Loss of Cyclin-Dependent Kinase 5 from Parvalbumin Interneurons Leads to Hyperinhibition, Decreased Anxiety, and Memory Impairment

Citation

As Published
http://dx.doi.org/10.1523/jneurosci.0969-14.2015

Publisher
Society for Neuroscience

Version
Final published version

Accessed
Wed Mar 06 13:17:03 EST 2019

Citable Link
http://hdl.handle.net/1721.1/98036

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Introduction
Parvalbumin (PV)-expressing interneurons constitute a major (up to 50%) component of inhibitory GABAergic neurons in the cortex (Kubota et al., 1994; Wonders and Anderson, 2006), as well as in other brain regions. For example, ~30% of all interneurons in the hippocampus (including CA1 region) express PV (Kosaka et al., 1987; Katsumaru et al., 1988; Bezaire and Soltesz, 2013). PV interneurons have unique fast-spiking action potential properties that allow for very efficient communication within neuronal networks (Bartos et al., 2007). PV-interneuronal activity is both necessary and sufficient for the generation of gamma oscillations in vivo (Cardin et al., 2009; Sohal et al., 2009). Gamma rhythms were shown to be involved in the regulation of perception, attention, and memory (Uhlhaas and Singer, 2010). Dysfunctions in PV interneurons have been implicated in the pathogenesis of epilepsy, anxiety disorders, and schizophrenia (Wonders and Anderson, 2006). Despite strong interest, the mechanisms regulating the PV interneuron-dependent inhibitory neurotransmission and the relevance of these mechanisms to behavior remain unclear.

Cyclin-dependent kinase 5 (Cdk5) is a proline-directed serine/threonine kinase expressed primarily in the CNS in mammals. Postsynaptic Cdk5 plays a critical role in homeostatic synaptic plasticity by controlling spine-associated RapGAP (SPAR) phosphorylation and degradation of SPAR protein (Pak et al., 2001; Seeburg et al., 2008). Presynaptically, Cdk5 also plays multiple roles, including regulation of synaptic vesicle exocytosis by modulating interactions between syntaxin1 and Sept5 (Tani-guchi et al., 2007) and by phosphorylation of Munc18 (Shuang et al., 1998) and Pctx1 (Cheng et al., 2002), as well as endocytosis by phosphorylating multiple dephosphoinositid proteins (Matsubara et al., 1996; Cousin et al., 2001; Lee et al., 2004). Importantly, a recent study (Kim and Ryan, 2010) demonstrated that a balance of the activities of Cdk5 and protein phosphatase calcineurin is a key regulatory mechanism determining the size of recycling vesicle pool and controlling neurotransmission. Altogether, Cdk5 is considered to be a major regulator of signaling that orchestrates plasticity at the synapse (Su and Tsai, 2010; Mitra et al., 2012). The critical presynaptic role of Cdk5 led us to hypothesize that

Behavioral/Cognitive

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Perturbations in fast-spiking parvalbumin (PV) interneurons are hypothesized to be a major component of various neuropsychiatric disorders; however, the mechanisms regulating PV interneurons remain mostly unknown. Recently, cyclin-dependent kinase 5 (Cdk5) has been shown to function as a major regulator of synaptic plasticity. Here, we demonstrate that genetic ablation of Cdk5 in PV interneurons in mouse brain leads to an increase in GABAergic neurotransmission and impaired synaptic plasticity. PVCre;fCdk5 mice display a range of behavioral abnormalities, including decreased anxiety and memory impairment. Our results reveal a central role of Cdk5 expressed in PV interneurons in gating inhibitory neurotransmission and underscore the importance of such regulation during behavioral tasks. Our findings suggest that Cdk5 can be considered a promising therapeutic target in a variety of conditions attributed to inhibitory interneuronal dysfunction, such as epilepsy, anxiety disorders, and schizophrenia.

Key words: anxiety; axon initial segment; cognition; cyclin-dependent kinase 5; fast-spiking (FS) parvalbumin (PV)-interneurons
removal of this enzyme from PV interneurons may allow to manipu-
late their synaptic properties and behavioral outputs. This, in turn, should provide an insight into the regulation of PV in-
terneurons and the roles of these cells in animal behavior.

Here, we demonstrate that selective ablation of Cdk5 from PV interneurons leads to an increase in the number of synaptic vesicles in PV cells and enhanced GABA release and causes alter-
tations in emotional and cognitive behavior. Our results demon-
strate that Cdk5 gates GABAergic function of PV interneurons regulating their behavioral outputs and thus can be considered a promising molecular target for impairments attributed to PV-
interneuronal dysfunction, such as epilepsy, anxiety disorders, and schizophrenia.

Materials and Methods

All experiments were performed according to the Guide for the Care and Use of Laboratory Animals and were approved by the National Institutes of Health and the Committee on Animal Care at the Massachusetts Institute of Technology.

Subjects. We generated PVCre;fCdk5 mice by crossing PVCre mice (B6;129P2-Pvalbtm1(cre)Arbr; The Jackson Laboratory) with mice carrying floxed alleles of Cdk5 (fCdk5) [B6.129S5(Cg)-Cdk5tm1.1Hlt; The Jackson Laboratory]. Both strains were maintained on the inbred C56BL/6 background for multiple generations. Three- to 4-month-old congenic PVCre;fCdk5 (homozygous mutant) and littermate fCdk5 (control) mice from the F2 generation were used for experiments. For con-
sistency purposes, only male mice were used in all experiments.

Histology and immunohistochemistry. Mice were transcardially per-
fused with PBS, followed by 4% paraformaldehyde (PFA) in 1× PBS. The brains were dissected and postfixed in 4% PFA in PBS at 4°C overnight. Free-floating vibratome coronal sections (40 μm) were incubated in a blocking solution of 10% normal donkey serum, 3% bovine serum albu-
mumin, 0.2% Triton X-100, and 0.02% sodium azide in 1× PBS, followed by 4% paraformaldehyde (PFA and 0.1% glutaraldehyde in PBS. The sections were then incubated with the appropriate Cy3-conjugated (1:1000; The Jackson Laboratory) and Alexa Fluor 488 (1:1000; Invitrogen) secondary antibodies for 2 h at RT. Twenty minute incubations with Hoechst dye (Invitrogen) at RT were performed to label cell nuclei. The following primary antibodies were used: PV-positive interneurons were identified with anti-PV rabbit polyclonal PV-25 antibody at 1:2000 [catalog #PV 25; Resource Research Identifier (RRID) AB10000344; Swant] and mouse monoclonal PV-235 antibody at 1:1000 [catalog #PV 235; RRID AB10000343; Swant]; rabbit polyclonal antibody against Cux I at 1.200 (M 22; catalog #sc-13024; RRID AB2261231; Santa Cruz Biotechnology) was used as a cortical layer marker; and Cdk5 immunoreactivity was identified with C8 rabbit polyclonal antibody at 1:100 (catalog #sc-173; RRID AB631224, Santa Cruz Biotechnology). The axon initial segment (AIS) was identi-
fied with rabbit polyclonal H-212 antibody against ankyyrin-G (AnkG) at 1:400 (catalog #sc-9167; RRID AB2140663; Santa Cruz Biotechnology) and mouse monoclonal S8809 antibody against pan-Na, at 1:200 (catalog #S8809; RRID AB477552; Sigma-Aldrich). Images were acquired using high-resolution multichannel scanning confocal microscopy (Zeiss LSM 510). PV cell numbers in the mutant and control mice were assessed by counting PV-positive cells in the hippocampus and cingulate cortex in every fourth section throughout the brain. The cortical layer distribution of PV cells was assessed by counting their relative numbers in layers II–IV and V–VI of every fourth section through the motor cortex. All the cell counts were performed blind to the genotype of the mice. Labeling of the AIS was performed with anti-AnkG and anti-pan-Na, antibodies. The evaluation of AIS length was performed using an antibody against AnkG and PV. Approximately 100 neurons were compared in corresponding sections of the CA1 area of the hippocampus from three pairs of litter-
mate PVCre;fCdk5 and fCdk5 mice. All PV-positive cells were excluded from AIS scoring. Scoring was performed blind to the genotype of the mice. All the images were acquired using high-resolution multichannel scanning confocal microscopy (Zeiss LSM 510) and quantified using NIH ImageJ software.

Immunogold labeling transmission electron microscopy. PVCre;fCdk5 and control mice (three animals per group) were transcardially perfused with PBS, followed by 4% PFA and 0.1% glutaraldehyde in PBS. The brains were dissected and postfixed in 4% PFA in PBS at 4°C overnight. Free-floating coronal sections (100 μm) were cut using a vibratome (Leica) and incubated in permeabilizing/blocking solution for 1 h at 4°C. Preembedding labeling with anti-PV antibody was conducted over-
night at 4°C. Brain sections were washed and incubated with rabbit anti-
mouse IgG (1:100; Cappel) for 1 h at RT. The section were then incubated with Protein A–gold 5 nm particles (Utrecht University Medical Center, Utrecht, The Netherlands) for 1 h at RT, washed, and fixed in 1% glutar-
aldehyde/PBS for 10 min. The sections were washed in 0.1 M cacodylate buffer and postfixed with 0.5% osmium tetroxide for 30 min, washed in water, and dehydrated in graded alcohols. The samples were then em-
bedded in TAAB Epon (Marivac Canada) between two sheets of Aclar film (order #50425; EMSdiusion.com) and polymerized at 60°C for 48 h. Imaging was performed using a transmission electron microscope. Images were recorded with an AMT 2k CCD camera (Advanced Microscopy Techniques) at 30,000× magnification. Imaging was conducted on three grids per animal in the stratum pyramidale and stratum radiatum areas of the hippocampal CA1 regions. Ultrastuctural analysis was performed for ~100 unequivocally detected PV/Protein A–gold-labeled boutons identified in the CA1 stratum pyra-
idale and stratum radiatum in the corresponding hippocampal sec-
tions from three pairs of control and PVCre;fCdk5 mice. The analyzed PV boutons were not selected for the target specificity. The diameter of the presynaptic area (two-dimensional area; two straight line measurements across the diameter per bouton) was calculated using AMT Image Capture Version 600.214 software (Advanced Microscopy Techniques). Scoring the number of synaptic vesicles was performed in the same PV boutons using NIH ImageJ software.

Mouse behavior. All the behavior experiments were performed using groups of 8–11 3- to 4-month-old littermate PVCre;fCdk5 (mutant) and fCdk5 (control) mice. Mice were housed in groups of three to five animals. Mice were left to acclimate in the testing rooms for 45 min before the experiments. If the same groups of mice were used in different behavioral experiments, the tests were separated by 1 week. The most stressful tests, the fear conditioning and Morris water maze paradigms, were performed last. All the experiments were done during the light phase, the second part of the day, with experimenters that were blind to the genotype and treatment of the mice.

Open field. Activity in a novel environment was measured in the 40 × 40 cm Plexiglas VersaMax chambers (Accuscan Instruments) using sets of 16 photoelectric arrays. During each 60 or 10 min session, the number of beam breaks (activity) was measured automatically.

Elevated plus maze. Anxiety-like behavior was assessed using the ele-
vated plus-maze paradigm. The maze consisted of the central platform (10 × 10 cm) with two opposing open arms (30 × 10 × 0.5 cm) and two arms enclosed by Plexiglas walls (30 × 10 × 30 cm) elevated 50 cm from the floor. The animal’s position was automatically determined, and the mouse was tracked for 10 min with VersaMax software (TSE Systems).

Light/dark exploration. The light/dark apparatus consisted of a trans-
parent Plexiglas open-field box (40 × 40 cm) containing a black Plexiglas box (20 × 20 cm) occupying half of the area. Experiments were con-
ducted in a room with the overhead light off and a bright 120 W lamp directed at the light part of the open-field area. A mouse was placed in the black Plexiglas box, and its behavior was video recorded for 10 min. The frequency of exits to the bright area and the time spent in the bright and the dark areas were scored.

Morris water maze. Spatial reference memory testing was performed in a circular tank (1.2 m) filled with opaque water, 22°C. The walls sur-
rounding the tank contained bright and contrasting shapes that served as
reference cues. The tank contained a fixed platform (10 cm diameter) in a target quadrant. On day 1 of the experiment, mice were placed into the water, guided toward a visible platform, and kept on the platform for 15 s. This procedure was repeated one more time. On the following days, the platform was submerged, and the mice were place into the maze at one of four points randomly facing the wall of the tank. Mice were allowed to search for a platform for 60 s, and, if they did not find a platform, they were gently guided to it and kept on the platform for 15 s. Two trials a day were conducted with 2 min intertrial intervals. Mouse behavior was video recorded and analyzed using Videomot 2 software (TSE Systems). On day 6 of the experiment, the platform was removed, and a probe trial was conducted. The time spent in each of the four quadrants was recorded automatically.

Prepulse inhibition. Testing was conducted using the Startle Monitor System (SM100; Hamilton Kinder). For the first 2 d, animals were habituated to the startle monitor apparatus. On day 3, six different types of trials were then performed on randomized intervals: no acoustic stimulus trials, trials with acoustic stimulus (120 dB, 40 ms) alone, and trials with four different prepulse stimuli (70, 75, 80, and 83 dB, 20 ms each) 100 ms later, followed by an acoustic startle stimulus (120 dB). Prepulse trials were performed in blocks of six in a pseudorandom order with intertrial intervals of 20–30 s. The prepulse inhibition (PPI) level was calculated as [100 – (response amplitude for prepulse stimulus with the startle stimulus/amplitude for the startle stimulus) × 100].

Slice electrophysiology. Transverse hippocampal slices were prepared from 2- to 3-month-old male littermates. The brain was rapidly removed and transferred to ice-cold, oxygenated (95% O2 and 5% CO2) low-Ca2+/high-Mg2+ cutting solution containing the following (in mM): 211 sucrose, 3.3 KCl, 1.3 NaH2PO4, 0.5 CaCl2, 10 MgCl2, 26 NaHCO3, and 11 glucose. Hippocampal slices were cut with a Leica VT1000S vibratome (Leica) and transferred for recovery to a holding chamber containing oxygenated artificial CSF (ACSF) consisting of 124 mM NaCl, 3.3 mM KCl, 1.3 mM NaH2PO4, 2.5 mM CaCl2, 1.5 mM MgCl2, 26 mM NaHCO3, and 11 mM glucose at 32–34°C for 30 min and then allowed to return to RT for at least another 60 min before recording. CA1 field EPSP (fEPSP) evoked by Schaffer–collateral (SC) stimulation were measured at 28–30°C as described previously (Oh et al., 2010). Long-term potentiation (LTP) was induced by four episodes of theta burst stimulation (TBS) with 10 s intervals. TBS consisted of 10 brief bursts of stimuli delivered at 70 ms after the stimulus artifact.

NMDAR/AMPAR ratio, picrotoxin was added to ACSF, and EPSCs were recorded at a holding potential of −70 mV (for AMPAR-mediated currents) or at a holding potential of +40 mV (for NMDAR-mediated currents). The amplitude for NMDAR-mediated current was measured 50 ms after the stimulus artifact.

Statistics. Student’s two-tailed t tests were used for analysis of two experimental groups. One-way ANOVA with Tukey’s post hoc test was used when more than two groups were compared. The graphs represent mean values, with error bars indicating SEM. All the statistical analysis was performed using GraphPad Prism (GraphPad Software).

Results
Selective ablation of Cdk5 from PV interneurons
To examine the role of Cdk5 in PV interneurons, we ablated Cdk5 specifically in PV cells by generating PVCre;fCdk5 mice obtained by crossing PVCre+/− animals (Hippenmeyer et al., 2005) with mice carrying loxp alleles of Cdk5 (Samuels et al., 2007; Guan et al., 2011). PVCre;Cdk5 mice are viable, fertile, bred according to Mendelian ratios, do not exhibit developmental disadvantages or anatomical abnormalities, and have normal general health (data not shown). Immunohistochemical examination demonstrated the complete loss of Cdk5 from all observed PV interneurons in various cortical and hippocampal areas in young adult mice (Fig. 1A,B). Characterization of the general brain architecture of the mutant animals did not reveal any obvious abnormalities. Survival, migration, and differentiation of PV interneurons were unaffected by the loss of Cdk5 (Fig. 1C–E).

Decreased anxiety-related behavior in PVCre;fCdk5 mice
We performed a general battery of tests to analyze behavior of the PVCre;Cdk5 mice. All the tests were conducted using groups of 3- to 4-month-old PVCre;Cdk5 and control fCdk5 male littermates, 8–11 animals per group. Examination of the novelty-induced locomotion and in the open-field arena revealed an increase in horizontal activity (p < 0.05; Fig. 2A) and total distance traveled by PVCre;Cdk5 mice (p < 0.05; Fig. 2A) compared with control animals.

To examine the influence of Cdk5 ablation on anxiety-related behavior, we conducted an elevated plus-maze test (Dawson and Tricklebank, 1995). Remarkably, PVCre;Cdk5 mice showed a significantly increased frequency of entries into the open arms (10.2 ± 2.4) and spent significantly more time in the open arms (63.9 ± 16.4 s) and less time in the closed arms (466.4 ± 21.9 s) than the control mice (3.0 ± 0.6, 6.2 ± 2.7, and 555.8 ± 23.7 s, respectively; p < 0.01, p < 0.05, and p < 0.01 respectively; Fig. 2B). These behaviors indicate decreased anxiety in the PVCre;fCdk5 mice.

To extend the observation of anxiety phenotype in the PVCre;fCdk5 mice, the light/dark exploration test was performed (Crawley and Goodwin, 1980; Crawley, 2007). Although the total time that PVCre;Cdk5 mice spent in the light area was not significantly different from the controls (164.2 ± 24.5 vs 106.6 ± 25.3 s; p = 0.09), PVCre;Cdk5 animals exhibited a significantly shortened latency to enter the brightly lit area (36.8 ± 8.9 vs 143.6 ± 37.4 s; p < 0.001) and an increased number of transitions to the light (14.4 ± 1.3 vs 9.6 ± 1.9; p < 0.05; Fig. 2C).

PVCre;fCdk5 mice exhibit impaired learning and memory
To evaluate cognitive function in the PVCre;Cdk5 mice, we performed pavlovian cued and contextual fear conditioning (Phillips and LeDoux, 1992). Both hippocampus- and amygdala-dependent contextual fear memory (62 ± 8 vs 41 ± 6.5% freezing events; p < 0.05) and amygdala-dependent cued fear memory (58 ± 7 vs 37 ± 4% freezing events; p < 0.01) were significantly decreased in PVCre;Cdk5 mice compared with control animals (Fig. 3A).

To extend our observations of memory impairment in PVCre;fCdk5 mice, we used the Morris water-maze paradigm to evaluate hippocampus-dependent spatial reference memory (Vorhees and Williams, 2006). During 5 d of training, control mice demonstrated a reduction in their escape latency from an average 50.33 ± 4.9 to 13.6 ± 8.7 s (Fig. 3B). In contrast, the PVCre;Cdk5
mice showed poor learning, with less reduction in their escape latency (46.06 ± 9.2 to 35 ± 17.6 s), which was significantly worse than in control mice (p = 0.023). A probe trial conducted 24 h after the last training session also revealed significant differences in target versus opposite quadrant preference between the mutant and control groups of animals (p < 0.05 for fCdk5 mice and p > 0.05 for PVCre;fCdk5 mice; Fig. 3B). Control experiments indicated that there was no significant difference in swim speed between fCdk5 and PVCre;fCdk5 mice (p > 0.05; data not shown).
Deficient PPI of acoustic startle reflex in *PVCre;fCdk5* mice

PPI of the startle reflex is a physiological measure of sensorimotor gating, i.e., the ability to process and filter sensory information. PPI was shown to significantly correlate with an operational measure of thought disorder in psychiatric patients and proposed to reflect cognitive fragmentation. An examination of PPI revealed a significant decrease of the inhibition at different prepulse intensities in the *PVCre;fCdk5* mice compared with controls (*p* < 0.05; Fig. 3C). We found no differences in the amplitude of the acoustic startle reflex across the whole range of acoustic startle stimulus intensities (70–120 dB) between *PVCre;fCdk5* and control mice, which indicates that the *PVCre;fCdk5* mice have normal hearing and motor reflexes (Fig. 3C).

Figure 2. *PVCre;fCdk5* mice exhibit decreased anxiety and hyperactivity. A, Horizontal activity and total distance traveled in the open field; movement velocity in the elevated plus maze. B, Frequency of entries into open arms (FOA), time spent in the open arms, frequency of entries to the open arms (FOA)/(frequency of entries to the open + closed arms), frequency of entries to the closed arms, time spent in the closed arms, and distance traveled during 10 min exploration in the elevated plus maze. C, Latency to emerge, the number of dark/light transitions, and the time spent in the light area in the light/dark exploration test. Black, Control mice; red, *PVCre;fCdk5* mice. *p* < 0.05, **p** < 0.01, ***p** < 0.001; error bars indicate ± SEM.
Impaired LTP and decreased basal synaptic transmission in PVCre;fCdk5 mice

To investigate the potential neurophysiological basis of cognitive impairment in PVCre;fCdk5 animals, we tested LTP in the hippocampus of PVCre;fCdk5 and control mice. CA1 fEPSPs were evoked by SC stimulation, and LTP was induced by four episodes of TBS with 10 s intervals. This stimulus induced robust LTP in control mice (148.4 ± 11.0%; however, LTP induction was significantly impaired in the PVCre;fCdk5 animals (108.1 ± 10.6%; p < 0.05; Fig. 4A).

Next, we evaluated basal synaptic transmission using hippocampal slices. The input–output curve was obtained by plotting the slopes of fEPSP against fiber volley amplitudes to calculate basal synaptic transmission. Short-term presynaptic release probability was assessed using the paired-pulse facilitation (PPF) ratio, which was plotted against various interstimulus intervals. We found a significant decrease in the average slope of fEPSP (p < 0.01; Fig. 4B), which suggests a downregulation of synaptic transmission. However, there was no difference in PPF between control and PVCre;fCdk5 mice (p > 0.05; Fig. 4C), which suggests normal presynaptic glutamate release probability.

To evaluate the potential contribution of inhibitory neurotransmission to the LTP impairment observed in PVCre;fCdk5 mice, we tested LTP induction in the hippocampus of PVCre;fCdk5 and control animals in the presence of picrotoxin, a non-competitive antagonist of GABA<sub>A</sub> receptors. LTP was induced in hippocampal slices using the protocol described previously. Remarkably, application of picrotoxin (100 μM) resulted in complete rescue of the LTP (165.4 ± 9.3 vs 157.1 ± 19.4% in controls;
Fig. 4 Impaired LTP and decreased basal synaptic transmission in PVCre;fCdk5 mice. A, LTP in CA1 in acute hippocampal slices from 3-month-old control (fCdk5; 5 animals, 8 slices) and littermate PVCre;fCdk5 (5 animals, 10 slices) mice. fEPSP slope is shown as percentage of baseline. The traces were taken 1 min before TBS (gray) and at the end of the recording (black). Calibration: 1 mV, 10 ms. A bar graph shows the average of fEPSP as a percentage of baseline during the last 10 min of recording. B, C, CA1 fEPSPs evoked by SC pathway stimulation in acute hippocampal slices. B, The input–output curve was obtained by plotting the slopes of fEPSP against fiber valley amplitudes. Calibration: 1 mV, 10 ms. C, PPF ratio was plotted against interstimulus interval (control, 3 animals, 11 slices; PVCre;fCdk5, 3 animals, 9 slices). Calibration: 1 mV, 250 ms. D, LTP in CA1 in acute hippocampal slices from 3-month-old control (4 animals, 8 slices) and littermate PVCre;fCdk5 (4 animals, 9 slices) mice in the presence of picrotoxin (PTX; 100 μM). The traces were taken 1 min before TBS (gray) and at the end of the recording (black). Calibration: 1 mV, 10 ms. A bar graph shows the average of fEPSP as a percentage of baseline during the last 10 min of recording. E, CA1 fEPSPs evoked by SC pathway stimulation in the presence of picrotoxin. The input–output curve was obtained by plotting the slopes of fEPSP against fiber valley amplitudes. Calibration: 1 mV, 10 ms. Black, Control mice; red, PVCre;fCdk5 mice. *p < 0.05, **p < 0.01; error bars indicate ±SEM.

$\rho > 0.05$; Fig. 4D) and basal synaptic transmission (Fig. 4E) deficit in PVCre;fCdk5 mice, suggesting that these impairments in PVCre;fCdk5 animals were attributable to an abnormal increase in GABAergic function.

Increase in GABAergic neurotransmission in PVCre;fCdk5 mice

GABAergic PV interneurons provide a main source of inhibitory control in the mammalian brain by exerting a hyperpolarizing
influence over pyramidal neurons (Glickfeld et al., 2009). To directly examine inhibitory neurotransmission in PVCre;fCdk5 and control animals, we conducted whole-cell recordings in CA1 pyramidal neurons. Whereas there was no significant difference in mEPSC between PVCre;fCdk5 and control mice (Fig. 5A,B), we observed that mIPSC frequency was approximately twofold greater in PVCre;fCdk5 (1.8 ± 0.3 Hz) than in control littersmates (0.94 ± 0.2 Hz; p < 0.05; Fig. 5C,D), which suggests an increased GABA release.

To further strengthen our conclusion of an increased inhibition in PVCre;fCdk5 mice, we measured IPsCs in CA1 pyramidal neurons during SC stimulation. To isolate IPsCs, the responses to activation in (and control mice. Because AnkG is a main protein of Cdk5 in afferent and control mice, respectively (0.33 ± 0.03 vs 0.23 ± 0.02; p < 0.01; Fig. 5G,H).

To further evaluate potential ultrastructural alterations that may lead to an increased GABA release in PV− Cdk5− interneurons, we performed electron microscopy analysis using transverse hippocampal floating brain sections. Remarkably, the measurements revealed an approximately twofold increase in average PV+ bouton diameter in PVCre;fCdk5 brains compared with controls (1.49 ± 0.12 vs 0.72 ± 0.04 μm; p < 0.0001; Fig. 5H). Estimation of the total number of synaptic vesicles in PV/Protein A–gold-labeled boutons detected unequivocally in the CA1 area of the hippocampus showed significantly increased number of vesicles in PVCre;fCdk5 mice compared with controls (102.74 ± 7.38 vs 55.67 ± 3.08 vesicles; p < 0.0001; Fig. 5I).

Increased length of AIS in excitatory neurons in PVCre;fCdk5 mice

The synapses formed by PV interneurons onto excitatory neurons are mostly located perisomatically or at the AIS. Recent findings demonstrate that the size of the AIS can be regulated by presynaptic activity and that this variability serves as a component of synaptic plasticity that fine-tunes neuronal excitability (Grubb and Burrone, 2010; Kuba et al., 2010). To determine the potential effect of an increased inhibitory synaptic activity on AIS parameters, we calculated the average length of the AIS in excitatory neurons in the CA1 area of the hippocampus of PVCre;fCdk5 and control mice. Because AnkG is a main protein component located at the AIS and is required for its maintenance (Hedstrom et al., 2008), we used AnkG immunoreactivity to identify the AIS in neuronal populations. The scoring revealed a highly significant increase in the size of the AIS in PVCre;fCdk5 animals compared with control littersmates (p < 0.0001; Fig. 6). Calculating the length of AnkG-delineated AIS in the cingulate cortex produced the results very similar to our hippocampal findings: a significant increase in the length of AIS in pyramidal neurons in PVCre;fCdk5 animals compared with controls (data not shown).

Discussion

Here we show that reduction of Cdk5, an important component of synaptic function, selectively in PV interneurons affects neurophysiological processes at cellular, network, and behavioral levels. PVCre;fCdk5 mice exhibit increased GABAergic neurotransmission, decreased anxiety, and cognitive impairment.

Evaluation of the potential mechanism of an enhanced GABAergic function in PVCre;fCdk5 animals indicated that Cdk5 ablation leads to an increased GABA release. Our results are in good agreement with recent findings that Cdk5 acts presynaptically as a major negative constraint on neurotransmission via regulating synaptic vesicle pools and release probability in excitatory neurons (Kim and Ryan, 2010, 2013), but our study is the first one to demonstrate such a role for Cdk5 specifically in PV interneurons. Furthermore, examination of the ultrastructural basis for an enhanced GABAergic function in PVCre;fCdk5 mice revealed a significant increase in the diameter of synaptic boutons and, importantly, the number of synaptic vesicles in PV− Cdk5− cells compared with controls. Such an increase in the number of vesicles may explain an observed increase in GABAergic neurotransmission and demonstrate a structural mechanism underlying the function of Cdk5 in neurotransmitter release in PV interneurons (Pierce and Lewin, 1994; Nüsser et al., 1998; Mackenzie et al., 1999; Kubota and Kawaguchi, 2000). We should note that neurotransmitter release could also be facilitated by presynaptic NMDARs (Beretta and Jones, 1996; Corlew et al., 2007). Therefore, our demonstration of an increased PV-interneuronal NMDAR/AMPAR ratio in PVCre;fCdk5 mice suggests that Cdk5 could control GABA release at least in part via regulating surface NMDARs in PV interneurons.

We showed that stimulation-induced inhibitory synaptic transmission is increased in PVCre;fCdk5 mice compared with controls. A significant decrease in the average slope of CA1 fEPSP, which is rescued by picrotoxin, and normal PPF and mEPSC in PVCre;fCdk5 mice suggest that the decrease in basal synaptic transmission is not attributable to an alteration in excitatory neurotransmission but is a result of an increased inhibition in these animals. The observed decrease in hippocampal LTP and rescue of this deficit by picrotoxin suggests that the main reason for an impaired LTP in PVCre;fCdk5 mice is an increased inhibition.

We demonstrated that PVCre;fCdk5 mice display specific emotional and cognitive deregulation. The most pronounced phenotype was a decrease in anxiety-related behaviors, which phenocopies the effect of the GABA_A agonist diazepam (Fraser et al., 2010) and can be explained by increased GABAergic neurotransmission in these animals. The observed PPI deficit in PVCre;fCdk5 mice is also likely a result of a hyperinhibition. The cognitive impairments observed in PVCre;fCdk5 mice appear to reflect a decreased hippocampus-dependent LTP.

It may be interesting to compare our current behavioral findings with our previous report (Su et al., 2013), describing ablation of Cdk5 in the forebrain excitatory neurons using aCaMKIICre (Cdk5f/f/CW2 mice). The most pronounced behavioral abnormality exhibited by Cdk5f/f/CW2 mice was a strong hyperactivity evident throughout multiple paradigms. Additionally, Cdk5f/f/CW2 animals displayed an impaired performance in fear conditioning and Morris water-maze tasks, light/dark exploration, forced swim, and acoustic startle tests. Although PVCre;fCdk5 animals also display a decrease in anxiety and cognitive impair-
Figure 5. **PVCre;fCdk5** mice display increased inhibitory neurotransmission. **A–F**, Whole-cell recordings from pyramidal cells in the CA1 area of acute hippocampal slices from control (fCdk5) and PVCre;fCdk5 mice. **A**, Representative traces of mEPSCs from control and PVCre;fCdk5 mice. Calibration: 20 pA, 200 ms. **B**, Cumulative probability distribution histograms and bar graphs showing average values of mEPSC frequency or amplitude from control (3 animals, 9 cells) and PVCre;fCdk5 mice (3 animals, 9 cells). **C**, Representative traces of mIPSCs from control and PVCre;fCdk5 mice. Calibration: 25 pA, 500 ms. **D**, Cumulative probability distribution histograms and bar graphs showing average values of mIPSC frequency and amplitude in control (4 animals, 7 cells) and PVCre;fCdk5 (5 animals, 9 cells) mice. **E**, mIPSCs recording from control (3 animals, 9 cells) and PVCre;fCdk5 (3 animals, 11 cells) mice. IPSCs amplitude plotted against stimulus intensity is shown. Calibration: 50 pA, 50 ms. **F**, Paired-pulse depression (PPD) of IPSCs was measured in control (3 animals, 5 slices) and PVCre;fCdk5 (3 animals, 7 slices) mice. Calibration: 50 pA, 50 ms. **G**, IPSCs were identified based on their morphological features and later confirmed by filling with Alexa Fluor 488 hydrazide combined with anti-PV antibody labeling. Scale bar, 15 μm. **H**, NMDAR- or AMPAR-mediated current was measured at PV interneurons in hippocampal CA1 region in control (3 animals, 8 cells) and PVCre;fCdk5 (3 animals, 9 cells) mice. Calibration: 50 pA, 50 ms. **I**, Synaptic boutons of PV interneurons were identified in control and PVCre;fCdk5 mice by preembedding immunogold labeling transmission electron microscopy in ultrathin hippocampal sections. Diameter of all unequivocally identified PV boutons and number of synaptic vesicles per bouton were measured in corresponding hippocampal sections in CA1 stratum pyramidale and stratum radiatum from control and PVCre;fCdk5 mice (3 animals and ~100 boutons per group). Scale bars, 100 nm. Red arrows, PV/Protein A gold particles; white contours, PV bouton outlines; black, control mice; red, PVCre;fCdk5 mice; ns, not significant. *p < 0.05, ** p < 0.01, ***p < 0.001; error bars indicate SEM.
ment, there are some obvious behavioral dissimilarities between two transgenic strains. They include deficient PPI and normal startle reflex in PVCre;fCdk5 animals but normal PPI and startle hyper-reactivity and decreased behavioral despair in Cdk5f/f/CW2 animals. Although it is not easy to directly compare the mutant strains analyzed because of partially non-overlapping brain areas affected by the Cre lines used, we speculate that some behavioral domains, such as learning and memory, may require normal Cdk5 expression and function in multiple neuronal types. Therefore, a loss of Cdk5 from either excitatory neurons or PV interneurons may lead to a cognitive abnormality. The differences in PPI between two strains could be attributable to a different contribution of excitatory forebrain neurons and inhibitory PV interneurons to a sensorimotor gating, which may be of particular relevance for disorders such as schizophrenia. Deregulation of pathways associated with mania could be responsible for mania-like features, such as hyper-reactivity and decreased behavioral despair in Cdk5f/f/CW2 mice (Prickaerts et al., 2006; Su et al., 2012). Cdk5 has been shown to regulate synaptic vesicle pathways by phosphorylating the dephosphin proteins, such as dynamin I and synaptojanin, that, in turn, are dephosphorylated by the protein phosphatase calcineurin (Matsubara et al., 1996; Cousin et al., 2001; Lee et al., 2004). Interestingly, Kim and Ryan (2010) have shown that a balance of the activities of Cdk5 and calcineurin serves as a key regulatory mechanism determining the size of recycling vesicle pool and controlling neurotransmission. Based on these findings, we would suggest that gating inhibitory neurotransmission by Cdk5 in PV interneurons may occur via phosphorylation of some of the presynaptic dephosphins. It is also feasible that Cdk5 could determine release probability at least in part through regulating voltage-gated calcium channels (Kim and Ryan, 2013). Additionally, our results suggest that the Cdk5 could negatively regulate GABA release from PV interneurons via downregulating surface NMDARs. Additional studies should help to decipher the network of molecular mechanisms underlying the critical role of Cdk5 in gating GABAergic function of PV cells.

Figure 6. An increase in the length of the AIS in pyramidal neurons in the hippocampus of PVCre;fCdk5 mice. A, AnkG (green) colocalizes with pan-Na+(red) immunoreactivity at the AIS. B, AnkG immunoreactivity (green) as a marker of AIS of the pyramidal neurons in the corresponding hippocampal sections from control (fCdk5) and PVCre;fCdk5 animals (blue, Hoechst). Scale bars: A, 10 μm; B, 50 μm. C, Quantification of the length (micrometers) of the AIS in the neurons in the CA1 area of the hippocampus revealed by AnkG immunoreactivity. Magnification: main images, 20×; insets, 100×. Approximately 100 neurons were scored in the corresponding hippocampal sections from three pairs of control and PVCre;fCdk5 3.5-month-old littermate mice. Note a significant increase in the average length of the AIS in PVCre;fCdk5 mice compared with controls. Black, Control mice; red, PVCre;fCdk5 mice. ***p < 0.001; error bars indicate ±SEM.
The hypofunction of PV interneurons is among the most consistent findings in patients with schizophrenia, a devastating major psychiatric disorder that is characterized by psychotic symptoms and cognitive impairments (Behrens and Sejnowski, 2009; Lewis et al., 2011). Moreover, decreased inhibition has been implicated in the pathobiology of a number of other neurologic and neuropsychiatric illnesses (Wonders and Anderson, 2006). Our finding that Cdk5 expressed in PV interneurons acts a critical negative regulator of inhibitory neurotransmission may suggest potential Cdk5-based treatment strategies for interneuronopathy-related disorders.

In summary, our results indicate that Cdk5 gates GABAergic function of PV interneurons regulating their behavioral outputs and thus can be considered a promising molecular target for therapeutic interventions in a variety of conditions attributed to PV-interneuronal dysfunction, such as epilepsy, anxiety, and schizophrenia.

References


