appearance of PV has been highly correlated with increased afferent drive into sensory cortices [23–25, 30–32]. Similarly, an increase of excitatory synaptic activity onto PV-positive interneurons occurs in parallel with a marked upregulation of PV protein expression in the PFC during adolescence [33].

The full picture of PV function in the nervous system is only beginning to be understood. The available data indicate that PV contributes to neurotransmitter release, broadly through regulation of calcium dynamics at the presynaptic site [11]. If true, the degree of PV expression could have a significant impact in all processes ascribed to PV-positive interneurons. This becomes especially important in the context of psychiatric disorders, particularly in schizophrenia where a reduction in PV expression has been found (see [34]). Of note, one report shows an approximate 20% reduction in PV mRNA expression without a loss in the number of PV-positive interneurons in the PFC of patients with schizophrenia [35]. Collectively, these results suggest that there is a threshold level of PV expression required to sustain adult PV-interneuron function below which deficits in cortical

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Received: 21 February 2020 Revised: 4 May 2020 Accepted: 8 May 2020
Published online: 13 May 2020

processing may occur. Here, we directly tested the hypothesis that peri-adolescent upregulation of PV in the PFC is necessary to sustain local inhibitory transmission and support normal prefrontal functions in adults.

MATERIALS AND METHODS

All experimental procedures were conducted according to the Guide for the Care and Use of Laboratory Animals, and approved by the UIC Animal Care Committee. Male Sprague Dawley rats (Envigo, IN) were group-housed (3 rats/cage), maintained at a constant temperature (21–23 °C), humidity, and light–dark cycle, and allowed to acclimate to the facility for at least 1 week before survival surgeries. Food and water were available *ad libitum*. See Supplemental Information for detailed methods.

Delivery of shRNA into the PFC

Commercially available rat PV- and scrambled-shRNA vectors (HuSH, Origene, Rockville, MD) were propagated using High Purity Plasmid Midiprep columns (Origene, Rockville, MD), and resuspended in DNAse, RNAse- free water (Sigma, St. Louis, MO). On the day of the surgery, plasmids were complexed with In Vivo Jet-PEI (Polyplus, France) and injected bilaterally into the prelimbic area (0.6 µL/side) for cohorts of rats subjected to electrophysiological and behavioral studies.

Assessment of PV downregulation

A separate cohort of rats injected unilaterally with shRNA at P34–38 were perfused after P65 with cold saline followed by 4% PFA in 0.1M PB. Brains were post-fixed for 24 h and kept in 30% sucrose/PB. At least six coronal sections (50 µm) anterior and six posterior to the injection site were utilized for PV immunohistochemistry as described before [33]. Images were acquired with a Nikon Eclipse Ni-E microscope (Nikon Instruments Inc., Melville, NY) using a 10× objective and analyzed using Image J.

In vivo recordings of local field potential (LFP) in the PFC

Changes in the pattern of LFP at 10, 20, and 40 Hz were determined as previously described [36, 37]. In another cohort of animals, a third bipolar concentric electrode was placed in the basolateral amygdala (BLA) to determine the effect of high-frequency stimulation (HFS)-induced facilitation of LFP in the PFC. After a period of stable baseline recording, a protocol of HFS consisting of four trains of 50 pulses/each at 100 Hz was delivered into the BLA as previously described [38]. Changes in the slope of the evoked LFP were measured after HFS. At minute 45, a second set of HFS was delivered into the ventral hippocampus and changes in amygdalar-evoked LFP were recorded for an additional 45 min. Each data point was computed by averaging the slope value of eight evoked LFPs from a 2 min window.

Ex vivo recordings of inhibitory synaptic currents in the PFC

All procedures were conducted as previously described [39, 40]. For each neuron, the mean inhibitory postsynaptic currents (IPSC) frequency was compared. In another set of neurons, locally-evoked IPSC was elicited by a teflon-coated bipolar electrode placed ~200 µm from the cell body along the apical dendrite. The stimulation intensity was titrated to elicit a monosynaptic IPSC response with a failure rate of ~50% using a paired-pulse protocol to reveal changes in the probability of neurotransmitter release.

Concurrent recordings of excitatory and inhibitory synaptic currents in the PFC

All recordings were conducted using an aCSF free of glutamate and GABA blockers, and a low-chloride-based internal solution to enable concurrent acquisition of excitatory and inhibitory synaptic currents at a single-cell level as previously described [40].

Ex vivo recordings of fast-spiking interneurons in the PFC

All recordings were obtained from layer V using a potassium-based internal solution, and changes spontaneous excitatory postsynaptic current events were compared as previously described [33]. Only cells that remained stable for at least 20 min after obtaining the whole-cell configuration were included.

Trace fear conditioning and extinction

We adapted a fear conditioning protocol modified from Zhang & Rosenkranz [41]. The trace fear conditioning phase consisted of a 120 s habituation period followed by five pairings of a neutral tone paired with a foot shock (0.4 mA) a delay of 20 s from the end of the tone. Conditioning trials were presented using a pseudo-random inter-trial interval of 240–280 s. After conditioning, rats were returned to their home cage for 24 h. Extinction trials began the next day in a visually and tactilely distinct chamber. Following a 120 s habituation period, the conditioned tone (20 s) was presented 14 times (60 s per trial) without foot shock to enable extinction of the fear memory. The acquisition of fear extinction is typically revealed by the level of conditioned freezing to the tone that diminishes over repeated trials. An infrared camera connected to the ANY-maze behavioral analysis software (Stoelting Co., IL) was used to record and quantify the time spent freezing per trial.

RESULTS

PV downregulation diminishes GABAergic transmission

Unlike previous genetic models where PV is completely ablated in the brain [42], we sought to retain peri-adolescent levels of the protein to assess whether the upregulation of PV occurring in the PFC during adolescence was necessary for normal adult functions. In order to prevent the upregulation of PV expression observed in adolescence, we injected an shRNA against PV into the medial PFC during postnatal days (P) 34–38 (Fig. 1a). All analyses of PV expression were compared with the scrambled (Scr)-shRNA control at ~P65 (Fig. 1a). Using this approach, we were able to achieve ~25% reduction in PV expression in the injected site compared with the contralateral side, as measured by fluorescence immunohistochemistry (Fig. 1b). The effect was noticeable in the neuropil, due to a reduction of PV-positive innervation, and in individual cells (Fig. 1c). PV knock-down also changed the appearance of "baskets", the PV-positive structures circumscribing the cell body of pyramidal neurons. While still present, these profiles displayed a marked reduction in the intensity of surrounding terminals exemplified by the heat plots (Fig. 1c).

Concomitant with the upregulation of PV expression observed from juveniles to adults [33], there is an increase in GABAergic transmission in the PFC as revealed by the number of spontaneous inhibitory postsynaptic current (IPSC) onto layer V pyramidal neurons [39]. Thus, we determined if the loss of PV achieved with the shRNA strategy had an effect on GABAergic transmission impinging upon pyramidal neurons. Whole-cell patch-clamp recordings obtained from PFC brain slices revealed that a reduction of PV expression below adult levels is sufficient to decrease the frequency of IPSC onto layer V pyramidal neurons (Fig. 1d) without altering the mean IPSC amplitude (Scr-shRNA: 15.9 ± 1.2 pA; PV-shRNA: 15.4 ± 0.8 pA). Moreover, the mean IPSC frequency obtained in the adult PFC of PV-shRNA-treated rats closely resembled that of peri-adolescent animals [39]. Data obtained from locally-evoked IPSC using a protocol of paired-pulse stimulation to elicit monosynaptic responses further revealed a presynaptic mechanism of GABAergic disruption (Fig. 1e). While pyramidal neurons recorded from Scr controls exhibited a typical paired-pulse suppression ($IPSC_2/IPSC_1 < 1.0$), a paired-pulse facilitation ($IPSC_2/IPSC_1 > 1.0$) emerged following PV downregulation (Fig. 1e). This increase in IPSC paired-pulse ratio indicates that the probability of GABA release is decreased.

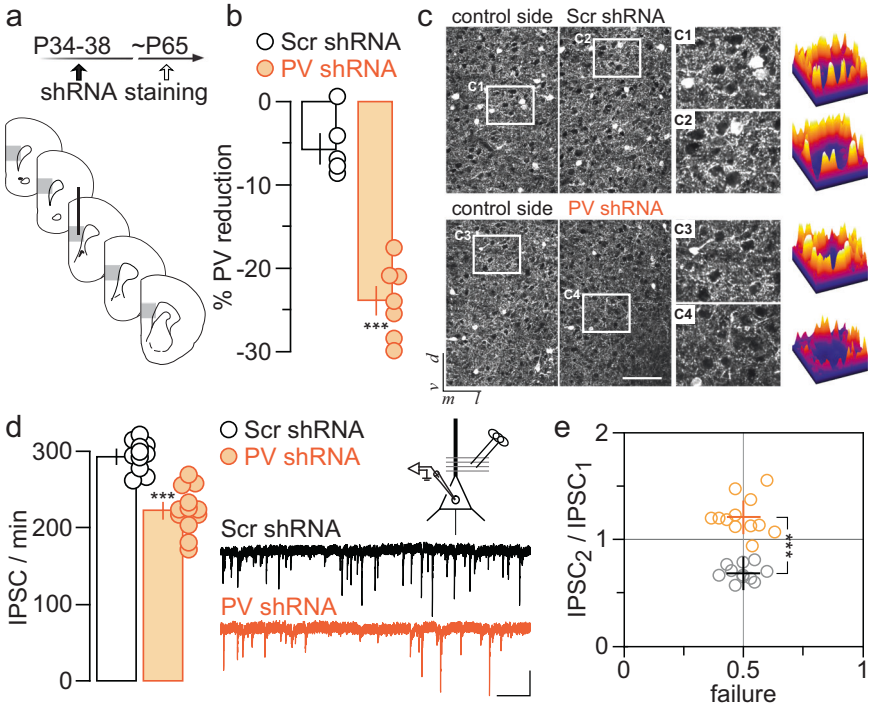

Fig. 1 PV downregulation modifies baskets and reduces GABAergic transmission in the PFC. a Diagram of experimental design for quantification of PV downregulation after unilateral, intra-PFC delivery of scrambled (Scr; $n = 5$) or PV shRNA ($n = 7$) during postnatal days (P) 34–38. Anti-PV staining was performed when rats reached ~P65 in 50 µm-thick sections 300 µm anterior and posterior to the injection site. **b** Relative to the uninjected side, a ~25% reduction of PV immunoreactivity can be measured in the PFC 30 days after PV shRNA injection (***$p < 0.0005$, unpaired *t*-test). **c** Representative images of Scr and PV shRNA-treated PFC displaying a pronounced reduction of PV expression in the neuropil and deep layer "baskets" (scale bar: 100 µm). Magnified insets (C1–C4) showing the degree of PV downregulation in deep layer baskets. *Right panels:* intensity heat plots of individually magnified baskets. **d** Bar-graph summarizing the effect of PV shRNA treatment on spontaneous inhibitory postsynaptic current (IPSC) recorded in the PFC at P65–85. Whole-cell patch-clamp recordings from layer V pyramidal neurons revealed a marked reduction of IPSC frequency (events/min) in the PV shRNA group (12 cells, 6 rats) compared with Scr controls (11 cells, 6 rats; ***$p < 0.0001$, unpaired *t*-test). Inset are examples traces of spontaneous IPSC recorded from layer V pyramidal neurons illustrating the effect of PV shRNA in the PFC (calibration: 15pA, 1s). **e** Summary of the data obtained from layer V pyramidal neurons using a paired-pulse protocol of minimal stimulation at 50 ms interval. Note that the intensity of stimulation was titrated to elicit monosynaptic IPSC responses at ~50% failure rate in both groups to enable the detection of any changes in the probability of GABA release. While pyramidal neurons recorded from Scr controls (10 cells, 5 rats) exhibited $IPSC_2/IPSC_1$ ratios <1.0, all neurons recorded from the PV-shRNA group (13 cells, 7 rats) showed $IPSC_2/IPSC_1$ ratios >1.0 (***$p < 0.001$ vs. Scr shRNA, unpaired *t*-test), indicating that the probability of GABA release is decreased following PV downregulation.

Altogether, these data indicate a presynaptic mechanism underlies the observed GABAergic deficit and demonstrate that the developmental upregulation of PV during adolescence is necessary to sustain normal levels of GABAergic transmission in the adult PFC.

PV downregulation disrupts the excitatory–inhibitory balance in the PFC

The negative impact of PV downregulation on GABAergic transmission is likely to alter the balance of excitatory–inhibitory (E–I) activity in the PFC only if local glutamatergic transmission remains unaltered. To test this hypothesis, we conducted whole-cell patch-clamp recordings from layer V pyramidal neurons using a protocol that enables the acquisition of GABAergic and glutamatergic synaptic activity within a single cell (Fig. 2a; [40]). The results showed both Scr- and PV-shRNA-treated PFC display nearly identical levels of postsynaptic current events recorded at −60 mV ($PSC_{-60\ mV}$; Fig. 2b), indicating PV-shRNA treatment does not affect the activity of glutamatergic synapses. In contrast, PV downregulation selectively diminished the GABAergic component of synaptic activity, as revealed by a marked reduction in $PSC_{+15\ mV}$ frequency (Fig. 2b). Consequently, a higher E/I ratio emerged in the PFC of PV-shRNA-treated animals (Fig. 2c). Notably, such a deficit was already detectable by P45, a time when adult levels of GABAergic activity are already attained (Fig. 2b). Further analyses revealed the increase in E/I ratio is correlated with the frequency of IPSC (Fig. 2d), indicating that the E–I imbalance induced results from a preferential disruption of PFC GABAergic transmission. Collectively, these results demonstrate that a modest down-regulation of PV expression can impact the E/I ratio of PFC output neurons, which in turn could have a detrimental effect in signal processing and integration.

PV downregulation prevents the normal facilitation of glutamatergic transmission onto FSI

The E–I imbalance observed in the PFC following adolescent PV downregulation could arise from a developmental deficit in the recruitment of fast-spiking interneurons (FSI) by excitatory inputs during adolescence [33]. To test this hypothesis, we conducted electrophysiological recordings to determine whether the characteristic facilitation of glutamatergic transmission onto FSI observed during adolescence [33] is disrupted following PV downregulation. FSI were identified by a non-adapting firing response to somatic depolarization and a prominent after-hyperpolarization potential as previously described [33]. Relative to Scr-shRNA controls, FSI recorded from the PV-shRNA-treated group showed a lower frequency of excitatory postsynaptic currents (EPSC) (Fig. 2f–h) without any detectable changes in the mean EPSC amplitude (Scr-shRNA: 15.8 ± 1.2 pA; PV-shRNA: 15.4 ± 0.8 pA). Such a deficit in EPSC transmission was detectable

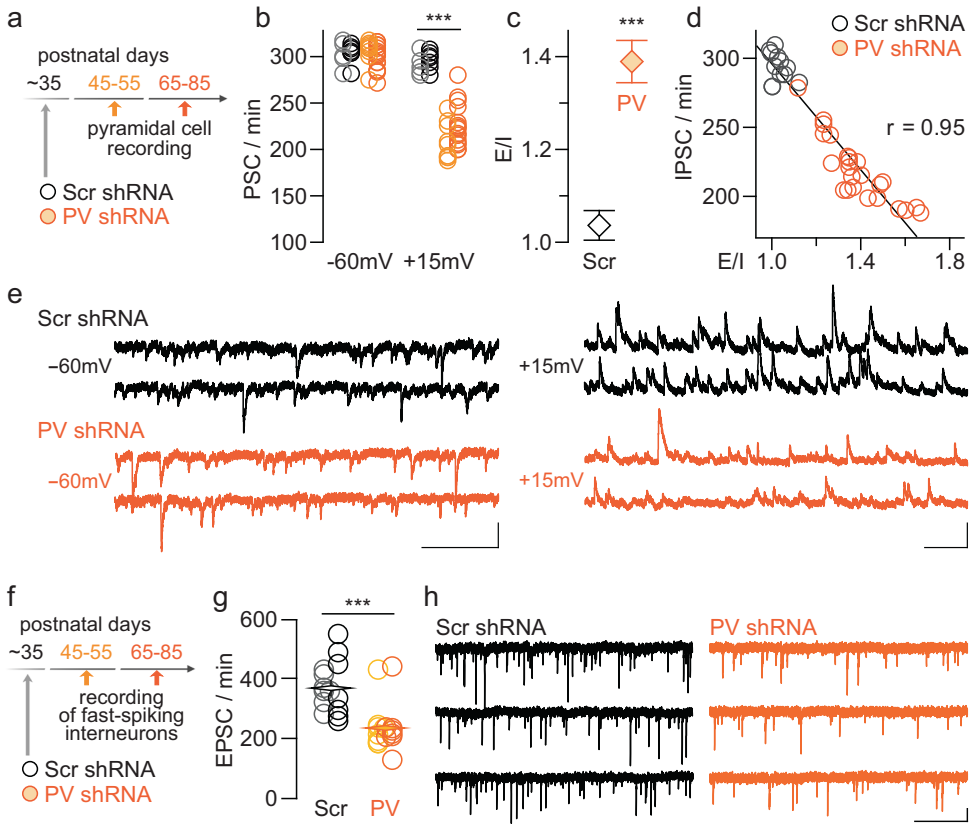

Fig. 2 Downregulation of PV increases the ratio of excitatory–inhibitory synaptic activity onto layer V pyramidal neurons and reduces the frequency of excitatory transmission onto fast-spiking interneurons. a Changes in excitatory (E)–inhibitory (I) balance of synaptic activity following intra-PFC delivery of Scr or PV shRNA at P35 were determined at P45–55 and P65–85. Data from the P45–55 group were collected to assess whether the functional impact of PV shRNA is already detectable at 10–15 days post-delivery**. b** All recordings were obtained from layer V pyramidal neurons in the PFC using a low-chloride-based internal solution that enables concurrent acquisition of inhibitory ($PSC_{+15\ mV}$) and excitatory ($PSC_{-60\ mV}$) postsynaptic currents (see Materials & Methods). Relative to Scr controls (P45–55: gray circles, 4 rats; P65–85: black circles, 5 rats), PV shRNA delivery did not alter the frequency of $PSC_{-60\ mV}$ events (PSC/min) at P45–55 (yellow circles, 4 rats) or P65–85 (orange circles, 5 rats). In contrast, intra-PFC delivery of PV shRNA diminished the frequency of $PSC_{+15\ mV}$ in layer V pyramidal neurons, an effect that can be detected at P45 (***$p < 0.0005$, unpaired *t*-test). **c** Calculation of the E/I ratio for each individual neuron recorded from P45–55 and P65–85. Data from both age groups were pooled. Relative to Scr controls, treatment with PV shRNA markedly increased the E/I ratio in layer V pyramidal neurons by more than 30% (***$p < 0.0005$, unpaired *t*-test). **d** Further analysis revealed a significant correlation ($p < 0.001$) between the E/I ratio and the frequency of $PSC_{+15\ mV}$ (i.e., IPSC) events. **e** Example traces of $PSC_{+15\ mV}$ and $PSC_{-60\ mV}$ recorded from layer V pyramidal neurons illustrating the effect of intra-PFC delivery of PV shRNA shown in b (calibration: 25 pA/0.5 s for $PSC_{-60\ mV}$ and 40 pA/0.5 s for $PSC_{+15\ mV}$). **f** Impact of intra-PFC delivery of PV shRNA (~P35) on fast-spiking interneurons' (FSI) excitatory postsynaptic (EPSC) events recorded at P45–55 and P65–85. **g** Relative to the Scr shRNA control group (P45–55: 4 rats; P65–85: 5 rats), a marked reduction in EPSC frequency was observed in FSI following adolescent PV downregulation (P45–55: 4 rats; P65–85: 5 rats; ***$p < 0.0001$, unpaired *t*-test). **h** Example traces of spontaneous EPSC recorded from FSI illustrating the impact of adolescent delivery of PV shRNA into the PFC shown in (**f**) (calibration: 10 pA, 500 ms).

by P45 (Fig. 2g, h), a time when adult levels of glutamatergic activity onto FSI are already attained [33]. Notably, the level of EPSC frequency observed following adolescent PV downregulation resembles that of naïve juvenile animals [33]. Of note, PV downregulation did not alter the excitability of FSI (resting membrane potential, mV: 65.6 ± 0.7 vs. 66.7 ± 1.0; input resistance, mOhm: 323.9 ± 26.2 vs. 323.2 ± 25.5; after-hyperpolarization, mV: 19.9 ± 1.2 vs. 19.7 ± 1.5; Scr-shRNA vs. PV-shRNA, respectively). Together, these results indicate that adult levels of PV expression are necessary for sustaining normal levels excitatory transmission onto FSI.

Disruption of hippocampal-PFC transmission following PV downregulation

To assess whether prefrontal processing of afferent signal is disrupted by local downregulation of PV expression, we conducted local field potential (LFP) recordings and examined the pattern of PFC responses to ventral hippocampal train stimulation at 10, 20, and 40 Hz. The ventral hippocampus is one of the main regions projecting to the PFC and its activation can reveal distinct developmental stages of prefrontal GABAergic function in vivo [37, 39, 43]. Using this approach, we found the normal pattern of LFP response at 10 Hz remained unaltered in the PFC of PV-shRNA-treated rats (Fig. 3a, e, f). At 20 Hz, Scr-shRNA animals showed the typical transient LFP suppression observed in the PFC of normal adults [36, 37], whereas the PV-shRNA-treated group displayed a pattern of LFP facilitation (Fig. 3b, e, f). Similarly, the level of LFP suppression upon hippocampal stimulation at 40 Hz was smaller in the PFC of PV-shRNA-treated rats relative to Scr-shRNA controls (Fig. 3c, e, f). Of note, the patterns of LFP facilitation (at 20 Hz) and attenuated suppression (at 40 Hz) observed following PV downregulation were indistinguishable from the responses obtained in juveniles (Fig. 3e; see also [37]) and those recorded in adults following PFC blockade of GABA-AR transmission [36, 37]. These results indicate that an optimal level of PV expression is needed to enable PFC GABAergic control of high-frequency afferent signal from the ventral hippocampus. Thus, the ability of PV-positive

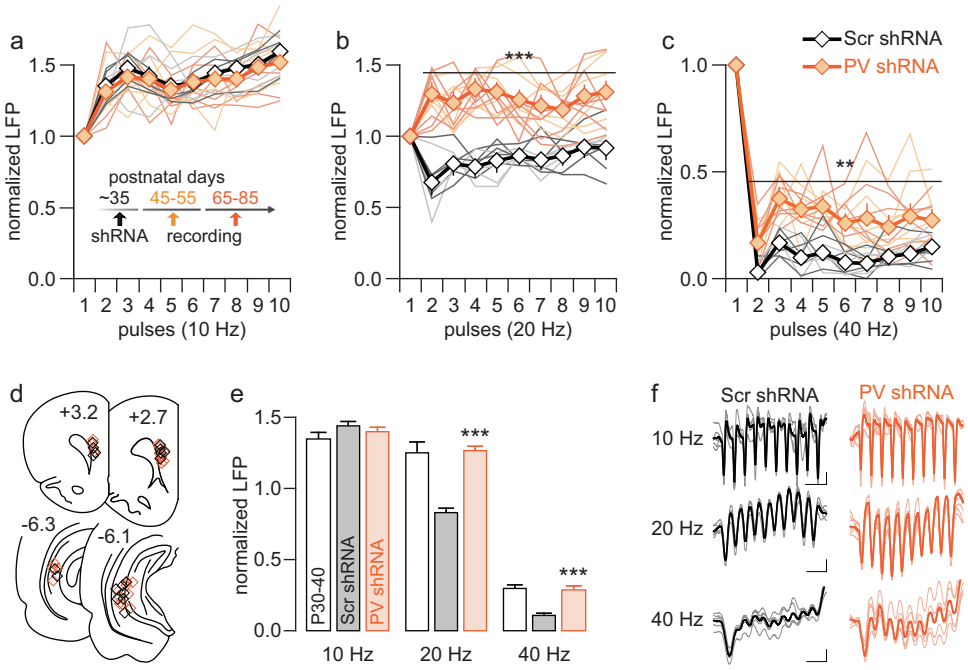

Fig. 3 Decreased levels of PV in the prefrontal cortex confer juvenile-like pattern of hippocampal-evoked response. a The pattern of ventral hippocampal-evoked (10 Hz) facilitation of local field potential (LFP) responses in the PFC was indistinguishable between Scr ($n = 9$) and PV shRNA ($n = 13$) groups. **b** Following hippocampal stimulation at 20 Hz, a normal transient suppression of LFP response was recorded in the PFC of Scr controls (P45–55: gray lines, 4 rats; P65–85: black lines, 5 rats) whereas a pattern of prefrontal LFP facilitation emerged in PV shRNA-treated animals (P45–55: yellow lines, 6 rats; P65–85: orange lines, 7 rats; treatment x pulse interaction, $F_{9200} = 6.1$, $^{***}p < 0.0001$; main treatment effect, $F_{1200} = 349.9$, $p < 0.0001$, two-way ANOVA). Note the abnormal facilitation of LFP response in the PFC of PV shRNA-treated animals is already apparent at P45 (yellow lines). **c** At 40 Hz, both Scr and PV shRNA groups exhibited similar patterns of LFP suppression in the PFC. However, the magnitude of prefrontal LFP suppression was markedly reduced following PV shRNA delivery (treatment × pulse interaction, $F_{9200} = 2.6$, $^{**}p < 0.005$; main treatment effect, $F_{1200} = 147.1$, $p < 0.0001$, two-way ANOVA). Such a disruption was also apparent at P45 (yellow lines). **d** Diagram of PFC (top) and hippocampal (bottom) coronal sections showing the placement for all recording and stimulating electrodes, respectively (black: Scr shRNA group; orange: PV shRNA group). **e** Bar-graph summarizing the impact of PV shRNA on prefrontal LFP responses presented in panels (**a–c**). Data from a cohort of naïve P30–40 rats ($n = 7$) were included for comparison. Note that the levels of LFP facilitation (at 20 Hz) and LFP suppression (at 40 Hz) recorded in the PFC of PV shRNA-treated group are equivalent to those observed in P30–40 animals ($^{***}p < 0.0005$ vs. P30–40 or PV shRNA, Tukey post-hoc test after significant one-way ANOVA; $F_{226} = 40.3$, $p < 0.0001$ for 20 Hz and $F_{226} = 37.1$, $p < 0.0001$ for 40 Hz). **f** Example traces of prefrontal LFP response to hippocampal train stimulation at 10, 20, and 40 Hz utilized for the analysis in panels (**a–c**) (calibration: 3 mV/200 ms at 10 Hz; 3 mV/100 ms at 20 Hz; 6 mV/50 ms at 40 Hz).

interneurons to handle high-frequency afferent drive relies in part on the expression of PV itself.

PV downregulation prevents the normal modulation of basolateral amygdala inputs by the ventral hippocampus in the PFC

The enduring E–I imbalance resulting from PV downregulation in the PFC could also disrupt the developmentally-regulated modulation of basolateral amygdala (BLA) inputs by the ventral hippocampus [38]. To test this hypothesis, we employed a protocol of high-frequency stimulation (HFS; Fig. 4) to induce potentiation of prefrontal LFP elicited from the BLA. Following potentiation of the BLA-evoked response, a second set of HFS was delivered into the ventral hippocampus to determine whether the degree of prefrontal depotentiation is disrupted by the PV shRNA treatment. We found the pattern of LFP potentiation resulting from HFS of the BLA remained unaltered following PV down-regulation in the PFC (Fig. 4a, b). This is consistent with previous data showing that plasticity of the BLA-to-PFC pathway does not rely on prefrontal GABAergic transmission [43]. However, the characteristic depotentiation of BLA-evoked LFP upon sequential HFS of the ventral hippocampus was only observed in the PFC of Scr-shRNA controls (Fig. 4b). In PV-shRNA animals, hippocampal HFS failed to disrupt the potentiated BLA-evoked LFP in the PFC (Fig. 4b, c), a pattern of response identical to that observed in naïve juveniles [38]. Together, these results support the premise that an optimal level of PV expression in the PFC is needed to enable the inhibitory control of BLA inputs by the ventral hippocampus.

PV downregulation impairs the level of extinction of a trace-fear memory

PFC processing of ventral hippocampal and BLA inputs is critical for the expression of cognitive functions [44, 45] including the acquisition and extinction of conditioned fear memories [46, 47]. In order to test whether PV downregulation in the PFC during adolescence impacts behavioral outcomes in adulthood, we utilized a trace-fear conditioning paradigm in which the cue (tone) and shock are separated by a 20 s-delay. This delay engages the hippocampal-PFC pathway and provides a behavioral readout of functional connectivity between the PFC, ventral hippocampus, and BLA [46].

Compared with Scr controls, bilateral downregulation of PV in the PFC does not disrupt the acquisition of cue-mediated fear response tested in adulthood (Fig. 5a). In fact, the behavioral response obtained from Scr- and PV-shRNA-treated groups were indistinguishable from each other, achieving more than 80% of freezing after 5 tone-shock presentations (Fig. 5a). Similarly, both groups exhibited similar levels of baseline freezing during habituation prior to extinction on day 2 (Scr-shRNA: 11.5 ± 1.8% vs. PV-shRNA: 11.4 ± 1.4%). During testing for the extinction of the fear memory, the Scr-shRNA group showed the typical gradual reduction of the freezing response to the tone over repeated trials (Fig. 5b). In contrast, the PV-shRNA group displayed increased

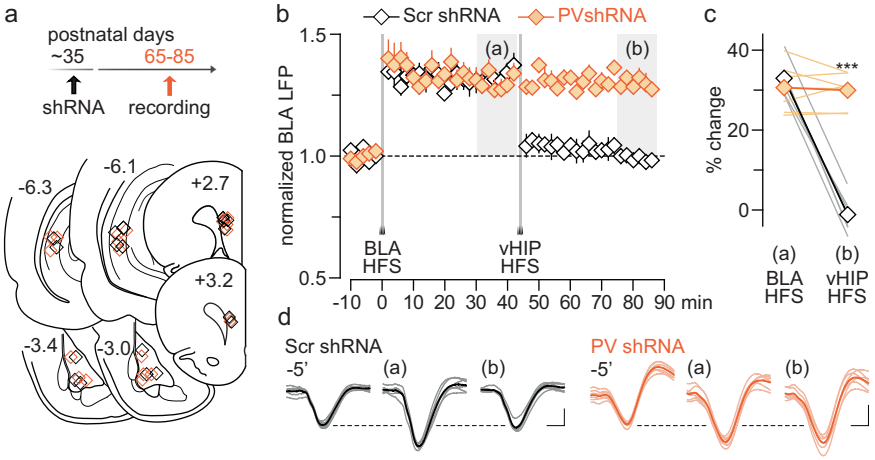

Fig. 4 PV downregulation impairs the inhibitory control of amygdalar inputs to the prefrontal cortex by the ventral hippocampus. **a** Timeline of the experimental design and diagrams of brain coronal sections showing the location for all PFC recording sites and placement of ventral hippocampal (vHIP) and basolateral amygdalar (BLA) stimulating electrodes used to collect the data shown in (**b**) (black: Scr shRNA group; orange: PV shRNA group). **b** PFC downregulation of PV did not alter the typical potentiation of BLA-evoked LFP induced by a protocol of high-frequency stimulation in the BLA (HFS; see Materials & Methods). However, only the Scr shRNA group showed the normal response to vHIP HFS applied 40 min post-BLA potentiation. While HFS of the vHIP resets the LFP facilitation driven by the BLA in Scr controls ($n = 6$ rats), the suppression of BLA-evoked LFP response is no longer observed in PV shRNA-treated animals ($n = 7$ rats). **c** Summary of the mean LFP response obtained from the last 10 min post-HFS of the BLA (**a**) and vHIP (**b**) shown in (**b**) (area marked in gray). Two-way ANOVA revealed main effects of input stimulation ($F_{122} = 43.6$, $p < 0.0001$), PFC shRNA treatment ($F_{122} = 29.7$, $p < 0.0005$), and input × treatment interaction ($F_{122} = 39.9$, $p < 0.0001$; ***$p < 0.0005$ vs. Scr shRNA, Tukey post-hoc test). **d** Traces of BLA-evoked LFP responses recorded from the PFC illustrating the impact of PV shRNA shown in (**b**) (−5′: baseline; (**a**): post-BLA HFS; (**b**) post-vHIP HFS; calibration: 5 mV, 20 ms).

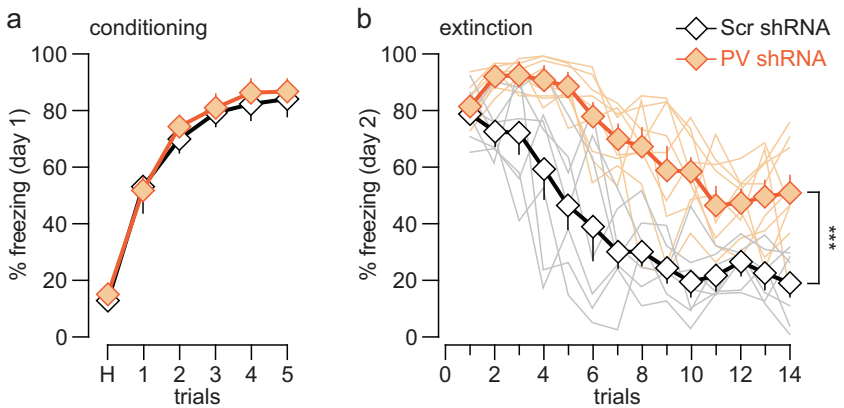

Fig. 5 Downregulation of PV in the prefrontal cortex increases the level of freezing response during extinction testing. **a** A "trace" fear conditioning paradigm in which the cue (tone) and the shock (1 s, 0.4 mA) are separated by 20 s was utilized to measure intact hippocampal-PFC connectivity. Following habituation (H) on day 1, both Scr-shRNA ($n = 7$ rats) and PV-shRNA ($n = 9$ rats) groups were conditioned to the tone as evidenced by increasing levels of freezing over five trials, with no differences in the rate of acquisition of the fear response. **b** Twenty-four hours later (day 2), extinction of the cue-induced freezing response was measured in both experimental groups. Relative to Scr controls, PV shRNA-treated rats exhibited a slower extinction rate (main effect of treatment, $F_{1196} = 192.2$, ***$p < 0.0005$; treatment × trial interaction, $F_{13196} = 2.0$, $p = 0.024$, two-way ANOVA).

freezing times relative to Scr controls, a difference which persisted until the last trial of cue presentation (Fig. 5b). Post-hoc analyses also revealed a difference in the level of freezing between the first and last trials of extinction for both Scr- and PV-shRNA-treated rats (Scr-shRNA: $p < 0.00005$, PV-shRNA: $p < 0.01$, Tukey post-hoc test). Altogether, these results indicate that an optimal level of PV expression in the PFC is critical for regulating the level of extinction learning.

DISCUSSION

While genetic ablation of PV-positive interneuron activity has provided proof of concept data on the importance of prefrontal PV interneurons in cognitive functions [48–53], it has yet to provide plausible biological mechanisms to explain the reduction of PV-positive interneuron activity commonly described in psychiatric disorders. Using an shRNA approach at the time PV expression is about to rise, we were able to detect the functional impact of PV-shRNA as early as 10 days post-delivery. The results presented herein show that a mere downregulation of PV to adolescent levels is sufficient to decrease GABAergic transmission and disrupt the E–I balance in the PFC, ultimately reducing the inhibitory control of afferent integration to levels indistinguishable from those in juvenile animals. The defect in afferent information processing is further manifested as an impairment in the level of extinction learning. Altogether, these findings provide a novel, biologically-relevant mechanism by which GABAergic transmission in the PFC can be regulated during adolescent development.

The upregulation of PV in multiple brain regions at specific time points [14, 17, 54, 55] is seemingly part of a developmental

program triggered by increased synaptic activity from specific inputs. Accordingly, PV expression in sensory cortices is dramatically reduced after blocking afferent drive [23–25, 30–32]. It remains to be defined whether a single or concerted glutamatergic activity from PFC afferent structures (e.g., ventral hippocampus, BLA, or mediodorsal thalamus) is responsible for eliciting the genetic/epigenetic program that results in PV upregulation within the developmental window studied here. Previous work in organotypic cultures suggests such a program may be established early on and may vary for each cortical layer [26, 56]. Interestingly, genetic ablation of the global transcriptional activator PGC-1α results in a striking loss of cortical PV among other proteins specifically associated with GABAergic interneurons [57, 58]. Nonetheless, our study indicates that preventing the developmental increase of PV alone is sufficient to halt the gain of prefrontal GABAergic transmission attained during adolescence, suggesting that even small changes in PV concentration in the PFC can affect local inhibitory control and disrupt its optimal computational capacity [59].

The continued increase in PV expression observed in the PFC during adolescence serves an integral role in GABA neurotransmission in adulthood possibly by allowing PV-positive interneurons tighter temporal control over inputs and/or by conferring increased calcium buffering capacity to sustain GABA release [11]. Irrespective of the synaptic mechanisms by which PV regulates GABAergic transmission, loss of PV results in a net increase in the E/I ratio, likely through the inability of GABAergic interneurons to sustain a high firing rate during periods of high cognitive demand. Such deficiencies would not be apparent until the protracted consolidation of long and short-term inputs occurring in the PFC during adolescence [36, 37, 59]. In support of this idea, previous work has demonstrated that a complete absence of PV preferentially alters synaptic activity of narrowly timed events [42, 60, 61], indicating PV function only becomes manifest upon certain type of inputs defined by their proximity and/or frequency. Our results expand this concept to demonstrate that only mature levels of cortical PV can exert proper inhibitory control of afferent drive received at high frequencies (i.e., >20 Hz). It remains to be understood why PV downregulation selectively affects PFC integration of hippocampal inputs, despite the amygdala also targets PV interneurons in the PFC [62, 63]. The input-specific impact of PV downregulation suggests that there are concurrent, perhaps conditional mechanisms in the PFC and hippocampus that are required for normal development and function of both structures. Supporting this idea, the ventral hippocampus also undergoes a protracted maturation in its PFC-projection domain during adolescence [64, 65]. Thus, the amount of PV within an interneuron needs to match the strength of input-specific glutamatergic transmission in order to enable an optimal synaptic response with sufficient temporal control of afferent drive.

Not surprisingly, the results presented herein diverge from the ones obtained in *full* PV knock-out mice [60, 61, 66]. Complete absence of PV has been reported to enhance the excitability of FSI [66] and facilitate GABAergic transmission as revealed by an increased in paired-pulse ratio [60]. Of note, these studies did not employ protocols of minimal stimulation to reveal presynaptic changes in transmitter release [67], leaving open the possibility that the increased paired-pulse ratio observed in *full* PV knock-out mice could arise from compensatory processes from the absence of PV since embryonic stages, thus rendering FSI hyperexcitable [66]. In contrast, partial PV downregulation during adolescence in the PFC did not change FSI excitability, but it markedly reduced the frequency of IPSC in conjunction with an increased paired-pulse ratio of evoked IPSC at minimal stimulation, all indicative of a presynaptic deficit in GABA release onto pyramidal neurons.

We propose the full range of PV function is the sum of a concentration and time-dependent increase of PV at different cellular compartments: 1) in dendrites where PV-positive interneurons receive most of their synaptic inputs, and 2) in synaptic terminals, where PV is positioned to regulate neurotransmitter release. The observation that the increase in PV occurs primarily at terminals at critical developmental points (documented as a prevalence of "basket" structures [18, 33]), suggests that the role of PV in regulating GABA release becomes pronounced only after PV concentration reaches a "critical mass" during development.

Although the retrieval of extinction memory was not tested here, our data indicate that small changes in PV expression in the PFC are sufficient to cause an impairment in the level of extinction learning. Similar alterations in neural circuits regulating extinction behavior have been documented in schizophrenia [68], and recapitulated in animal models of the disease [69]. Interestingly, a small but consistent reduction in prefrontal PV expression (~20%) has been observed in schizophrenia [35, 70], suggesting that reduced PV levels may explain some, but certainly not all the pathophysiology of the disease in which more than one brain structure is compromised. Whether the reduction in PV observed in schizophrenia is the result of increased oxidative stress [71, 72], a lack of developmental upregulation [1], or a combination of both, a mismatch between prefrontal PV expression and afferent drive is enough to render the PFC hypofunctional. Future studies are warranted to determine the extent of behavioral changes resulting from such a discrete disruption of PV expression in the PFC during adolescence.

CONCLUSIONS

The experimental observations presented above together with the computational model proposed by Eggermann and Jonas [73], indicate that the ultimate role of PV can only be examined and understood under a developmental "lens" as its function is highly dependent on the absolute amount present at any given developmental window, which itself is a function of afferent activity. In light of the results presented here, a re-evaluation of the role of PV based on the developmental status of each region studied is warranted. Our results provide unequivocal demonstration that the protracted expression of PV in the PFC during adolescence is a biologically relevant process that must take place to attain mature levels of inhibitory transmission and support adult PFC-dependent behaviors.

FUNDING AND DISCLOSURE

This work was supported by grants R01-MH086507 and R01-MH105488 to KYT. The authors have no financial disclosures or conflict of interest to disclose.

ACKNOWLEDGEMENTS

We thank Ms. Yuliya Zayats for assistance with tissue processing. This project was initiated at Rosalind Franklin University—Chicago Medical School.

AUTHOR CONTRIBUTIONS

AC and KYT designed the study, wrote the paper, and prepared the figures. AC, EFB, and DRT performed the experiments and analyzed the data under supervision of KYT.

ADDITIONAL INFORMATION

Supplementary Information accompanies this paper at (https://doi.org/10.1038/s41386-020-0709-9).

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