Distinct Physiological Maturation of Parvalbumin-Positive Neuron Subtypes in Mouse Prefrontal Cortex

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Parvalbumin-positive (PV⁺) neurons control the timing of pyramidal cell output in cortical neuron networks. In the prefrontal cortex (PFC), PV⁺ neuron activity is involved in cognitive function, suggesting that PV⁺ neuron maturation is critical for cognitive development. The two major PV⁺ neuron subtypes found in the PFC, chandelier cells (ChCs) and basket cells (BCs), are thought to play different roles in cortical circuits, but the trajectories of their physiological maturation have not been compared. Using two separate mouse lines, we found that in the mature PFC, both ChCs and BCs are abundant in superficial layer 2, but only BCs are present in deeper laminar locations. This distinctive laminar distribution was observed by postnatal day 12 (P12), when we first identified ChCs by the presence of axon cartridges. Electrophysiology analysis of excitatory synapse development, starting at P12, showed that excitatory drive remains low throughout development in ChCs, but increases rapidly before puberty in BCs, with an earlier time course in deeper-layer BCs. Consistent with a role of excitatory synaptic drive in the maturation of PV⁺ neuron firing properties, the fast-spiking phenotype showed different maturation trajectories between ChCs and BCs, and between superficial versus deep-layer BCs. ChC and BC maturation was nearly completed, via different trajectories, before the onset of puberty. These findings suggest that ChC and BC maturation may contribute differentially to the emergence of cognitive function, primarily during prepubertal development.

Key words: action potential; basket neuron; chandelier neuron; parvalbumin; prefrontal cortex; synaptic current

Significance Statement

Parvalbumin-positive (PV⁺) neurons tightly control pyramidal cell output. Thus PV⁺ neuron maturation in the prefrontal cortex (PFC) is crucial for cognitive development. However, the relative physiological maturation of the two major subtypes of PV⁺ neurons, chandelier cells (ChCs) and basket cells (BCs), has not been determined. We assessed the maturation of ChCs and BCs in different layers of the mouse PFC, and found that, from early postnatal age, ChCs and BCs differ in laminar location. Excitatory synapses and fast-spiking properties matured before the onset of puberty in both cell types, but following cell type-specific developmental trajectories. Hence, the physiological maturation of ChCs and BCs may contribute to the emergence of cognitive function differentially, and predominantly during prepubertal development.

Introduction

Parvalbumin-positive (PV⁺) neurons tightly control the timing of pyramidal cell output in cortical neuron networks (Sohal et al., 2009; Roux and Buzsáki, 2015). Consistent with this essential role, PV⁺ neuron activity in the prefrontal cortex (PFC) is di-

rectly involved in cognitive function (Cho et al., 2015; Kim et al., 2016; Lagler et al., 2016), and PV⁺ neuron alterations in the PFC contribute to the disturbances of oscillatory network synchrony thought to underlie cognitive deficits in schizophrenia (Gonzalez-Burgos et al., 2015). Given their importance for PFC function, the postnatal maturation of PV⁺ neurons could contribute significantly to the emergence of PFC-dependent cognitive abilities that improve substantially during childhood and adolescence (Luna et al., 2015), and are impaired in schizophrenia. Thus, investigating the postnatal maturation of PV⁺ neurons in the PFC is crucial to elucidate their role in cognitive development.

The two major subtypes of cortical PV⁺ neurons, basket cells (BCs) and chandelier cells (ChCs), are distinguished by morphological features of their axons, which target separate compartments of the postsynaptic cell membrane. Specifically, ChC axons display abundant vertical arrays of synaptic boutons, termed cartridges, which innervate the initial segment of the pyramidal neu-

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ron axon (DeFelipe et al., 2013). In contrast, BC axons lack cartridges, and target the pyramidal cell body and proximal dendrites (DeFelipe et al., 2013). Interestingly, ChCs may be excitatory, instead of inhibitory, depending on the network activity state (Szabadics et al., 2006; Woodruff et al., 2009, 2011). Moreover, *in vivo*, ChCs fire spikes with different timing than BCs (Zhu et al., 2004; Klausberger and Somogyi, 2008; Massi et al., 2012). These notable differences, and the proximity of ChC synapses to the site of action potential (AP) initiation (Kole and Stuart, 2012), suggest that ChCs and BCs play different roles in cortical circuit function. Therefore, ChC and BC maturation may contribute differently to the development of PFC circuits and cognitive function. To date, however, the time course of physiological maturation of ChCs and BCs has not been compared in any cortical region.

Previous studies suggested that the unique fast-spiking (FS) electrophysiological phenotype of PV⁺ neurons, which is essential for their role in cortical networks (Buzsáki and Wang, 2012; Hu et al., 2014), develops in an activity-dependent manner (Miller et al., 2011; Dehorter et al., 2015). Therefore, excitatory drive from glutamate synapses may shape the maturation the FS phenotype, and thus excitatory synapses and FS properties may display similar maturation time courses. In addition, although in adult cortex PV⁺ neurons are present in layers 2–6 (Gabbott et al., 1997; Chattopadhyaya et al., 2004; Rymar and Sadikot, 2007; Bartolini et al., 2013), PV⁺ cells populate the deep layers first during early development (Rymar and Sadikot, 2007; Bartolini et al., 2013). Thus, the maturation time course of PV⁺ neurons may differ between layers.

To test these ideas, we investigated the functional maturation of excitatory synapses and of FS properties in ChCs and BCs across cortical layers in the PFC. We found, in two independent mouse strains, that BCs are positioned throughout layers 2–5, whereas ChC somata are found only near the layer 1–2 (L1/2) border. Developmental analysis of PV⁺ neurons from postnatal day (P) 12 revealed that excitatory synaptic drive did not change with age in L1/2 ChCs, but increased rapidly in BCs, reaching levels ~4 times higher than in L1/2 ChCs, and possibly with an earlier onset in BCs of layers 3–5 (L3/5) compared with L1/2 BCs. Consistent with a role of excitatory synaptic drive in the maturation of the FS phenotype, FS properties developed with the slowest rate in L1/2 ChCs, and possibly with earlier onset in L3/5 BCs than in L1/2 BCs. Importantly, independent of subtype-dependent and layer-dependent differences, the maturation of all PV⁺ neurons in mouse PFC was rapid and almost completed by the onset of puberty. Thus, PV⁺ neuron maturation may contribute to the emergence of cognitive function primarily during prepubertal development.

Materials and Methods

Brain-slice preparation

Coronal brain slices (300 μ m thick) containing various subregions of the medial PFC (medial orbital, infralimbic, prelimbic, and dorsal anterior cingulate) were prepared from the frontal cortex of mice of either sex, ranging in age from P8 to P73. Animal housing and all procedures followed National Institutes of Health guidelines, approved by the University of Pittsburgh Institutional Animal Care and Use Committee. Under deep isoflurane anesthesia, the mice were decapitated. Then a tissue block was dissected and glued to the stage of a vibrating microtome (Leica Microsystems, VT1000). Slices were cut in ice-cold slicing solution containing the following (in mM): 210 sucrose, 10 NaCl, 1.9 KCl, 1.2 Na₂HPO₄, 33 NaHCO₃, 20 glucose, 1.3 ascorbate, 2.4 pyruvate, 6 MgCl₂, 0.5 CaCl₂, pH 7.3–7.4 when bubbled with 95% O₂ and 5% CO₂. Before recording, slices were incubated at room temperature (20–24°C) for ≥ 60 min in a holding chamber filled with artificial CSF (ACSF) containing the following (in mM): 125 NaCl, 2.5 KCl, 1.25 Na₂HPO₄, 10 glucose, 25

NaHCO₃, 0.4 ascorbate, 1 MgCl₂, 2 CaCl₂, pH 7.3–7.4 when gassed with 95% O₂ and 5% CO₂. In most experiments, slices were from G42 mice (The Jackson Laboratory, stock no. 007677), a transgenic line expressing green fluorescence protein (GFP) exclusively in PV⁺ neurons (Chattopadhyaya et al., 2004). In some experiments, we used mice expressing cre recombinase (Cre) and the red fluorescent protein tdTomato in PV⁺ neurons, derived from crossing *Pvalb*^{Cre} mice, a knock-in strain expressing Cre in PV⁺ neurons (The Jackson Laboratory, stock no. 008069), with mice of the tdTomato reporter knock-in line *Ai14* (The Jackson Laboratory, stock no. 007908). Because tdTomato expression is very low in immature PV⁺ neurons labeled in *Pvalb*^{Cre} mice (Carlén et al., 2012), the *Pvalb*^{Cre}; *Ai14* line was used only to study PV⁺ neurons at age P ≥ 30 .

Electrophysiological recording and data analysis

For recording, the slices were transferred to a submersion chamber superfused at 2 ml/min with oxygenated ACSF solution at 30–32°C, containing 10 μ M gabazine, a GABA_A receptor antagonist, to block inhibitory synaptic currents. Tight-seal whole-cell recordings were obtained from PV⁺ neurons identified by the GFP or tdTomato fluorescence, using Olympus or Zeiss microscopes equipped with epifluorescence, infrared illumination, differential interference contrast, and CCD video cameras (EXi Aqua, Q-Imaging). Pipettes pulled from borosilicate glass (resistance, 3–6 M Ω) were filled with the following solution (mM): 120 potassium gluconate, 10 KCl, 10 HEPES, 0.2 EGTA, 4.5 MgATP, 0.3 NaGTP, 14 sodium phosphocreatine. The pH was adjusted to 7.2–7.4 using KOH. Biocytin (0.4–0.5%) was freshly added to the pipette solution to fill the PV⁺ cells for later morphological identification. Recordings were obtained with Multiclamp 700B amplifiers (Molecular Devices). Signals were low-pass filtered at 6 kHz, and digitized at 10 or 20 kHz using Power 1401 data acquisition interfaces (Cambridge Electronic Design). Data acquisition and analysis were performed using Signal 5 software (Cambridge Electronic Design), running custom-made scripts.

Voltage-clamp experiments. We compensated the pipette capacitance (C_p) and continuously monitored the series resistance (R_s), but did not use R_s compensation. R_s was estimated as $R_s = 5/I(0)$, where $I(0)$ is the initial amplitude of the capacitive current evoked by a 5 mV voltage step, excluding the current due to charging of C_p . To estimate $I(0)$ minimizing errors from variability in the accuracy of C_p compensation, we use a script written in Signal software that fits, to the capacitive current, a double exponential function as follows (Eq. 1):

$$I(t) = A + B * \exp\left(-\frac{t}{\tau_B}\right) + C * \exp\left(-\frac{t}{\tau_C}\right)$$

To exclude the current due to charging of C_p , the first millisecond of current was omitted from the fits (Langdon et al., 1995). At $t = 0$, $I(0) = A + B + C$, thus R_s (M Ω) = 5 (mV)/ $A + B + C$ (nA). Only recordings with an initial $R_s < 20$ M Ω were used for analysis. The R_s values (mean $\pm$ SD) were 10.3 $\pm$ 3.5 M Ω ($n = 60$), 10.6 $\pm$ 3.3 M Ω ($n = 37$), and 10.5 $\pm$ 2.7 M Ω ($n = 43$) for recordings from L1/2 BCs, L1/2 ChCs, and L3/5 BCs, respectively.

EPSCs were recorded holding the PV⁺ neurons at -70 mV, near their average resting membrane potential, which ranged between -60.8 and -79.7 mV (see Figs. 8, 9, 11, 12). The stability of R_s was measured by the current evoked by a 50 ms -5 mV voltage step, delivered every 10 s. EPSC data analysis was completed in a short time window with small changes in R_s , which increased on average by 1.3, 4.1, and 1.4%, in L1/2 BC, L1/2 ChC, and L3/5 BC recordings, respectively. Importantly, the initial R_s (mean $\pm$ SD) did not differ with age in the recordings from L1/2 BCs (P12: 10.9 $\pm$ 4.9 M Ω ; P20: 9.9 $\pm$ 3.2 M Ω ; P30–P40: 11.6 $\pm$ 1.3 M Ω ; $F_{(2,17)} = 0.199$, $p = 0.821$), L1/2 ChCs (P12: 10.9 $\pm$ 4.6 M Ω ; P20: 9.7 $\pm$ 2.1 M Ω ; P30–P40: 10.7 $\pm$ 2.5 M Ω ; $F_{(2,17)} = 0.136$, $p = 0.874$), or L3/5 BCs (P12: 10.4 $\pm$ 3.6 M Ω ; P20: 10.6 $\pm$ 1.7 M Ω ; P30–P40: 9.8 $\pm$ 3.2 M Ω ; $F_{(2,23)} = 0.142$, $p = 0.867$). Therefore, age-related differences in sEPSC properties were unlikely to reflect different voltage-clamp quality due to differences in R_s .

For each recorded PV⁺ neuron, 100–200 sEPSCs were detected and analyzed using Mini Analysis software (Synaptosoft), starting ~ 1 min after the beginning of whole-cell recordings. For EPSC detection, the

amplitude threshold was 4–6 pA (two times the root mean square of the baseline noise, typically 2–3 pA). The EPSC area threshold was 4 pA/ms; the average baseline before EPSC onset was set as 2–10 ms. The sEPSCs detected were inspected visually, and all detected events were used to estimate the mean peak sEPSC amplitude and sEPSC frequency. The mean sEPSC amplitude reported for each neuron is the average of the amplitudes measured for each sEPSC detected for each cell. The sEPSC frequency reported is the mean number of sEPSCs detected per second, thus expressed in hertz.

EPSC trains (see Fig. 4) were evoked using electrodes pulled from borosilicate theta capillary glass, filled with oxygenated extracellular solution and connected to a stimulation unit via silver wires. Pulses of 100 μ s duration had amplitudes (10–100 μ A) that in each neuron evoked EPSCs reliably (see Fig. 4A). When testing the effect of tetrodotoxin (TTX) on sEPSCs (see Fig. 4), we monitored the block of APs produced by voltage escape during large depolarizing commands (sometimes termed action currents).

Current-clamp experiments. Only cells with an initial resting membrane potential of -60 to -80 mV were included in this study. R_s and C_p were monitored and cancelled using the bridge balance and capacitance neutralization circuits. The input resistance (R_{in}) was estimated as the slope of the linear region of the relation between hyperpolarizing current step amplitude (-50 to -10 pA, 500 ms, three repeats) injected via the recording electrode, and membrane potential during the last 50 ms of the step. The membrane time constant (τ_m) was estimated fitting a single exponential function to the decay of the membrane potential response produced by hyperpolarizing steps of -30 to -10 pA (three repeats per step). This measure is an approximation of the actual τ_m , which, in a passive neuron with complex geometry, is the time constant of the slowest component of a multiexponential time course (Spruston et al., 1994). The voltage threshold to fire an AP (AP threshold), AP duration at half maximal amplitude (AP half-width), afterhyperpolarization (AHP) amplitude, and delay to fire the first AP (first AP delay), were all estimated from single APs (≥ 3 replicates per neuron), evoked by stimulation at or near the current threshold (I threshold). The AP threshold was estimated using derivatives of $V_m(t)$, as described previously (Henze et al., 2000). The AHP amplitude was estimated as the difference between the AP threshold and the voltage at the AHP trough. I threshold was the smallest current step amplitude eliciting ≥ 1 AP in three repetitions of that step amplitude. The slope of the relation between mean firing frequency and current step amplitude (f – I slope) was estimated from the linear region of the plots of mean firing frequency versus stimulus amplitude. The mean firing frequency was calculated from the number of APs evoked per 500 ms stimulus, averaged for the three repetitions of each stimulus amplitude. Spike frequency adaptation was estimated as the ratio between the last and first interspike interval (ISI): ISI ratio, $ISI_{(last)}/ISI_{(first)}$. For stimulus currents between I threshold and ~ 100 pA above, PV⁺ neurons fired single (see Fig. 6A) or a few APs with highly variable ISIs (data not shown). Between 100 and 200 pA above I threshold, the ISI ratio was stable for BCs, but increased progressively in ChCs. The ISI ratio values reported are the average from steps between 100 and 200 pA above I threshold. For several neurons reported in Figures 8, 10, 11, and 13, current-clamp recordings were obtained after voltage-clamp data were acquired, which are reported in Figures 3 and 5.

Histological processing and morphological reconstruction of biocytin-filled neurons

The PV⁺ neurons were filled with 0.4–0.5% biocytin during recordings, and then slices were immersed in 4% p-formaldehyde in 0.1 M PBS for 24–72 h at 4°C. The slices were cryoprotected (33% glycerol, 33% ethylene glycol, in 0.1 M PBS) and stored at -80°C until processed. To visualize biocytin, the slices were resectioned at 60 μ m, incubated with 1% H_2O_2 , and immersed in blocking serum containing 0.5% Triton X-100 for 2–3 h at room temperature. The tissue was then rinsed and incubated with the avidin–biotin–peroxidase complex (1:100; Vector Laboratories) in PBS for 4 h at room temperature. Sections were rinsed, stained with the nickel-enhanced 3,3'-diaminobenzidine chromogen, mounted on gelatin-coated glass slides, dehydrated, and coverslipped. Three-

dimensional reconstructions were performed using the NeuroLucida tracing system (MBF Bioscience).

ChCs are morphologically defined by an axonal arbor with abundant cartridges, which are short rows of boutons aligned vertically and connected by thin axonal segments (Somogyi et al., 1998; DeFelipe et al., 2013). In the PFC of mice $P \geq 28$, ChCs could be identified both by the presence of cartridges, and their overall axo-dendritic features, as described in previous studies (Woodruff et al., 2011; Inan et al., 2013). ChC axons in mouse somatosensory cortex display adult-like properties since P18, by frequently displaying four (range, 2–9) boutons per cartridge (Inan et al., 2013). With differential interference contrast imaging of the biocytin-filled axonal arbors, we could identify cartridges with ≥ 4 axonal boutons only in PV⁺ neurons from $P \geq 12$ mice. In many cases, the biocytin-labeled boutons in cartridges were in apposition with the unlabeled axon initial segment of pyramidal neurons. However, PV⁺ cells from the P8–P11 PFC lacked axon cartridges, although many of them displayed axo-dendritic features consistent with those of ChCs. Thus, we identified a neuron as ChC when ≥ 2 –3 cartridges (with ≥ 4 boutons per cartridge) were detected in the axonal arbor, and the global axo-dendritic features were consistent with mature ChCs. Using this criteria, ChCs could be identified starting at P12. Because many cartridges in mature ChC axons have < 4 boutons (Inan et al., 2013), our approach may have excluded some immature ChCs.

Figure 1E reports the distance between center of the soma and pial surface, measured, using the NeuroLucida software, over a line nearly perpendicular to the pial surface and the L1/2 border, for 109 GFP⁺ neurons (66 BCs, 43 ChCs) from $P \geq 28$ mice. These include cells with electrophysiology data displayed in Figures 3, 4, 5, 8, and 9, and GFP⁺ neurons filled with biocytin during recordings for which the electrophysiology data quality did not meet criteria for inclusion in analysis. For many of the ChCs and BCs with electrophysiology data in this study, the soma-to-pia distance was not measured, but microscope inspection showed that all ChC somata were localized exclusively near the L1/2 border, whereas BCs were found throughout all layers. Figure 1F reports data from biocytin-filled neurons from *Pvalb^{Cre};Ai14* mice. For some of these mice we report electrophysiological data in this study (see Figs. 9, 11).

Statistical analysis

Basic statistics. In the figures, the data were expressed as means $\pm$ SD, unless otherwise indicated. To determine whether postnatal age and neuron subtype had significant effects on the electrophysiological properties (excitatory synaptic function and FS phenotype), we employed Student's t tests or ANOVA. To assess normality of the data distribution, we constructed quantile–quantile (Q–Q) plots of the residuals for each variable, followed by Shapiro–Wilk tests of normality. Some variables were natural log transformed to meet the criteria of normality assumption, as indicated in each figure legend. In the figures, these variables are shown without transformation.

Exponential curve fitting and comparisons. To characterize the time course of maturation of the electrophysiological properties, we employed nonlinear curve fitting of single exponential functions of the following form (Eq. 2): $y(x) = y_0 + A * \exp\left(-\frac{x - 12}{\tau_{dev}}\right)$, where y is the physiological variable, and x is mouse age in days. The parameter y_0 (plateau value), represents the mature state, and A sets the value, relative to y_0 , at age P12, thus indicating the amount of change between P12 and mature state (for some variables, A is a negative number). The exponential constant τ_{dev} defines the rate of maturation, and is the time needed for y to decay by 0.63^*A . We chose a single exponential decay as the model function given that, for the variables more strongly regulated by age (for example, AP half-width, R_{in} , and τ_m), a single exponential decay function fit the data better than a linear regression or a logistic function, as judged by the coefficient of determination R^2 , and a double exponential decay did not substantially improve the fits. Interestingly, a previous study of PV⁺ BCs (Doischer et al., 2008) showed that the development of several of the electrophysiological variables studied here was also well fit by single exponential functions.

We compared the maturation time course between L1/2 BCs and L1/2 ChCs, or between L1/2 BCs and L3/5 BCs, using a combined exponential curve-fitting approach applied to the electrophysiological variables that,

Figure 1. Morphological features of PV⁺ neurons in the PFC of P ≥ 28 G42 mice. **A**, Neurolucida reconstructions of two examples of ChCs (left, P63; right, P28), filled with biocytin during recordings near the L1/2 border. The axon is shown in blue; soma and dendrites are in red. Note the presence of abundant cartridges of axonal boutons, some of which (red boxes) are highlighted in the insets (green boxes). Here, and in **B**, the gray lines illustrate the approximate location of the L1/2 border. As noted in the examples, many of the ChCs had prominent axonal projections descending into deeper cortical layers. Calibration bars for the insets, 10 μ m. **B**, Neurolucida reconstructions of two examples of L1/2 BCs (left, P62; right, P28). **C**, Neurolucida reconstructions of the somata and dendritic tree for examples of L1/2 ChCs and L1/2 BCs. Right, Polar plots of the distribution of dendritic length relative to the soma, positioned at the center, averaged for two samples of L1/2 ChCs ($n = 8$) and L1/2 BCs ($n = 7$). Shown are means $\pm$ SEM. **D**, Neurolucida reconstructions of examples of L3/5 BCs. **E**, Plots of distance between the center of the soma and the pia surface for PV⁺ neurons recorded from the PFC of G42 mice. **F**, Plots of distance between the center of the soma and pia surface for PV⁺ neurons recorded in the PFC of *Pvalb*^{Cre};Ai14 mice. **G**, Approximate localization of the cell bodies, across subregions of the mouse frontal cortex, of a subset of the PV⁺ ChCs and BCs reported in **E**. PV⁺ neurons from G42 or *Pvalb*^{Cre};Ai14 mice not shown in **G** were found in the same cytoarchitectonic regions. Red and gray dots represent, respectively, somata of individual ChCs and BCs. The numbers below the schematics, show the anterior–posterior (A–P) coordinates of the coronal sections. These coordinates are approximate, since the A–P location of the most rostral section varied between experiments. Cg, Anterior cingulate; PL, prelimbic; MO, medial orbital; IL/DP, infralimbic/dorsal peduncular. Abbreviations of cytoarchitectonic regions as defined in the 2001 atlas of Paxinos and Franklin (2001).

by t test, showed effect of age within each subtype under consideration. For the L1/2 ChC versus L1/2 BC comparison, the variables were as follows: R_{in} : L1/2 ChC $p = 0.006$, L1/2 BC $p = 0.0001$; AP half-width: L1/2 ChC $p < 0.00001$, L1/2 BC $p < 0.00001$; AP threshold: L1/2 ChC $p = 0.013$, L1/2 BC $p = 0.029$; τ_m : L1/2 ChC $p = 0.00028$, L1/2 BC $p < 0.00001$; $f-I$ slope: L1/2 ChC $p = 0.011$, L1/2 BC $p = 0.00022$. For the L1/2 BC versus L3/5 BC comparison, the variables were as follows: AP

half-width: L3/5 BCs $p = 0.000024$; τ_m : L3/5 BCs $p < 0.00001$; I threshold: L1/2 BCs $p = 0.000013$, L3/5 BCs $p = 0.00058$; R_{in} : L3/5 BCs $p = 0.00002$; $f-I$ slope: L3/5 BCs $p = 0.00039$. The combined exponential curve-fit approach was also employed to compare the maturation rate of sEPSC frequency between L1/2 BCs and L3/5 BCs.

Using R 3.1.0 software (The R Project for Statistical Computing, <https://www.r-project.org/>), we applied exponential curve fits to the data

Figure 2. Morphological properties of PV⁺ neurons in the developing mouse PFC. **A**, Neurolucida reconstructions of examples of L1/2 BCs from P8–P12 G42 mice. The axon is shown in blue; soma and dendrites are shown in red. **B**, Neurolucida reconstructions of examples of L1/2 ChCs from P9–P25 G42 mice. Note the presence of cartridges of axonal boutons in a P12 L1/2 ChC, highlighted in the insets (green boxes). Calibration bars for the insets, 10 μ m. **C**, Individual polar plots of the distribution of dendritic length relative to the soma, positioned at the center, for two L1/2 BCs (P8 and P12) and four L1/2 ChCs (P12–P15). Calibration bars, 40 μ m. **D**, Neurolucida reconstructions of examples of L3/5 BCs in P8–P16 G42 mice. **E**, Plots of distance between the center of the soma and the pia surface for PV⁺ neurons recorded in the PFC of P12–P17 G42 mice. **F**, Distribution of the recorded PV⁺ ChCs and BCs across subregions of the medial PFC of P $\leq$ 20 G42 mice. Cg, Anterior cingulate; PL, prelimbic; MO, medial orbital; IL/DP, infralimbic/dorsal peduncular. Red and gray dots represent, respectively, somata of individual ChCs and BCs. The calibration and anterior–posterior coordinates of the coronal section schematics are approximate, given that the brains of mice younger than P14 have slightly smaller dimensions, and that the anterior–posterior location of the most rostral section varied between experiments.

Figure 3. Developmental changes of EPSCs in PV⁺ neurons of the L1/2 border in mouse PFC. **A**, Left, Representative examples of sEPSCs recorded from a L1/2 BC in the continuous presence of 10 μ M gabazine, a GABA_A receptor antagonist. Right, Addition of 10 μ M CNQX, an AMPA receptor antagonist, blocked the sEPSCs. **B**, Consecutive sEPSC_{AMPA} traces superimposed and aligned (gray traces, 130 sEPSCs), together with their average, for a P12 L1/2 BC (black thick trace) and a P12 L1/2 ChC (red thick trace). **C**, Cumulative frequency distribution of sEPSC_{AMPA} amplitude for P12 ($n = 9$) and P35–P50 ($n = 14$) L1/2 BCs. Here and in **D**, the insets show binned histograms of the relative frequency (y -axis) of sEPSC_{AMPA} amplitude (x -axis) for each sample. **D**, Cumulative frequency distribution of sEPSC_{AMPA} peak amplitude for P12–P13 ($n = 6$) and P35–P50 ($n = 11$) L1/2 ChCs. Insets as in **C**. **E**, Mean sEPSC_{AMPA} peak amplitude in P12 ($n = 9$) or P > 30 ($n = 14$) L1/2 BCs (gray symbols), and in P12–P13 ($n = 6$) or P > 30 ($n = 11$) L1/2 ChCs (red symbols). Two-factor ANOVAs revealed lack of effect of age ($F_{(1,36)} = 0.0103$, $p = 0.899$) and significant effect of cell type ($F_{(1,36)} = 6.159$, $p = 0.0212$). At P > 30, the sEPSC_{AMPA} amplitude was significantly smaller in L1/2 ChCs ($\#p = 0.044$, Fisher's least significant difference test). **F**, Plot of mean sEPSC_{AMPA} peak amplitude as a function of postnatal age for L1/2 BCs (gray symbols) or L1/2 ChCs (red symbols). Each data point represents a single PV⁺ neuron. **G**, Mean sEPSC frequency (Figure legend continues.)

from each neuron subtype. For all the variables indicated above, we obtained a curve fit (see Fig. 10, 13), with the exception of R_{in} in L1/2 ChCs, for which the fitting algorithm did not converge, despite testing many combinations of initial values for the fit parameters (we programmed a loop to search over a dense grid with 50 values for each parameter), indicating that there is no unique solution for the curve fit. Overall, either the absence of age effect or the lack of fit in the data plots for one or both of the PV⁺ neuron subtypes under consideration precluded comparing some variables. The variables for which we compared the exponential fits were, between L1/2 BCs and L1/2 ChCs: AP half-width, τ_m , AP threshold, and $f-I$ slope, and between L1/2 BCs and L3/5 BCs: AP half-width, τ_m , I threshold, R_{in} , and $f-I$ slope.

To contrast the rate of development between PV⁺ neuron samples, we combined the data points for a given electrophysiological variable from the two samples under consideration, creating a dummy variable z , 0 for L1/2 BCs, and 1 for L1/2 ChCs (or L3/5 BCs). The general model fitted had two predictors (x and z) and six parameters ($y_0, y_0', A, A', \tau_{dev}, \tau_{dev}'$) as follows (Eq. 3):

$$y(x) = (y_0 + y_0' * z) + (A + A' * z) * \exp\left(-\frac{x - 12}{\tau_{dev} + \tau_{dev}' * z}\right),$$

where non-zero values of y_0', A', τ_{dev}' indicate these parameters are different between the two samples under comparison. Whereas y_0, y_0', A , and A' may be estimated from the experimental data (via the $P > 30$ and $P12$ values, respectively), the rate of development can only be estimated via τ_{dev} from the curve fits. Thus, our approach focused on comparing τ_{dev} (i.e., testing whether $\tau_{dev}' > 0$). To this aim, whenever there was no evidence of difference in $P > 30$ and $P12$ values, y_0' and A' were omitted, and the model thus had two predictors (x and z) and four parameters (y_0, A, τ_{dev} , and τ_{dev}'). This allowed us to focus the comparisons on τ_{dev} because the statistical power is greater when the number of parameters in the model is reduced. For the comparison between L1/2 BCs and L1/2 ChCs, both y_0' and A' were omitted in the combined curve fit, except that for τ_m (see Fig. 8), we kept y_0' . For the comparison between L1/2 BCs and L3/5 BCs, y_0' was omitted in all combined curve fits excepting $f-I$ slope, and A' was kept in the model for all compared variables, since all the experimental values at $P12$ differed between L1/2 BCs and L3/5 BCs. For each electrophysiological variable compared, the nls function in R software generates a p value for the test of $\tau_{dev}' > 0$, using a t statistic $\hat{\tau}/se(\hat{\tau})$, where $\hat{\tau}$ is the estimated value of τ_{dev}' and se is the SE of the estimator (approximated based on nonlinear least-squares theories). The statistic asymptotically follows a t distribution under the null hypothesis, thus producing a test similar to a t test for the coefficients in a linear regression model (Bates, 1988, 1992).

After performing the combined curve fits, we obtained a global comparison across physiological variables of the time constants of development τ_{dev} by pooling the information from all the variables compared between a given pair of PV⁺ neuron subtypes. For this global comparison, we used Fisher's method to combine p values as follows: $X^2 = -2\sum_{i=1}^k \log P_i$ (Fisher, 1950; Won et al., 2009). Assuming independence of the individual tests, under the null hypothesis of no differences in τ_{dev} for all k variables, the combined statistic follows a χ^2 distribution with degrees of freedom $2k$. To account for correlations in individual tests, we adjusted Fisher's method based on a theoretical approximation of the null distribution, using a

scaled χ^2 distribution as originally described by Brown (Brown, 1975; Moskvina et al., 2011), with the correlations among all pairs of tests approximated from the original data.

Results

ChCs and BCs have different laminar distribution since early postnatal development

We targeted for recording GFP⁺ neurons in acute slices from the PFC of G42 mice, in which GFP is expressed exclusively in PV⁺ neurons (Chattopadhyaya et al., 2004), and ~90% of the PV⁺ cells in layers 2/3 are GFP⁺ (Chattopadhyaya et al., 2004; Buchanan et al., 2012; Sipky and Yuste, 2013). In G42 mice, GFP⁺ neurons are detected by P0 (Chattopadhyaya et al., 2004), significantly earlier than the late postnatal onset of PV expression (Soriano et al., 1992; Del Río et al., 1994; Huang et al., 1999). In contrast, recombination in *Pvalb*^{Cre} mice labels only ~5% of the PV⁺ neurons by P13, and ~30% by P19 (Carlén et al., 2012), paralleling the protracted developmental expression of PV. G42 mice are therefore a suitable model to assess PV⁺ neuron development independent of age-dependent or activity-dependent changes in PV expression. We investigated PV⁺ neurons starting at P8, because ChC migration from the ganglionic eminence, which appears to be delayed relative to BC migration (Inan et al., 2012; Taniguchi et al., 2013), is not complete until ~P7 (Taniguchi et al., 2013). To assess PV⁺ neuron development, including the onset of puberty at ~P21 (Nelson et al., 1990; Laviola et al., 2003) and into adolescence, we studied G42 mice between P8 and ~P70.

In the adult cortex, PV⁺ neuron somata are found throughout layers 2–6 (Gabbott et al., 1997; Chattopadhyaya et al., 2004; Rymar and Sadikot, 2007; Bartolini et al., 2013). Because the laminar distribution of BCs and ChCs in the mouse PFC was not compared previously, we examined the morphology of GFP⁺ neurons with somata positioned across layers 2–5, filled with biocytin during recordings of excitatory synaptic currents, of intrinsic membrane properties, or both. We focused first on the PFC of $P \geq 28$ mice, when ChCs and BCs are well-developed (Inan et al., 2012, 2013; Taniguchi et al., 2013; Tai et al., 2014). We found that 49 of 94 PV⁺ neurons with soma near the L1/2 border were ChCs (Fig. 1A) displaying abundant vertical cartridges of boutons (Fig. 1A, insets) in axonal arbors that branched densely below the soma, descending into layers 2 and 3, and, in some ChCs, also reaching layer 5 (Fig. 1A). All other L1/2 border PV⁺ neurons (45 of 94) were BCs, with axonal arbors projecting mainly horizontally, nearly parallel to the L1/2 border, and with a few branches projecting into deeper layers in some BCs (Fig. 1B). The dendrites of the L1/2 ChCs mainly extended into layer 1 above the soma (Fig. 1C), whereas for most L1/2 BCs, the dendritic tree was more evenly distributed between layers 1 and 2 (Fig. 1C), as in the sensory cortex (Woodruff et al., 2011).

After finding that in the PFC of $P \geq 28$ mice, both ChCs and BCs are present near the L1/2 border, we assessed the morphology of PV⁺ neurons of layers 3 and 5. In previous work, embryonic recombination in *Nkx2.1*^{CreER} mice labeled numerous ChCs in deep cortical layers, which, however, were mostly PV-negative (PV[−]; Taniguchi et al., 2013). In G42 mice, we found that all the PV⁺ neurons recorded in PFC layers 3 and 5 ($n = 25$) were BCs (Fig. 1D). Measurements of the distance between the center of the soma and the pial surface for 109 GFP⁺ neurons (Fig. 1E) indicated that ChC somata are restricted to a short range of distances closely matching the L1/2 border, and are sometimes localized in deep layer 1, but excluded from deep layer 2, and from layers 3 and 5. In contrast, BCs are positioned throughout layers 2–5 (Fig.

←

(Figure legend continued.) sEPSC_{AMPAR} frequency in L1/2 BCs and L1/2 ChCs from P12–P13 and $P > 30$ mice. Symbols the same as in E. Two-factor ANOVAs revealed a significant effect of age ($F_{(1,36)} = 14.826, p = 0.0047$) and cell type ($F_{(1,36)} = 13.647, p = 0.00073$). Since Q–Q plot and Shapiro–Wilk analysis revealed a deviation from normality of the sEPSC_{AMPAR} frequency data, ANOVA was performed after log transformation of the data (Shapiro–Wilk $p = 0.748$). At $P > 30$, the sEPSC_{AMPAR} frequency was significantly lower in L1/2 ChCs versus $P > 30$ L1/2 BCs ($\#p = 0.00004$, Fisher's least significant difference test). H, Mean sEPSC_{AMPAR} frequency plotted as a function of postnatal age. The BC plot (gray symbols) was well fit by a single exponential function (thick black line), with a time constant τ_{dev} of 3.35 ± 1.94 d (mean $\pm$ SE from the nonlinear regression fit to the data). Shown are the 95% confidence bands of the curve fit for the L1/2 BC data. The plot for ChCs (red symbols) could not be fit by an exponential function.

Figure 4. Short-term EPSC depression and effects of blocking network activity on sEPSCs recorded from L1/2 BCs and L1/2 ChCs. **A**, Left, Examples of EPSC trains illustrating short-term depression. Shown are consecutive traces superimposed (gray) and their average (red). The EPSC trains were evoked in a L1/2 BC by focal extracellular stimulation applied to layer 1 (five stimuli at 40 Hz). Right, Plots of EPSC amplitude for each stimulus number, normalized to the amplitude of EPSC₍₁₎ in each train. Shown are means $\pm$ SEM. The EPSC₍₅₎/EPSC₍₁₎ values showed deviation from normality based on Q-Q plot and Shapiro-Wilk test analysis. EPSC₍₅₎/EPSC₍₁₎ were compared with Student's *t* tests performed after log transformation of the data (Shapiro-Wilk $p = 0.555$). L1/2 BCs, $t_{(9)} = 0.139$, $p = 0.892$; L1/2 ChCs, $t_{(5)} = 0.475$, $p = 0.657$. **B**, Left, Example of recordings of sEPSCs from a L1/2 BC (P42) before (top) and after (bottom) the addition of 1 μ M TTX. Right, Cumulative frequency distribution of sEPSC_{AMPAR} peak amplitude before (control) and after application of 1 μ M TTX for L1/2 BCs and L1/2 ChCs. Insets show binned histograms as in Figure 3. **C**, Summary plots of sEPSC_{AMPAR} amplitude before and after TTX application to L1/2 BCs (left) and L1/2 ChCs (right). Shown are the p values from paired Student's *t* tests comparing sEPSC amplitude before and after TTX application. L1/2 BCs, $t_{(5)} = 3.3$, $p = 0.021$; L1/2 ChCs, $t_{(6)} = 0.55$, $p = 0.601$. **D**, Summary plots of sEPSC_{AMPAR} frequency before and after TTX application to L1/2 BCs (left) and L1/2 ChCs (right). Shown are the p values from paired *t* tests comparing sEPSC amplitude before and after TTX application. L1/2 BCs, $t_{(5)} = 0.58$, $p = 0.589$; L1/2 ChCs, $t_{(6)} = 2.38$, $p = 0.054$.

1E). While these data suggest that PV⁺ ChCs and BCs have markedly different laminar distributions, it is possible that PV⁺ ChCs were not targeted for recording in layers 3 and 5, because not all PV⁺ neurons are GFP⁺ in the G42 mouse cortex (Chattopadhyaya et al., 2004; Buchanan et al., 2012; Sippy and Yuste, 2013). Hence, to assess the laminar distribution of PV⁺ ChCs and BCs independent of the incomplete GFP labeling in G42 mice, we crossed *Pvalb*^{Cre} mice with the *Ai14* knock-in reporter line, to derive *Pvalb*^{Cre}; *Ai14* mice, which express the red fluorescent pro-

tein tdTomato in PV⁺ cells. In the PFC of $P \geq 28$ *Pvalb*^{Cre}; *Ai14* mice, the laminar distribution of tdTomato-positive ChCs and BCs (Fig. 1F) was very similar to the distribution of GFP⁺ ChCs and BCs in $P \geq 28$ G42 mice (Fig. 1E). Thus, data from two separate mouse lines indicate that in the PFC of $P \geq 28$ mice, PV⁺ BCs populate layers 2–5, but ChCs are found only near the L1/2 border. The somata of the L1/2 border ChCs and BCs (L1/2 ChCs and L1/2 BCs, respectively), and of the layers 3 and 5 BCs (L3/5 BCs) were distributed across cytoarchitectonic regions of the medial frontal cortex (Fig. 1G), suggesting the distinct laminar distribution of ChCs versus BCs is common to all areas.

After characterizing the laminar distribution of BCs and ChCs in the PFC of $P \geq 28$ mice, we assessed the morphology of developing PV⁺ neurons, starting at P8, and focusing first on GFP⁺ neurons with soma near the L1/2 border. We found that many of these PV⁺ neurons had properties resembling L1/2 BCs, with axonal arbors extending horizontally (Fig. 2A), and dendrites evenly distributed between layers 1 and 2 (Fig. 2C). The other immature L1/2 border PV⁺ neurons had axo-dendritic tree features quite distinct from those of BCs, and similar to L1/2 ChCs. However, in most of the L1/2 ChC-like immature PV⁺ neurons, axon cartridges were absent, and cartridges were detected starting at P12, although in small numbers (Fig. 2B). The cartridge number increased markedly by P13–P15, and continued developing through the first 4 postnatal weeks (Fig. 2B).

Next, we characterized the morphology of GFP⁺ cells in layers 3 and 5 of the P8–P17 PFC, and found that all the PV⁺ neurons in these layers (28 of 28) had features consistent with BCs (Fig. 2D), and did not display axon cartridges. Measures of the distance between the pia and the soma of all the PV⁺ neurons from the developing PFC (P12–P17) revealed laminar distributions (Fig. 2E) similar to those observed in the $P \geq 28$ PFC (Fig. 1E, F). Moreover, the distribution of ChCs and BCs across subregions of the developing PFC (Fig. 2F) was consistent with that observed in the PFC of $P \geq 28$ mice (Fig. 1G).

Since ChCs were identified by the presence of axon cartridges first by P12, and were restricted to the L1/2 border, we focused on L1/2 border PV⁺ neurons in the PFC of $P \geq 12$ mice to compare the physiological maturation of ChCs and BCs independent of potential layer-related differences. To investigate whether BC maturation differs between layers, we compared L1/2 BCs with L3/5 BCs.

Excitatory synaptic inputs follow different maturation trajectories in ChCs and BCs

To contrast the maturation trajectory of excitatory synapses in L1/2 ChCs with L1/2 BCs, we recorded spontaneous EPSCs (sEPSCs). In both PV⁺ cell subtypes, the sEPSCs recorded while holding the membrane potential near rest (-70 mV) were blocked by CNQX, an AMPA receptor antagonist (Fig. 3A). Moreover, the AMPA receptor-mediated sEPSCs (sEPSC_{AMPAR}) had very similar features in L1/2 ChCs and L1/2 BCs (Fig. 3B). Specifically, the sEPSC_{AMPAR} peak amplitude did not change with age in L1/2 BCs or L1/2 ChCs (Fig. 3C–F), and it was slightly but significantly smaller in L1/2 ChCs (Fig. 3E). In L1/2 BCs, the sEPSC_{AMPAR} frequency was low at P12, and increased significantly with age (Fig. 3G). In contrast, L1/2 ChCs also had low sEPSC_{AMPAR} frequency at P12, but the frequency did not change with age (Fig. 3G). The increase of sEPSC_{AMPAR} frequency in L1/2 BCs followed a rapid time course well fit by a single exponential decay function (see Materials and Methods), with a developmental time constant τ_{dev} of 3.35 ± 1.94 d (mean $\pm$ SE from the nonlinear regression), reaching plateau levels by $\sim$ P20 (Fig. 3H). The plateau levels of

Figure 5. Developmental changes of EPSCs in PV⁺ L3/5 BCs. **A**, Cumulative frequency distribution of sEPSC_{AMPA} peak amplitude for P12 ($n = 8$) and P36–P45 ($n = 8$) L3/5 BCs. Here, as in Figure 3, the insets show binned histograms of the relative frequency (y -axis) of sEPSC_{AMPA} amplitude (x -axis) for each sample. **B**, Left, Mean sEPSC_{AMPA} amplitude in L3/5 BCs from P12 and P > 30 mice. Student's t test analysis showed absence of difference between age groups ($t_{(20)} = 0.955$, $p = 0.350$). Right, Plot of mean sEPSC_{AMPA} amplitude as a function of postnatal age. Each data point represents a single PV neuron. **C**, Left, Mean sEPSC_{AMPA} frequency in BCs and ChCs from P12 and P35–P38 mice. Student's t test analysis showed a significant increase with age ($t_{(20)} = 3.251$, $p = 0.0040$). Q–Q plot and Shapiro–Wilk analysis revealed a deviation from normality of the sEPSC_{AMPA} frequency. Thus, the t test was performed after log transformation (Shapiro–Wilk $p = 0.283$). Right, Plot of mean sEPSC_{AMPA} frequency as a function of postnatal age. The plot was well fit by an exponential function (thick black line) with time constant τ_{dev} of 1.29 ± 1.90 d ($\pm$ SE from the nonlinear regression). Shown are the 95% confidence bands of the curve fit.

sEPSC_{AMPA} frequency were 3–4 times higher in L1/2 BCs compared with L1/2 ChCs (Fig. 3*G,H*). These data show that divergent trajectories of early development produce significant differences in excitatory input onto L1/2 ChCs versus L1/2 BCs, which are already established by ~P20, near the onset of puberty.

The age-related increase in sEPSC_{AMPA} frequency in L1/2 BCs may be due to higher glutamate release probability (Pr), higher activity in a local network evoking EPSCs selectively in L1/2 BCs, or to increasing numbers of glutamate synapses in L1/2 BCs. To examine if Pr changes with age in L1/2 BC synapses, we measured short-term depression of EPSCs evoked by repetitive presynaptic stimulation. At synapses on PV⁺ neurons, Pr and EPSC depression are positively correlated (Koester and Johnston, 2005; Yang et al., 2013; Lu et al., 2014). Hence, if at synapses on L1/2 BCs Pr increases with age, then EPSC depression should increase with age as well. To test this prediction, we measured short-term depression of EPSCs evoked in L1/2 BCs or L1/2 ChCs by focal extracellular stimulation (five stimuli at 40 Hz) with electrodes placed in layer 1, to activate excitatory inputs predominantly in this layer, where both L1/2 BC and L1/2 ChC dendrites project substantially. We found that EPSCs displayed short-term depression in both L1/2 BCs and L1/2 ChCs in the PFC of P12–P13 or P45–P60 G42 mice (Fig. 4*A*). However, the magnitude of EPSC depression did not increase with age (Fig. 4*A*), indeed showing a decreasing trend, as reported for sensory cortex PV⁺ neurons (Miao et al., 2016). These results therefore argue against an age-related increase of Pr at L1/2 BC synapses.

If the age-related increase of sEPSC_{AMPA} frequency in L1/2 BCs depends on increasing levels of network activity, then the sEPSC_{AMPA} frequency in L1/2 BCs should be reduced when network activity is blocked. To test this prediction, in the PFC of P28–P42 G42 mice, when sEPSC_{AMPA} frequency reached age-related plateau (Fig. 3*G,H*), we recorded sEPSC_{AMPA} from L1/2 BCs or L1/2 ChCs before and after applying 1 μ M TTX to inhibit network activity (Fig. 4*B*). We found that in L1/2 BCs and L1/2 ChCs, TTX had small effects on sEPSC_{AMPA} amplitude (Fig. 4*C*) and frequency (Fig. 4*D*). Moreover, the frequency of miniature EPSC_{AMPA}, recorded in the presence of TTX, was higher in L1/2 BCs compared with L1/2 ChCs (Fig. 4*D*). These data thus suggest that the increase of sEPSC_{AMPA} frequency in L1/2 BCs is unrelated to an age-related increase in network activity.

We next assessed excitatory synapse maturation in L3/5 BCs, and found age-related changes in sEPSC_{AMPA} very similar to those observed in L1/2 BCs. For example, the mean sEPSC_{AMPA} amplitude did not change with age (Fig. 5*A*), and, moreover, did not differ from those of L1/2 BCs at P12 ($t_{(17)} = 0.186$, $p = 0.854$)

Figure 6. Intrinsic physiological properties of mature L1/2 BCs and L1/2 ChCs. *A*, Examples of membrane potential responses in L1/2 BCs (black traces) and L1/2 ChCs (red traces), elicited by injection of four current steps of increasing amplitude, shown in blue at the bottom. Note the longer delay to fire the first AP in the L1/2 BC compared with the L1/2 ChC at threshold (second traces from bottom). *B*, Superimposed traces of single APs, showing nearly identical duration, and smaller AHP in the L1/2 ChC compared with the L1/2 BC. *C*, Examples illustrating the first ISI (top) and last ISI (bottom) for examples of L1/2 BCs and L1/2 ChCs. Note the substantially longer duration of the last ISI compared with the first ISI in the L1/2 ChC.

or at $P \geq 28$ ($t_{(24)} = 0.885$, $p = 0.385$). The sEPSC_{AMPA} frequency in L3/5 BCs was low at P12, but was ~66% higher compared with P12 L1/2 BCs ($t_{(17)} = 1.80$, $p = 0.044$). After P12, the sEPSC_{AMPA} frequency in L3/5 BCs increased rapidly with age (Fig. 5*B*), reaching plateau levels that did not differ ($t_{(24)} = 0.320$, $p = 0.751$) from those in L1/2 BCs (Fig. 3*E,G*). The rapid increase of sEPSC_{AMPA} frequency in L3/5 BCs was well fit by an exponential function (Fig. 5*B*), with a time constant that had a smaller value ($\tau_{\text{dev}} = 1.29 \pm 1.90$ d) compared with L1/2 BCs ($\tau_{\text{dev}} = 3.35 \pm 1.94$ d), although both parameters displayed substantial SE. To test whether the sEPSC_{AMPA} frequency changed between P12 and plateau value at different rates in L1/2 versus L3/5 BCs, we assessed the significance of differences in τ_{dev} . To test whether the time constants τ_{dev} of two separate exponential decay functions fit to the data are significantly different (see Materials and Methods), we used a statistical model that combines in a single curve fitting session the data from the two samples under comparison. With this approach, we found that τ_{dev} of sEPSC_{AMPA} frequency did not differ between L1/2 and L3/5 BCs ($t_{(105)} = 1.306$, $p = 0.097$). These data show that the sEPSC frequency increases with similar exponential rates in L1/2 and L3/5 BCs, but

Figure 7. Development of the intrinsic physiological properties of L1/2 BCs and L1/2 ChCs. **A**, Examples of subthreshold membrane potential responses of P12 (top) and mature (bottom) L1/2 BCs (black) and L1/2 ChCs (red). Identical families of current steps (blue) were injected into each PV⁺ neuron. **B**, Example recordings showing differences between L1/2 BCs and L1/2 ChCs in the delay to fire the first AP at P12. **C**, Superimposed traces of single APs recorded from P12 (thin lines) and mature (thick lines) L1/2 BCs (black) and L1/2 ChCs (red). Note that in both PV⁺ neuron subtypes, the AP duration shortened significantly with age. **D**, f - I plots for P12 and mature L1/2 BCs and L1/2 ChCs. Note the increase with age in the slope of the linear portion of the f - I relation for both PV⁺ neuron subtypes. Shown are means $\pm$ SEM.

starting from a P12 value closer to the plateau in L3/5 BCs than in L1/2 BCs. Since the developmental increase in sEPSC_{AMPA} frequency likely starts before P12, the higher EPSC frequency in L3/5 BCs at P12 is consistent with an earlier onset of excitatory synapse maturation in L3/5 versus L1/2 BCs. Our analysis of the developmental trajectory of excitatory inputs therefore suggests that plateau levels of excitatory drive are reached around P20, before or very close to the onset of puberty, in all PV⁺ neurons across layers 2–5.

Intrinsic membrane properties follow distinct maturation trajectories in ChCs and BCs

If the time course of excitatory synaptic input development reflects the overall maturation trajectory of each PV⁺ neuron subtype, then the FS phenotype should mature earlier in L1/2 ChCs, in which excitatory inputs show mature-like properties by P12, compared with $\sim$ P20 in L1/2 and L3/5 BCs. However, in L1/2 ChCs the excitatory drive did not increase with age, contrasting with the rapid increase in BCs. Because FS properties are thought to develop in an activity-dependent manner (Miller et al., 2011; Okaty and Nelson, 2013; Dehorte et al., 2015), the developmental increase of excitatory drive in BCs may produce an earlier maturation of FS properties in BCs compared with ChCs. Hence, we assessed 10 electrophysiological variables contributing to the FS phenotype in PV⁺ neurons from the PFC of G42 P $\geq$ 12 mice.

First, we defined the physiological properties of L1/2 border PV⁺ neurons at P $\geq$ 30, when sEPSC features reached age-related plateau (Fig. 3). We found that both L1/2 BCs and L1/2 ChCs displayed APs with short duration at half maximal amplitude (AP half-width), large AHP, and sustained high-frequency firing with small spike-frequency adaptation, a crucial feature of the FS phenotype (Fig. 6A,B). However, some variables differed between L1/2 BCs and L1/2 ChCs, as shown previously (Woodruff et al., 2009; Povysheva et al., 2013; Taniguchi et al., 2013). For instance, compared with L1/2 ChCs, L1/2 BCs had lower R_{in} , higher I threshold, and delayed firing of the first AP (first AP delay) with stimulation near I threshold (Fig. 6A). Moreover, L1/2 ChCs had smaller AHP amplitude (Fig. 6B) and stronger spike-frequency adaptation (Fig. 6C).

Next, we assessed the electrophysiological properties of PV⁺ cells at P12, and compared them with those of P $\geq$ 30 neurons. We found that, at P12, most features were inconsistent with the FS phenotype, showing that FS properties emerge after P12. Moreover, at P12, L1/2 BCs and L1/2 ChCs had very similar electrophysiological properties (Fig.

7), and only the first AP delay differed between subtypes at this age (Fig. 7B). Most properties changed with age, including the AP half-width (Fig. 7C) and the f - I slope (Fig. 7D), which did not differ between mature L1/2 BCs and L1/2 ChCs. Two-factor ANOVA and *post hoc* comparison analysis (Fig. 8) revealed significant changes with age for most variables and divergent developmental changes, since only one parameter differed between L1/2 BCs and L1/2 ChCs at P12, but seven parameters differed at P $\geq$ 30 (Fig. 8).

Figure 8. Developmental changes in the intrinsic membrane properties of L1/2 BCs and L1/2 ChCs. The graphs summarize (mean $\pm$ SD) the data of 10 electrophysiological variables contributing to the FS phenotype of L1/2 BCs and L1/2 ChCs, measured from a sample of GFP⁺ PV⁺ neurons from P12 and P > 30 G42 mice, when all variables reach age-related plateaus. Shown above each graph are the *p* values from two-factor ANOVA tests of the effect of age and cell type. The *F* statistic values for age and cell type were, respectively, as follows: resting membrane potential (RMP): $F_{(1,30)} = 7.25, 8.84$; τ_m : $F_{(1,33)} = 119.7, 1.551$; R_{in} : $F_{(1,32)} = 30.3, 1.36$; I-threshold: $F_{(1,33)} = 25.6, 8.63$; AP threshold: $F_{(1,33)} = 12.4, 0.563$; first AP delay: $F_{(1,33)} = 19.52, 46.22$; AP half-width: $F_{(1,33)} = 166, 0.0045$; AHP amplitude: $F_{(1,33)} = 0.050, 10.7$; ISI ratio: $F_{(1,33)} = 4.53, 6.50$; *f*-I slope: $F_{(1,33)} = 41.2, 0.8666$. *, Significant difference between P12 L1/2 BCs and L1/2 ChCs; #, significant difference between P > 30 L1/2 BCs and L1/2 ChCs, Fisher's least significant difference *post hoc* tests. Q-Q plot and Shapiro-Wilk analysis revealed deviation from normality of four variables, for which the ANOVA was performed after log transformation (Shapiro-Wilk: $\tau_m, p = 0.357$; first AP delay, $p = 0.164$; ISI ratio, $p = 0.017$; *f*-I slope, $p = 0.190$). For the ISI ratio, we performed the ANOVA on the data ranks, with the following results: age, $F_{(1,33)} = 3.214, p = 0.082$; cell type: $F_{(1,33)} = 6.788, p = 0.014$. The *p* values of the Fisher's least significant difference tests were as follows: RMP, P12 $p = 0.1929$, P > 30 $p = 0.003$; τ_m , P12 $p = 0.570$, P > 30 $p = 0.00002$; R_{in} , P12 $p = 0.767$, P > 30 $p = 0.019$; I-threshold, P12 $p = 0.481$, P > 30 $p < 0.00001$; AP threshold, P12 $p = 0.165$, P > 30 $p = 0.456$; first AP delay, P12 $p = 0.0015$, P > 30 $p < 0.00001$; AP half-width, P12 $p = 0.984$, P > 30 $p = 0.928$; AHP amplitude, P12 $p = 0.570$, P > 30 $p = 0.00002$; ISI ratio, P12 $p = 0.952$, P > 30 $p = 0.000041$; *f*-I slope, P12 $p = 0.862$, P > 30 $p = 0.330$.

We studied L1/2 BC and L1/2 ChC development in G42 mice, because very few PV⁺ neurons are labeled before P20 by, for instance, Cre-mediated recombination in *Pvalb*^{Cre} mice (Carlén et al., 2012). However, we also recorded from some mature L1/2 BCs and L1/2 ChCs from *Pvalb*^{Cre}; *Ai14* mice (Fig. 1F). Thus, we compared the physiological properties of L1/2 BCs and L1/2 ChCs from *Pvalb*^{Cre}; *Ai14* and G42 mice, comparing our *Pvalb*^{Cre}; *Ai14* neuron sample with a subset of PV⁺ neurons from G42 mice of matching age range. As shown in Figure 9, two-factor ANOVA revealed significant effects of cell type (L1/2 BCs vs L1/2 ChCs) that were comparable to those found in G42 mice (Fig. 8). Moreover, mouse line (G42 vs *Pvalb*^{Cre}; *Ai14*) showed a lack of effect for most (9 of 10) electrophysiological variables (Fig. 9). These results, together with data from other mouse lines (Woodruff et al., 2009; Taniguchi et al., 2013), and from rat PFC (Povysheva et al., 2013), suggest that several physiological properties distinguish mature L1/2 BCs and L1/2 ChCs in general, and not specifically in particular mouse lines.

The data in Figure 8 show that most variables contributing to the FS phenotype change significantly between P12 and P > 30, but do not assess the maturation rate of these variables, nor whether the rate differs between L1/2 BCs and L1/2 ChCs. Thus, next, we assessed the developmental trajectories of the electrophysiological variables showing significant age effect, applying single exponential curve fitting (Fig. 10). Some variables, such as the AP half-width and τ_m , seemed more closely regulated by age, showing rapid developmental trajectories well fit by the exponential functions (Fig. 10). In contrast, other variables measured from the same sample of neurons, such as the *f*-I slope, R_{in} , and I threshold, showed larger variability, and some of these other variables could not be fit by exponential functions (Fig. 10). Hence, to compare the maturation rate of the FS phenotype in L1/2 BCs and L1/2 ChCs, assessed via the exponential time constant τ_{dev} , we focused on the four variables that showed age-related changes, and were fit by single exponential functions in both PV⁺ neuron subtypes (Fig. 10). For these variables, except τ_m , the values at

Figure 9. Intrinsic membrane properties of mature L1/2 BCs and L1/2 ChCs from G42 versus *Pvalb^{Cre};Ai14* mice (Pv). The graphs summarize (mean ± SD) the data of 10 electrophysiological variables contributing to the FS phenotype of L1/2 BCs and L1/2 ChCs measured from a sample of PV⁺ neurons from *Pvalb^{Cre};Ai14* mice (L1/2 BCs: P35–P37; L1/2 ChCs: P33–P42), and compared with a subset of the GFP⁺ PV⁺ neurons matched by age (L1/2 BCs: P34–P50; L1/2 ChCs: P33–P43). Shown above each graph are the *p* values from two-factor ANOVA tests of the effect of mouse line and cell type. For each variable, the *F* statistic values for mouse line and cell type were, respectively, as follows: resting membrane potential (RMP): $F_{(1,25)} = 1.62, 2.18$; τ_m : $F_{(1,25)} = 0.13, 16.2$; R_{in} : $F_{(1,25)} = 0.26, 35.9$; I-threshold: $F_{(1,25)} = 0.0006, 17.8$; AP threshold: $F_{(1,25)} = 0.57, 0.129$; first AP delay: $F_{(1,23)} = 13.1, 42.9$; AP half-width: $F_{(1,25)} = 0.0015, 3.39$; AHP amplitude: $F_{(1,23)} = 0.63, 10.8$; ISI ratio: $F_{(1,25)} = 0.12, 37.8$; *f*-*I* slope: $F_{(1,24)} = 0.74, 0.74$. Q-Q plot and Shapiro–Wilk analysis revealed a deviation from normality of ISI ratio and *f*-*I* slope, for which the ANOVA was performed after log transformation (Shapiro–Wilk: ISI ratio, *p* = 0.094; *f*-*I* slope, *p* = 0.372).

$P > 30$, which estimate the parameter y_0 of the exponential curve fits, did not differ between the PV⁺ neuron subtypes (Fig. 8). Moreover, the P12 values, which estimate the parameter A , did not differ between L1/2 BCs and L1/2 ChCs (Fig. 8). Thus, the maturation of FS properties in L1/2 BCs versus L1/2 ChCs may be distinguished mainly by the rate of development, τ_{dev} , which for each variable had consistently larger values in L1/2 ChCs (Fig. 10), suggesting a slower rate of maturation compared with L1/2 BCs. We next tested the significance of the differences in τ_{dev} , using the combined curve fit statistical model employed to compare the sEPSC frequency in BCs of different layers. We found that the difference in τ_{dev} between L1/2 BCs and L1/2 ChCs was significant in the individual tests for AP half-width ($t_{(110)} = 1.9151, p = 0.029$) and *f*-*I* slope ($t_{(97)} = 1.498, p = 0.037$), but not for the other two parameters (AP-threshold, $t_{(109)} = 0.7663, p = 0.223$; τ_m , $t_{(105)} = 0.9556, p = 0.153$). Given that the four τ_{dev} constants were consistently longer in L1/2 ChCs, we applied a global test using Fisher's method to combine *p* values (Fisher, 1950; Won et al., 2009), to pool the information from the four comparisons. This approach produced an overall *p* value of 0.020,

assuming independence of the four individual tests, and a *p* value of 0.040, after using Brown's method to estimate correlations between individual tests (see Materials and Methods). Moreover, assuming an average correlation among tests of 0.5, which is considered very high for tests of different variables, the resulting conservative combined *p* value is 0.07. Thus, the global comparison of τ_{dev} for the four parameters suggested that FS properties have slower maturation rate in L1/2 ChCs than in L1/2 BCs.

To investigate the development of FS properties in L3/5 BCs, we measured the same 10 variables assessed in PV⁺ neurons from the L1/2 border. At P12, the L3/5 BCs displayed an underdeveloped FS phenotype, similar to that of immature L1/2 BCs, as also reported for layers 3–5 PV⁺ neurons of immature somatosensory cortex (Goldberg et al., 2008, 2011). Comparing the properties of L3/5 BCs between P12 and $P > 28$ revealed that most parameters changed significantly with age, following the same trend observed in L1/2 BCs (Fig. 11). Because we recorded from some mature L3/5 BCs in slices of *Pvalb^{Cre};Ai14* mice, we compared their electrophysiological properties with those of L3/5 BCs from G42 mice. As observed for L1/2 border PV⁺ neurons, most electro-

Figure 10. Developmental trajectory of the intrinsic membrane properties contributing to the FS phenotype of L1/2 BCs and L1/2 ChCs. Shown are plots of the values of each parameter as a function of postnatal age. Each data point represents a single PV⁺ neuron. The plots for four of the parameters, AP half-width, AP threshold, τ_m , and f-I slope, were well fit by an exponential function (thick line) in both the L1/2 BC and L1/2 ChC plots. For R_{in} in L1/2 ChCs, the exponential curve fit did not converge (see Materials and Methods, Statistical analysis), whereas I-threshold did not show a significant effect of age in L1/2 ChCs (Fig. 8). In addition to the fitted curves, the 95% confidence bands of the curve fits are shown, along with the time constants of the single exponential τ_{dev} ($\pm$ SE from the nonlinear regressions).

Figure 11. Developmental changes in the intrinsic membrane properties of L3/5 BCs. The graphs summarize (mean $\pm$ SD) the data of 10 electrophysiological variables contributing to the FS phenotype of L3/5 BCs, measured from a sample of PV⁺ neurons from P12 and P > 28 G42 mice, when all variables reach age-related plateau. Shown above each graph are the t statistics and p values from Student's t tests for each variable. Q–Q plots and Shapiro–Wilk analyses revealed a deviation from normality for AP half-width and ISI ratio, for which the t tests were performed after log transformation (Shapiro–Wilk AP half-width $p = 0.560$; ISI ratio $p = 0.131$).

physiological variables assessed in mature L3/5 BCs (7 of 10) did not differ significantly between mouse lines (Fig. 12). While our data seem to reveal general physiological properties of PV⁺ neurons of all cortical layers independent of specific mouse lines, we cannot rule out the possibility that some PV⁺ neuron subtypes are not labeled in G42 or *Pvalb*^{Cre}; *Ai14* mice. Moreover, additional studies are necessary to determine whether the time course of PV⁺ neuron maturation described here only in G42 mice is a general feature of PV⁺ neurons and thus similarly observed in other mouse lines.

To assess the developmental trajectories of FS properties in L3/5 BCs, we applied exponential curve fitting for the five electrophysiological variables that changed significantly with age, and were fit by single exponential functions in both L1/2 and L3/5 BCs. As in L1/2 BCs, the curve fits showed a rapid time course of development, and for AP half-width, R_{in} , I threshold, and f –I slope, the exponential τ_{dev} constants had shorter values in L3/5 BCs (Fig. 13), compared with L1/2 BCs (Fig. 10). However, for τ_m , the value of τ_{dev} was greater in L3/5 BCs than in L1/2 BCs.

Moreover, in all cases the differences in τ_{dev} between L3/5 and L1/2 BCs were small, and the SEs for τ_{dev} from the nonlinear regressions, were relatively large. Therefore, to assess whether FS properties have different maturation rates in L1/2 BCs than in L3/5 BCs, we tested the significance of the differences in τ_{dev} using combined curve fitting. Importantly, for all variables, except f –I slope, the values at P $\geq$ 30, which estimate y_0 , did not differ between L1/2 and L3/5 BCs ($p = 0.307$, $p = 0.073$, $p = 0.067$, $p = 0.859$), whereas the values at P12, which estimate A , differed significantly ($p = 0.00153$, $p = 0.000023$, $p = 0.0099$, $p = 0.000067$), and were consistently closer to the mature values for each variable in L3/5 BCs. The individual contrasts showed that the τ_{dev} values did not differ significantly for any of the five variables (AP half-width, $t_{(130)} = 1.838$, $p = 0.0683$; R_{in} , $t_{(126)} = 0.516$, $p = 0.6067$; I threshold, $t_{(129)} = 0.384$, $p = 0.7018$; τ_m , $t_{(124)} = 1.1256$, $p = 0.2625$; f –I slope, $t_{(117)} = 0.0498$, $p = 0.9603$), suggesting that in L1/2 and L3/5 BCs, FS properties mature at similar exponential rates. In addition, because it is likely that developmental changes in FS properties start before P12, we conclude that the differences in

Figure 12. Intrinsic membrane properties of mature L3/5 BCs from G42 versus *Pvalb^{Cre};Ai14* mice (Pv). The graphs summarize (mean $\pm$ SD) the data of 10 electrophysiological variables contributing to the FS phenotype of L3/5 BCs, measured from a sample of PV⁺ neurons from *Pvalb^{Cre};Ai14* mice (P35–P37), and compared with a subset of the GFP⁺ PV⁺ neurons matched by age (P38–P45). Shown above each graph are the t statistics and p values from Student's t tests for each variable. Q–Q plots and Shapiro–Wilk analyses revealed a deviation from normality of first AP delay and τ_m , for which the t tests were performed after log transformation (Shapiro–Wilk first AP delay $p = 0.125$; τ_m $p = 0.723$).

P12 values are consistent with an earlier onset of maturation of FS properties in L3/5 BCs than in L1/2 BCs.

Discussion

The distribution of PV⁺ BCs and ChCs across cortical layers differs beginning early in postnatal development

In two mouse lines with PV⁺ neurons labeled via different genetic mechanisms, the laminar distribution of BCs in PFC is consistent with the presence of PV⁺ neuron somata throughout layers 2–6 (Gabbott et al., 1997; Chattopadhyaya et al., 2004; Rymar and Sadikot, 2007; Bartolini et al., 2013). In contrast, PV⁺ ChC somata were exclusively positioned near the L1/2 border, in agreement with our previous report that all FS neurons in PFC layer 5 of C57BL/6 mice were BCs (Rotaru et al., 2011). Moreover, in Sprague Dawley rats, PV⁺ BCs are found across PFC layers 2–5, but PV⁺ ChC somata are found only near the L1/2 border (Massi et al., 2012).

Previous work showed that embryonic recombination in *Nkx2.1^{CreER}* mice labeled numerous ChCs in layer 5 (Taniguchi et al., 2013), which, however, were mostly PV[−] (Taniguchi et al.,

2013), and thus may represent a subpopulation not studied here. It appears, therefore, that two ChC subpopulations, PV⁺ and PV[−] ChCs, are found in the cortex, but PV⁺ ChCs are present only near the L1/2 border. Additional work is necessary to determine whether deep-layer ChCs represent a PV[−] subtype or contain very low PV levels, undetectable with current methods. Since PV expression is activity-dependent (Patz et al., 2004), and we found that L1/2 ChCs have low excitatory drive, it is possible that ChCs are less active than BCs, and therefore express lower PV levels. Indeed, in rat PFCs, ChCs have weaker PV immunoreactivity compared with BCs (Massi et al., 2012). Independent of the mechanisms that determine PV levels in ChCs, we found that the low abundance of PV⁺ ChCs in deep layers of PFCs is observed as early as P12.

ChCs and BCs have rapid and cell-type-dependent trajectories of physiological maturation

We found that during development after P12, glutamate synapse strength, as revealed by the peak sEPSC_{AMPA} amplitude, does not change with age in PFC PV⁺ neurons. In contrast, the

Figure 13. Developmental trajectory of intrinsic electrophysiology variables in L3/5 BCs. Shown are plots of the values of each parameter as a function of postnatal age. Each data point represents a single PV⁺ neuron. Four of the plots (AP duration, R_{in} , τ_m , and I threshold) were well fit by an exponential function (thick line). For the AP threshold there was no significant age effect (Fig. 11). In addition to the fitted curves, 95% confidence bands of the curve fits are shown, as well as the exponential constant values τ_{dev} ($\pm$ SEs from the nonlinear regressions).

sEPSC_{AMPA} frequency increased significantly with age in BCs, markedly enhancing the excitatory drive, but did not change in ChCs. Our data also suggest that the increase in sEPSC_{AMPA} frequency is not related to a developmental increase in network activity, as assessed in acute slices, or in glutamate release probability. Consistent with the idea that the higher EPSC frequency reflects increasing numbers of excitatory synapses in BCs, the density of VGlut1-containing puncta, in apposition to PV⁺ neuron somata in PFC layers 5–6, increases significantly between P14 and P40 (Yang et al., 2013). Since deeper-layer PV⁺ neurons migrate and populate their target layer earlier (Rymar and Sadikot, 2007), it is possible that an earlier laminar positioning determines an earlier trajectory of excitatory synaptogenesis in L3/5 versus L1/2 BCs, consistent with the higher sEPSC_{AMPA} frequency observed here in P12 L3/5 BCs. Similarly, the later migration and laminar positioning of ChCs compared with BCs suggested by previous work (Inan et al., 2012; Taniguchi et al., 2013) may underlie a different trajectory of synaptogenesis in ChCs.

Because the maturation of FS properties is thought to be activity-dependent, the marked differences in excitatory input development would suggest that in ChCs, which have lower excitatory drive throughout development, the FS phenotype has a slower maturation rate. Moreover, our data suggest that an earlier maturation of excitatory inputs in L3/5 BCs may correlate with an earlier maturation of FS properties in L3/5 BCs than in L1/2 BCs. Although we did not test a causal relation between maturation of excitatory inputs and FS properties, we assessed the developmental trajectories of multiple electrophysiological

properties contributing to the FS phenotype. We found that several of these properties changed with age following trajectories that were well fit by exponential functions indicating that FS properties mature at faster rate in L1/2 BCs than in L1/2 ChCs. In contrast, the FS phenotype matured at similar rates in L3/5 and L1/2 BCs, but possibly with an earlier onset in L3/5 BCs.

Estimates from the exponential curve fits showed that by ~P21 FS properties were at 96.1–99.9% of plateau in L3/5 BCs, 88.6–99.6% in L1/2 BCs, and 66.5–96.2% in L1/2 ChCs, suggesting that at P21, near the onset of puberty (Nelson et al., 1990; Laviola et al., 2003), PV⁺ neurons reached a mature state. Thus, despite the differences in developmental trajectories revealed by our study, all PV⁺ neurons across PFC layers 2–5 exhibit physiological properties of an advanced maturation state by the onset of puberty.

The PV⁺ neuron maturation trajectory has implications for the development of PFC circuit function and the pathogenesis of schizophrenia

Although we did not perform tests of a causal relation between the development of excitatory inputs and FS properties, our results are consistent with the idea that excitatory drive from glutamate synapses shapes the maturation of the FS phenotype. The age-related changes in the membrane properties of BCs and ChCs reported here are consistent with previous work showing that PV⁺ neuron maturation enables fast physiological signaling (Doischer et al., 2008; Goldberg et al., 2011). These

include a decrease in R_{in} and shortening of τ_m , which contribute to the short summation time window, and fast dendrosomatic propagation, of synaptic potentials (Goldberg et al., 2011). AP firing similarly developed faster signaling mode, since spikes turned briefer, and low spike-frequency adaptation emerged after P12. Some of these changes were previously attributed to changes in potassium channel gene expression (Okaty et al., 2009), including the Kv3, Kir2, and TASK/TWIK subfamilies of two-pore domain weakly rectifying K^+ channels (Goldberg et al., 2011).

BCs developed properties consistent with the FS phenotype crucial for their role in cortical circuits (Buzsáki and Wang, 2012; Hu et al., 2014). However, mature ChCs differed from BCs in some features, confirming previous reports (Woodruff et al., 2009; Povysheva et al., 2013; Taniguchi et al., 2013). Particularly, ChCs had higher R_{in} , longer τ_m , and greater spike-frequency adaptation, suggesting that ChC physiology somewhat differs from the classical FS properties thought to be necessary to produce gamma band synchrony (Pike et al., 2000; Bartos et al., 2007; Hu et al., 2011). Indeed, unlike BCs, ChCs may not contribute rhythmic inhibition during gamma oscillations (Gulyás et al., 2010; Dugladze et al., 2012; Massi et al., 2012).

Our data suggest that the FS phenotype matures later in PV^+ cells of superficial layers, where corticocortical projection pyramidal cells are more abundant (DeFelipe and Fariñas, 1992). Thus, PV^+ neuron-dependent control may mature later for corticocortical than corticosubcortical output, conveyed via deep-layer pyramidal cells. Our data also indicate that excitatory inputs and intrinsic physiology have mature features in all PFC PV^+ neurons at or before the onset of puberty. Hence, PV^+ neuron maturation may not contribute directly to the improvement of PFC-dependent cognition during adolescence and early adult life. However, we did not study the GABA synapses by which PV^+ neurons control pyramidal cell activity. These synapses could show a protracted development into adolescence and young adulthood.

Our results may have implications for understanding how PV^+ neurons are altered in schizophrenia. First, our data suggest that the deficit of excitatory synapses in PV^+ neurons in schizophrenia (Chung et al., 2016), if produced during development, may disrupt the FS phenotype. Indeed, voltage-dependent potassium channels normally expressed by PV^+ neurons (Rudy and McBain, 2001; Okaty et al., 2009; Georgiev et al., 2012) show altered expression in schizophrenia (Georgiev et al., 2014; Yanagi et al., 2014). Second, our data indicate that BCs may be particularly susceptible to the effects of schizophrenia risk factors before puberty, when excitatory drive increases markedly. Indeed, ablation of the NMDA receptor subunit GluN1, starting at $\sim P7$, affects PV^+ cell function, producing schizophrenia-like behavioral disturbances in adulthood (Belforte et al., 2010). Conversely, GluN1 deletion starting by $\sim P60$, when PV^+ cells are physiologically mature, does not produce significant effects (Belforte et al., 2010). Interestingly, PV^+ cells develop normally in the PFC of *Dlx5/6*-deficient mice until $\sim P50$, but then show altered physiology, correlating with cognitive deficits, by $\sim P63$ – $P80$ (Cho et al., 2015). Similarly, in *ErbB4*-deficient mice, excitatory synapses on PFC PV^+ cells develop normally until P20 (Yang et al., 2013), but show alterations at $P \geq 40$ (Ting et al., 2011; Yang et al., 2013). Thus, PV^+ neurons may be sensitive to the disruptive effects of schizophrenia risk alleles and environmental risk factors, not only during prepubertal development,

but also during later periods, when they display adult-like physiology.

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