Reduced Chemical and Electrical Connections of Fast-Spiking Interneurons
in Experimental Cortical Dysplasia

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Running Head: Synaptic and electrical connections in cortical dysplasia

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ABSTRACT

Aberrant neural connections are regarded as a principal factor contributing to epileptogenesis. This study examined chemical and electrical connections between fast-spiking (FS), parvalbumin (PV) immunoreactive (Ir, FS-PV) interneurons and regular spiking (RS) neurons (pyramidal neurons or spiny stellate neurons) in a rat model of prenatal irradiation-induced cortical dysplasia. Presynaptic action potentials were evoked by current injection and the elicited unitary inhibitory or excitatory postsynaptic potentials (uIPSPs or uEPSPs) were recorded in the postsynaptic cell. In dysplastic cortex, connection rates between presynaptic FS-PV interneurons and postsynaptic RS neurons and FS-PV interneurons, and uIPSP amplitudes were significantly smaller than controls. But both failure rates and coefficient of variation of uIPSP amplitudes were larger than controls. In contrast, connection rates from RS neurons to FS-PV interneurons and uEPSPs amplitude were similar in the two groups. Assessment of the paired pulse ratio showed a significant decrease in synaptic release probability at FS-PV interneuronal terminals and the density of terminal boutons on axons of biocytin-filled FS-PV interneurons was also decreased, suggesting presynaptic dysfunction in chemical synapses formed by FS-PV interneurons. Electrical connections were observed between FS-PV interneurons, and the connection rates and coupling coefficients were smaller in dysplastic cortex than controls. In dysplastic cortex, we found a reduced synaptic efficiency for uIPSPs originating from FS-PV interneurons regardless of the type of target cell and impaired electrical connections between FS-PV interneurons. This expands our understanding of the fundamental impairment of inhibition in this model and may have relevance for certain types of human cortical dysplasia.

KEY WORDS: epilepsy, inhibition, irradiation, neocortex, regular spiking
INTRODUCTION

Cortical dysplasia (CD) is a major cause of intractable epilepsy across all age groups. In spite of intensive studies for over 20 years, we are only now beginning to arrive at a better understanding of the different types of focal CD (FCD) and their clinical manifestations. Even less is known about the etiology and mechanisms of pathogenesis for the different types of FCD. In 2011, the latest classification system for FCD was presented (Blümcke et al. 2011). It includes Type I for disorders primarily of cytoarchitecture, Type II for abnormalities of cellular size and shape, and Type III for FCD associated with other potentially epileptogenic lesions. Type I FCD shows loss of the normal 6-layered arrangement of the neocortex along with neurons that may be hypertrophic and show disorientation with respect to the pial surface. Although Type II FCD has received much attention based on the presence of bizarre cellular abnormalities such as giant, dysmorphic neurons (Type IIa) and balloon cells (Type IIb), many clinical reports have shown that Type I FCD is just as prevalent (or more so) and creates epilepsy syndromes that are just as severe as Type II FCD (Krsek et al. 2008; 2009; Tassi et al. 2010). Therefore, it is clear that advances in treatment for dysplasia-associated human epilepsy demand a better understanding of the pathogenetic forces at play in Type I FCD.

One of the factors limiting the study of FCD is the paucity of animal models for the different types of FCD. The in utero irradiated rat model has been used to study CD and it most closely resembles FCD Type Ib in humans (Roper et al. 1995; Palmini et al. 2004; Zhou and Roper 2011). Type Ib CD is defined by abnormal tangential cortical lamination which can result in complete absence of recognizable layering outside of layer 1, often with thinning of the cortical mantle (Blumcke et al. 2011). Rats exposed to a low dose of external radiation on gestational day 17 (E17) develop cortical abnormalities that include thinning of the cortex, loss of lamination of the neocortex, disoriented and sometimes hypertrophic neurons in the neocortex, masses of subcortical and periventricular heterotopic gray matter, and accumulations of ectopic neurons in the hippocampus (Cowan and Geller 1960; Roper...
et al. 1995, Marín-Padilla et al. 2003). These animals show spontaneous seizures (Kondo et al. 2001; Kellinghaus et al. 2004) and increased susceptibility to a number of convulsant agents (Roper et al. 1997; Oghlakian et al. 2009). Previous studies have demonstrated a relative loss of inhibitory interneurons in irradiated rats (Roper et al. 1999; Deukmedjian et al. 2004) and a concomitant reduction in inhibitory postsynaptic currents (IPSCs) in pyramidal neurons in the neocortex of irradiated rats (Zhu and Roper 2000). In addition there is a reduction in excitatory postsynaptic currents (EPSCs) in the surviving neocortical interneurons (Zhou et al. 2009a). This reduction of excitatory drive renders the interneurons relatively quiescent when spontaneous action potentials are recorded (Zhou and Roper 2011).

But the recording of spontaneous EPSCs and IPSCs in target cells tells us little about the presynaptic sources of those currents. Fast-spiking cells, in contrast to other types, represent a well-defined subtype of GABAergic interneurons with their characteristic firing pattern and selective expression of parvalbumin (McCormick et al. 1985; Galarreta and Hestrin 2002; Zhou et al. 2009a, Zhou and Roper 2011). They comprise more than half of all GABAergic interneurons in the neocortex (Amitai et al. 2002; Zhou and Roper 2011) and provide powerful inhibitory control of adjacent excitatory and inhibitory cells (Markram et al. 1997; Galarreta and Hestrin 1998; DeFelipe 1999; Gibson et al. 1999; Beierlein et al. 2003; Sun et al. 2006). This study was designed to directly test the strength of synaptic and electrical connections of an important and well-defined subclass of inhibitory neurons (fast-spiking, parvalbumin-immunoreactive; FS-PV) onto neighboring excitatory and inhibitory neurons in the irradiated rat model of CD. We hypothesized that the impaired inhibition shown in our previous work (Zhu and Roper 2000; Zhou and Roper 2010) would also be seen as impaired synaptic strength of FS-PV interneurons onto postsynaptic targets. In addition, we wanted to directly examine the excitatory synaptic contacts that FS-PV interneurons receive from their excitatory neighbors. Based on previous data (Zhou et al. 2009a; Zhou and Roper 2011), we predicted that there...
would be a reduced synaptic strength of excitatory contacts onto FS-PV interneurons. This study used simultaneous whole-cell recordings from pairs of fast-spiking interneurons and excitatory neurons to better understand the local connectivity of those cells in somatosensory cortex in a model of cortical dysplasia. We found reduced synaptic efficiency for inhibitory postsynaptic potentials (IPSPs) originating from the FS-PV interneurons regardless of whether the target cell was excitatory (pyramidal neuron or spiny stellate neuron) or another inhibitory, FS-PV interneuron. In contrast to our predictions, synaptic efficiency of excitatory neurons onto inhibitory interneurons was not altered in the dysplastic cortex. This expands on our understanding of the fundamental impairment of inhibition that is present in this model of CD. Further implications with respect to previous findings are discussed.

MATERIALS AND METHODS

Animals and irradiation

Timed pregnant Sprague-Dawley rats were obtained from Harlan Sprague Dawley Inc. (Indianapolis, Indiana). Unanesthetized, pregnant rats received 225 cGy of external X-irradiation or sham-irradiation on embryonic day 17 (E17). Four- to five-week-old male offspring (62 from 15 control pregnant rats and 46 from 12 irradiated pregnant rats) were used for experiments. The rats were housed in cages under controlled environmental conditions with an artificial light regimen (light on from 7:00 to 19:00). Both food and water were available ad libitum. All animal care and experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Florida.

Preparation of cortical slices

Slices were prepared as described previously (Zhou and Roper 2011). Rats were deeply anesthetized and transcardially perfused with ice-cold cutting solution. After decapitation, the skull was opened and
the whole brain was removed quickly. Coronal slices from somatosensory cortex (400 µm) were cut using a Vibratome (Leica VT1000 s, Leica Microsystems, Wetzlar, Germany). Slices were cut between the level of the appearance of anterior commissure (~9 and ~6 mm anterior to the interaural line, respectively, in control and irradiated rats) and the level of the appearance of hippocampus on the ventral side (~5 and ~3 mm anterior to the interaural line, respectively, in control and irradiated rats) (Paxinos and Watson 1986), which included somatosensory cortex (Lehohla et al. 2001). Slices were kept at room temperature (~ 23°C) in extracellular solution for at least 1 h before moving to the recording chamber. The cutting solution contained (in mM) 220 sucrose, 3.5 KCl, 1.25 NaH2PO4, 26 NaHCO3, 0.5 CaCl2, 7 MgCl2 and 15 D-glucose (osmolarity, 350-360 mOsm); the extracellular solution contained (in mM): 125 NaCl, 3.5 KCl, 1.25 NaH2PO4, 26 NaHCO3, 2 CaCl2, 1 MgCl2 and 15 D-glucose (305-315 mOsm). These solutions were adjusted to pH 7.4 using NaOH and equilibrated with 95% O2-5% CO2.

**Electrophysiology**

Whole cell recordings were performed as described previously (Zhou et al. 2008). Slices were submerged in the recording chamber and perfused with extracellular solution at a constant rate of 3 ml/min. Cortical laminae and individual cells in slices were identified using infrared differential interference contrast microscopy on an inverted microscope (Nikon Eclipse E600FN). Recordings were performed in neurons in visually identified layer IV and V of control and in the middle region of irradiated rat somatosensory cortex. The approximate laminar locations of recorded sites were further identified histologically according to the distribution PV-immunoreactive (Ir) interneurons. PV-Ir interneurons were unevenly distributed throughout layers with a highest density in layer IV and a second in layer V in control cortex; the distribution of PV-Ir interneurons did not show any laminar pattern in dysplastic cortex, but they were relatively dense in the middle region (Zhou and Roper
We could not identify layers in dysplastic cortex due to the loss of lamination that is a hallmark of this model. We recorded most pairs of FS-PV interneuron-spiny stellate neuron in layer IV, FS-PV interneuron-pyramidal neuron in layer V, and FS-PV interneuron- FS-PV interneuron in both layer IV and V in control cortex. We recorded those pairs in the middle region of dysplastic cortex. We compared connectivity from control to dysplastic cortex. Somata of neurons located deep within the slices (~150 - 250 μm from the surface) were chosen for recording so that their dendritic and axonal processes were less likely to be cut by the slicing process and their connectivity could be better preserved. To test connections of fast-spiking, parvalbumin-immunoreactive (FS-PV) interneurons and other neurons, we chose two adjacent somata for dual somatic whole cell recordings.

The recordings pipettes were pulled from Wiretrol II capillary glass (Drummond Scientific, Broomall, PA) using a Model P-87 Flaming/Brown Micropipette Puller (Sutter Instrument Company, Novato, CA). Pipette resistances were 5 - 7 MΩ in extracellular bath solution when filled with pipette (intracellular) solution that consisted of (in mM) 130 K-gluconate, 3 KCl, 1.5 NaCl, 10 HEPES, 4 MgATP, 0.3 Na₃GTP, 0.2 EGTA, 4 Na₂-phosphocreatine, and 0.1% biocytin. The solution was adjusted to a pH of 7.25 with KOH and had an osmolarity of 285-290 mOsm. Recordings were performed at 30 °C. Signals were amplified using a MultiClamp 700B (Molecular Devices, Union City, CA). Data acquisition was performed using Clampex 10.1 software via 16-bit data acquisition system Digidata 1320A (Molecular Devices, Union City, CA). Recordings were digitally sampled at 10 - 50 kHz and stored for off-line analysis using Clampfit 10.2 software.

To determine chemical coupling, a presynaptic action potential (AP) was evoked by a brief depolarizing current pulse (5 ms, 200-400 pA) from one cell every 5 s and the elicited unitary inhibitory or excitatory postsynaptic potentials (uIPSPs or uEPSPs) were recorded in the postsynaptic cell at the resting membrane potentials. To obtain paired pulse ratio (PPR), two consecutive presynaptic FS-PV action potentials were evoked at an interstimulus interval of 100 ms and repetition
rate of 0.1 Hz (Zhou et al. 2009). To determine electrical connections between two FS-PV cells, subthreshold depolarizing or hyperpolarizing current injections (300 ms duration, –300 to +300 pA) in the presynaptic cell were used to observe the changes of membrane potential in the postsynaptic cell.

**Immunohistochemistry**

Staining was performed as described previously (Zhou et al. 2009b). Slices with biocytin-injected neurons were fixed in 4% paraformaldehyde solution in phosphate buffer saline (PBS) for 48 h. After washing twice for 5 min each in 0.5% Triton-100 in PBS, each section was incubated with mouse anti-PV monoclonal antibody (1:1500, Sigma) at 4°C for 72 h with gentle shaking. After washing, sections were then incubated in the secondary antibodies (488 goat anti-mouse IgG, 1:400, used for visualizing PV, and 594 conjugated streptavidin, 1:500, used for visualizing biocytin; Invitrogen, Carlsbad, California, USA) at ~23°C for 2.5 h. The primary and secondary antibodies were diluted in 1% bovine serum albumin, 2% normal goat serum and 0.5% Triton-100 in PBS. After staining, sections were mounted on glass slides in Vectashield mounting medium H-1000 (Vector Labs, Burlingame, CA), cover-slipped and sealed with clear nail polish for imaging. Series images were taken with an Olympus IX81-DSU Spinning Disk Confocal Microscope (Olympus America, Melville, NY). Z-axis image stacks were prepared from the series images. We performed all image processing using Slidebook 4.2 software (Intelligent Imaging Innovations, Inc., Denver, CO).

**Analysis of electrophysiological data**

The PSP (including EPSPs and IPSPs) shape parameters were measured from an average of thirty trials of postsynaptic responses. The PSP peak amplitude was obtained by subtracting the voltage at baseline from the voltage at PSP peak. The latency of the PSP was measured from the peak time of triggered AP to the onset of the PSP. Presynaptic APs were not aligned. The PSP half-width was defined as the
time interval between the rise and decay of the PSP at the 50% peak amplitude of the PSP. The PSP rise time was determined as the time taken for PSP to rise from 10% - 90% of the peak amplitude.

Coefficient of variation (CV) of PSP amplitude was calculated as the standard deviation/mean of 30 successive PSPs. The paired pulse ratio was defined as uIPSP2/uIPSP1 amplitude that was measured from an average of twenty trials of postsynaptic responses. Except for calculation of failure rate, we measured the other parameters without sweeps containing synaptic transmission failures.

The strength of electrical coupling in FS-PV : FS-PV pairs was quantified by calculation of the coupling coefficient, defined as the ratio of the potential change in the postsynaptic cell to the potential change in the current-injected presynaptic cell. The data for the coupling coefficient included connections from cell 1 to cell 2 and from cell 2 to cell 1 and were averaged from both directions. The baseline noise level was determined and response measurements that were smaller than $1.5 \times \text{noise}$ were considered failures of synaptic or electrical coupling.

For intrinsic properties of neurons, the membrane time constant was computed by the monoexponential curve fitting of voltage responses to hyperpolarizing current pulses. AP threshold was determined from a first derivative plot where the $dV/dt$ abruptly increased (5 V s$^{-1}$). Spike widths were measured at half amplitude of APs evoked by depolarizing current injection (300 ms, 300 pA) and were averaged. AP amplitude was measured from the threshold to peak. The slope (in Hz/nA) of the linear regression was determined by the relationship between injected current intensity and firing rates ($f – I$); AP adaptation was defined as the ratio of the first interspike interval (ISI) to the last ISI of APs. The membrane potentials were not corrected for liquid junction potentials that ranged from 9.9 to 10.4 mV (bath solution-patch pipette solution).

We analyzed axon terminals or terminal boutons/swellings, which are GABAergic boutons (Ma and Prince DA 2012), in well biocytin filled FS-PV axonal arbors from stacked image taken with 60 ×
objective. Three terminal axonal segments (each for 100 μm long) from each of FS-PV interneurons in layer IV and V of control cortex and in the middle region of dysplastic cortex were selected randomly and the number of terminal boutons were counted. Bouton density was expressed as bouton number/μm.

Statistical analysis

For all data, mean ± standard error of the mean were given. For comparison of data sets, one-way ANOVA was used to compare group data obtained in different groups unless otherwise stated. Significance level was set at p = 0.05.

RESULTS

1. Identification of cell Types

Neurons with large, triangular somata and a single, main apical dendritic trunk were initially chosen for recording as pyramidal neurons. Neurons without a main apical dendritic trunk (most of them with relatively small and round soma) were initially chosen for recording as spiny stellate neurons or FS interneurons (Fig. 1A). Neurons were further identified by electrophysiological characteristics. Excitatory neurons (pyramidal and spiny stellate neurons) discharged at low firing frequencies with obvious frequency adaptation, but the FS interneurons fired a train of APs at higher frequencies with little or no adaptation when a depolarizing current was applied (Fig. 1B). Excitatory spiny neurons in layer IV were classified as spiny stellate neurons and pyramidal neurons (Schubert et al. 2003); the latter can be subclassified as pyramidal neurons and star pyramidal neurons according to their somatodendritic morphology reported in some previous studies (Staiger et al. 2004), but we did not subclassify the pyramidal neurons in this study due to very similar electrophysiological properties of pyramidal neurons and star pyramidal neurons. Several intrinsic properties (e.g. input resistance) were
significantly different between excitatory and inhibitory interneurons, and this helped confirm the identity of the different cell types (Table 1). There were no significant differences for firing patterns and other intrinsic properties between control and irradiated rats for a given cell type (Table 1). In connected cells, excitatory neurons were also identified during recordings by their ability to elicit uEPSPs in the postsynaptic cell and inhibitory interneurons were identified by their ability to elicit uIPSPs.

The identity of recorded cells was also confirmed using histological characteristics. Pyramidal neurons had a triangular soma, a main apical dendrite and multiple thick primary dendrites (Fig. 2); most FS neurons (235/258 in control cortex and 147/169 in dysplastic cortex) were PV immunoreactive (Ir). PV-Ir interneurons had round or ovoid somata with multiple thin primary dendrites in control and dysplastic cortex (Figs. 2,3). Only FS, PV-Ir interneurons (FS-PV interneurons, \( n = 382 \)) were included for analysis. Spiny stellate neurons were characterized by the absence of an apical dendrite and numerous primary dendrites in control and dysplastic cortex (Fig. 3). Both pyramidal and stellate neurons lacked PV immunoreactivity and they were easily distinguished from FS-PV interneurons (Figs. 2,3). Pairs with at least one FS-PV interneuron were chosen for analysis. Therefore the possible pairs included FS-PV : pyramidal neuron, FS-PV : stellate neuron and FS-PV : FS-PV.

2. Connections between FS-PV interneurons and pyramidal neurons

We recorded from pairs of neighboring FS-PV interneurons and pyramidal neurons in the somatosensory cortex from control and irradiated offspring (Fig. 2). The averaged distance between the two cell somata was 40.6 ± 4.0 \( \mu m \) (\( n = 81 \)) in control and 42.1 ± 4.5 \( \mu m \) (\( n = 52 \)) in irradiated rats (\( P > 0.05 \)). To study chemical synaptic coupling, we generated presynaptic APs by injecting current and recorded the postsynaptic responses.
2.1. Lower inhibitory synaptic connectivity from FS-PV interneurons to pyramidal neurons in dysplastic cortex

In 27 of 81 (33.3%) pairs in control tissue and 8 of 52 (15.4%) pairs in dysplastic cortex, presynaptic APs in FS-PV interneurons produced uIPSPs in postsynaptic pyramidal neurons (Fig. 4), showing that FS-PV-to-pyramid connectivity was significantly lower in irradiated rats than in controls ($P < 0.05$, two-tailed $\chi^2$ test; Table 2). These results suggest that FS-PV interneurons of irradiated rats are less likely to communicate synaptically with neighboring pyramidal neurons compared to controls.

2.2. Weaker inhibitory synaptic connections from FS-PV interneurons to pyramidal neuron in dysplastic cortex

The amplitude of uIPSPs for FS-PV : pyramid pairs in irradiated rats was $0.94 \pm 0.13$ mV ($n = 8$), which was significantly smaller than control values with a mean value of $1.74 \pm 0.19$ mV ($n = 27$, $P < 0.05$; Fig. 4A, Table 2). These results suggest that inhibitory synaptic strength from FS-PV interneurons to pyramidal neurons is weaker in the cortex of irradiated rats. Other uIPSP kinetics including rise time, half-width and latency were similar between control and irradiated rats (Table 2), suggesting that the inhibitory synapses along with somatodendritic domains of the pyramidal neurons and postsynaptic receptors have similar properties.

2.3. Unchanged excitatory synaptic connections from pyramidal neurons to FS-PV interneurons in dysplastic cortex

Presynaptic APs in pyramidal neurons produced uEPSPs in postsynaptic FS-PV interneurons (Fig. 4B). The connection rates and amplitude of uEPSPs from pyramidal neurons to FS-PV cells were similar
between control and irradiated rats \((P > 0.05; \text{Fig. } 4B, \text{Table } 2)\). These results suggest that excitatory connections from pyramidal neurons to FS-PV interneurons are similarly distributed and strong in the two groups. Nine of 81 FS-PV : pyramidal pairs (11.1\%) in controls had reciprocal connections while 2 of 52 pairs (3.8\%) in irradiated rats had reciprocal connections.

3. Connections between FS-PV interneurons and spiny stellate neurons

We made 72 paired recordings in control and 45 pairs in irradiated rats, in which one cell was a spiny stellate neuron and the other was an FS-PV interneuron (Figs. 3 and 5). The distance between the two cell somata was \(41.2 \pm 4.6 \, \mu m\) in control cortex and \(43.4 \pm 5.9 \, \mu m\) in dysplastic cortex \((P > 0.05)\). As shown in Table 2 and Fig. 5, the connection rate from FS-PV interneurons to stellate neurons was significantly lower and the amplitude of uIPSPs was significantly smaller in irradiated rats than those in the controls \((P < 0.05; \text{Fig } 5A)\). However, irradiation did not alter the connection rates or the uEPSP amplitude from stellate neurons to FS-PV interneurons \((P > 0.05; \text{Fig. } 5B)\). In addition, rise time, half-width and latency of both uIPSPs and uEPSPs were not different between the two groups (Table 2).

Eight of 72 pairs (11.1\%) of FS-PV : stellate recordings in control and 1 of 45 pairs (2.2\%) in irradiated rats had reciprocal synaptic connections. The results are similar to those from connections between FS-PV cells and pyramidal neurons.

4. Connections between two FS-PV interneurons

4.1. Chemical synaptic coupling of two FS-PV interneurons

4.1.1. Hypo-connectivity between FS-PV interneurons in dysplastic cortex

We recorded 41 FS-PV interneuron-interneuron pairs in control rats and 25 pairs in irradiated rats (Fig. 6A, Table 2). The distance between the two cell somata was \(35.7 \pm 6.1 \, \mu m\) in control and \(46.9 \pm 7.5 \, \mu m\) in
μm in irradiated rats ($P > 0.05$). In control rats, of 41 pairs, 29 were connected, of which 15 were reciprocal; these pairs yielded 44 chemical connections. In irradiated rats, of 25 pairs, 9 were connected, of which 2 were reciprocal; these pairs yielded 11 uIPSPs (Table 3). These results indicate that dysplastic interneurons are significantly less interconnected than the controls ($P < 0.01$, two-tailed $\chi^2$ test; Table 2). They suggest that the probability of FS-PV interneuron inhibiting a neighboring interneuron was significantly decreased in the cortex of irradiated rats.

4.1.2. Weaker interneuronal synaptic connections in dysplastic cortex

The mean peak amplitude of uIPSPs was $1.97 \pm 0.18$ mV ($n = 44$ uIPSPs) in control rats and $1.01 \pm 0.13$ mV ($n = 11$ uIPSPs) in irradiated rats; they were significantly different ($P < 0.01$; Table 2, Fig. 6B). The mean rise time, half-width and delay of uIPSPs were similar between the two groups ($P > 0.05$; Table 2).

4.2. Electrical Coupling of FS-PV interneurons

4.2.1. Lower rate of electrical connectivity in dysplastic cortex

To test the presence of electrical coupling, we detected the changes in the membrane potential of one neuron in response to the injecting pulses of subthreshold depolarizing or hyperpolarizing current into the other neuron. We found that electrical coupling occurred in 61% (25 of 41 pairs) of control pairs of FS-PV interneurons; however, in irradiated rats, it occurred in only 24% (6 of 25 pairs) of pairs ($P < 0.01$, two-tailed $\chi^2$ test; Fig. 6A,C; Table 3).

4.2.2. Weaker electrical connectivity in dysplastic cortex

We calculated the coupling coefficient to estimate the strength of the electrical coupling. In controls, the coupling coefficient ranged from 0.55% to 5.21% with an average value of $2.25 \pm 0.21\%$ ($n = 50$;
note that data from both directions were included); however, it was significantly reduced in irradiated offspring, ranging from 0.33% to 2.01% with an average value of $0.97 \pm 0.16\%$ in irradiated rats ($n = 12$; $P < 0.05$; Fig. 6C,D). Electrical coupling was always reciprocal and had similar coupling coefficient in both directions in the two groups that were averaged (Fig. 6D). These results suggest that the number and strength of electrical synapses among FS-PV interneurons were dramatically reduced in irradiated rats. Some pairs were connected via both electrically and chemically coupled in the two groups (Table 3, Fig. 6). Electrical coupling was not observed between FS-PV interneurons and excitatory neurons.

5. Impaired efficiency of presynaptic mechanism in FS-PV interneurons in dysplastic cortex

For synaptic connections of FS-PV interneurons to pyramidal neurons, spiny stellate neurons and other FS-PV interneurons, both failure rate and coefficient of variation of uIPSP amplitudes were significantly increased in dysplastic cortex; however, connections from both pyramidal neurons and spiny stellate neurons to FS-PV interneurons (where uEPSPs were elicited) were not significantly changed in dysplastic cortex (Table 2). We also obtained PPR, the ratio of amplitude of IPSPs (IPSP2/IPSP1), as an index of synaptic release probability. In postsynaptic pyramidal neurons, stellate neurons and FS-PV interneurons, we found paired-pulse depression of IPSPs in controls and facilitation of IPSPs in dysplastic cortex in response to paired pulse stimulation in presynaptic FS-PV interneurons; resulting in an increased PPR in dysplastic cortex (Fig. 7). These results suggest that the probability of inhibitory neurotransmitter release by activation of presynaptic terminals from FS-PV interneuron is decreased in dysplastic cortex while the release probability of excitatory terminals from pyramidal neurons and spiny stellate neurons onto FS-PV interneurons is not changed in dysplastic cortex.
A reduction in the number of terminal boutons formed by FS-PV interneurons might also contribute to the reduced FS-PV mediated inhibition (Ma and Prince DA 2012). To address this possibility, we analyzed terminal boutons in well biocytin filled FS-PV axonal arbors. We found that the bouton density was significantly reduced in dysplastic cortex (Fig. 8). The reduced number of synapses formed by single FS-PV interneuron could also contribute to the impaired efficiency of presynaptic mechanisms in FS-PV interneurons in dysplastic cortex.

**DISCUSSION**

Dual recording has demonstrated a reduced chemical synaptic efficiency for uIPSPs originating from FS-PV interneurons to their target excitatory neurons and other FS-PV interneurons; however, the synaptic efficiency of excitatory neurons onto FS-PV interneurons was not altered in dysplastic cortex. Electrical coupling between FS-PV interneurons was also impaired in dysplastic cortex.

*Chemical coupling from FS-PV interneurons to adjacent neurons is impaired in dysplastic cortex*

Synaptic inhibition mediated by GABAergic interneurons exerts a fast and powerful inhibitory control over excitatory neurons and other interneurons in cortex (Tamás et al. 1998; Woo and Lu 2006) and plays an important role in synchronization of normal and abnormal cortical activity (Whittington and Traub 2003; Bartos et al. 2007). Impaired inhibition is observed in many types of human and experimental epilepsy (Cossart et al. 2005; Ma and Prince 2012), and some types of human CD (Calcagnotto et al. 2005). Abnormalities resulting in impaired GABAergic inhibition would make the affected cortex more susceptible to seizure activity. In cortex, GABAergic interneurons make up about 15 - 30% of the total neuronal population (Galarreta and Hestrin 2002). They display distinct morphologies, physiological properties, connectivity patterns, and gene expression profiles (Woo and Lu 2006; DeFelipe et al. 2013). FS interneurons, a well-defined subtype of GABAergic interneurons,
can be reliably identified by their firing pattern and their selective expression of PV as confirmed in
this study and others (McCormick et al. 1985; Galarreta and Hestrin 2002; Zhou et al. 2009a, Zhou and
Roper 2011). FS-PV interneurons are the most prevalent GABAergic neurons in the neocortex (Cauli
et al. 1997; Gonchar and Burkhalter 1997; Pangratz-Fuehrer and Hestrin 2011) and account for more
than half of all GABAergic interneurons (Amitai et al. 2002; Zhou and Roper 2011). They are densest
in the middle region of somatosensory cortex in control (layer IV and V) and irradiated rats (Amitai et
al. 2002; Zhou and Roper 2010). They make very dense contacts on the soma and proximal dendrites
of other neurons (Woodruff and Sah 2007) to provide powerful inhibitory control of adjacent
excitatory and inhibitory cells (Markram et al. 1997; Galarreta and Hestrin 1998; DeFelipe 1999;
Gibson et al. 1999; Beierlein et al. 2003; Sun et al. 2006). Regular-spiking excitatory neurons include
pyramidal neurons in all layers and spiny stellate neurons in layer IV of the neocortex (Markram et al.
1997). Therefore, FS-PV interneurons were chosen in this study to elucidate the alterations of neuronal
connections to adjacent neurons in dysplastic cortex and help us better understand underlying
mechanisms of epileptogenesis in this model.

Most studies of direct neuronal connections have been completed in control, but not pathological,
tissues. This study examined the neuronal connections in both control and dysplastic cortex. The
connection rate among FS-PV interneurons and excitatory or inhibitory neurons in control cortex was
similar to previous studies (Tarczy-Hornoch et al. 1998; Galarreta and Hestrin 2002; Beierlein et al.
2003; Woodruff and Sah 2007); the amplitude of both uIPSPs in excitatory neurons and uEPSPs in
inhibitory interneurons vary widely among previous studies (Holmgren et al. 2003; Ali et al. 2007;
Thomson and Lamy 2007). The amplitudes reported in the present study are well within the range of
previous reports. The amplitudes of uIPSPs and uEPSPs vary at different membrane potentials
(holding or resting). The uIPSPs and uEPSPs may have been recorded at different membrane potentials
from previous studies, and so most of results in previous studies are not directly comparable with data
of this study. Importantly, the present study demonstrated a significant impairment of chemical synaptic efficiency for uIPSPs originating from FS-PV interneurons regardless of whether the target cells was excitatory (pyramidal neuron or spiny stellate neuron) or another FS-PV interneuron in dysplastic cortex.

These results are generally consistent with previous studies in this model of CD showing a reduction in the frequency and amplitude of sIPSCs in pyramidal cells (Zhu and Roper 2000) and in the frequency of IPSCs in interneurons (Xiang et al. 2006; Zhou et al. 2009a). This could result from an impaired neurotransmitter release mechanism in the presynaptic inhibitory terminals (Zhou et al. 2009a). A reduction in number of inhibitory synaptic contacts by FS-PV interneurons onto adjacent excitatory neurons could also provide an explanation, because amplitude of uIPSPs are closely correlated with the numbers of synaptic contacts (Thomson et al. 1996; Xiang et al. 2002), and the smaller number of contacts would lead to a reduced chemical synaptic efficiency in the dysplastic cortex. This explanation is supported by previous studies that have revealed a dramatic loss of PV-ir interneurons and presynaptic terminals in dysplastic cortex (Roper et al. 1999; Zhou and Roper 2010). The reduced number of synapses formed by single FS cells may also contribute to impaired chemical synaptic efficiency observed in this study. However, this issue is difficult to access precisely. Instead, we analyzed the number of terminal boutons formed by FS-PV interneurons and found a reduced terminal bouton density in dysplastic cortex.

Reduced synaptic release probability in GABAergic terminals from FS-PV interneurons could also contribute to the reduced synaptic strength that we found in this study. Failure rates of synaptic transmission were higher in FS-PV interneurons in CD compared to control tissue. We also found paired-pulse depression of uIPSPs in control and facilitation of uIPSPs in dysplastic cortex in response to the paired pulse stimuli in presynaptic FS-PV interneurons. This increased PPR suggests a decreased release probability in dysplastic cortex (Stevens and Wang 1995). These results would also contribute
to the decreased amplitude of uIPSPs in dysplastic cortex. Reduced quantal size could also contribute
to the reduced uIPSPs in CD in this study; however, we have no data that directly addresses this
question.

Another possible explanation involves postsynaptic mechanisms that still remain to be addressed
in this model, such as potential alterations in GABA_A receptors as reported in other epilepsy models
and in some types of human FCD (Brooks-Kayal et al. 1998; DeFazio and Hablitz 1999; Redecker et
al. 2000; Crino et al. 2001). However, this explanation is less likely since the amplitude and kinetics of
sIPSCs and mIPSCs are not altered in irradiated rats (Zhu and Roper 2000). Reduced neuronal
connections from FS-PV interneurons may dramatically decrease inhibitory control of adjacent
neurons, and thereby disrupt the balance of excitatory and inhibitory synaptic inputs to those neurons
and may play a crucial role in epilepsy.

In the present study, we did not find any difference between control and irradiated rats in
chemical synaptic efficiency for uEPSPs originating from excitatory neurons onto FS-PV interneurons.
This was unexpected because previous studies have shown a reduced frequency of sEPSCs and
mEPSCs in the surviving cortical interneurons in this model (Xiang et al. 2006; Zhou et al. 2009a).
This may suggest that the reduced EPSCs in the prior studies are the result of lower numbers of
excitatory connections from subcortical presynaptic neurons (such as thalamic neurons) and/or
intracortical interlaminae (such as layer III excitatory neurons) that were not included in the current
study. Previous studies have demonstrated that thalamic excitatory inputs target cortical layer IV and V
FS-PV interneurons in thalamocortical slices (Sun et al. 2006). However, our previous studies (Xiang
et al. 2006; Zhou et al. 2009a) and the present study have used coronal cortical slices and the
thalamocortical connections would not be well preserved. It is not clear to what extent the severed
axons and terminals from thalamic neurons could contribute to spontaneous and miniature EPSCs that
were reported in our previous studies but one suspects that they would be minor compared to the intact
intracortical connections. Intracortical interlaminar excitatory connections from other layers, such as layer III, to FS-PV interneurons in layers IV and V have also been reported (Thomson et al. 2002). Therefore, decreased interlaminar connections (outside of layers that were studied in the current experiments) may contribute to decreased EPSCs observed in our previous studies. Different underlying molecular mechanisms of single, AP-evoked and spontaneous excitatory neurotransmitter release may also account for this inconsistency (Song et al. 2005).

Interneuronal electrical coupling is impaired in dysplastic cortex

Most neurons communicate via chemical synapses, but neurons can also form direct electrical connections via electrical synapses, also called gap junctions (Connors and Long 2004; Hestrin and Galarreta 2005). Chemical and electrical connections can cooperate in generating a patterned circuit output (LeBeau et al. 2003; Traub et al. 2003; Hestrin 2011). Electrical connections appear to be a cell-type specific feature and have not been observed between different cell-types (Galarreta and Hestrin 1999). Gap junctions play a key role in rhythmic, synchronous population activity, such as gamma frequency (30–80 Hz) oscillations and ultrafast sharp-wave ripples (80–200 Hz) in mammalian brains (Cobb et al. 1995; Whittington et al. 1995; Ylinen et al. 1995; Draguhn et al. 1998; Bartos et al. 2007). These oscillations are thought to play an important role in proper brain functions and the alteration of expression of gap junctions could influence network oscillations and cause neurological disorders, especially epilepsy (MacVicar and Dudek 1981; Perez Velazquez and Carlen 2000; LeBeau et al. 2003; Volman et al. 2011). Gap junctions are formed by connexins in the plasma membrane that can be dynamically modulated leading to changes in neuronal network interactions (Rouach et al. 2002). The present study found that, in control cortex, the incidence of electrical connections between pairs of FS-PV interneurons is about 60% and the coupling coefficient (a measure of the strength of electrical coupling) is about 2%. These results are consistent with previous studies (Gibson et al. 1999; Galarreta...
and Hestrin 2002). The current study has demonstrated that both the incidence and strength of electrical coupling are reduced in FS-PV interneurons in dysplastic cortex. This could result from impairment in the function and expression of connexins in interneurons (Connors, 2009; Deans et al. 2001; Hormuzdi et al. 2001).

**Decreased inhibition in focal cortical dysplasia and epilepsy**

FCD is highly and intrinsically epileptogenic as revealed by intraoperative electrocorticography in patient (Palmini et al. 1995). In vitro studies have also demonstrated that slices from FCD specimens exhibits repetitive, seizure-like discharges in the presence of 4-aminopyridine (Mattia et al. 1995). Although the precise mechanisms of epileptogenesis in CD are unknown, impaired inhibition is observed in tissue slices obtained from patients with Type II FCD (Calcagnotto et al. 2005), and it has been suggested to play an essential role in the epileptogenesis in patients with FCD. Animals with irradiation-induced CD demonstrate spontaneous seizures in vivo (Kondo et al. 2001; Kellinghaus et al. 2004) and increased epileptiform activity in vitro (Roper et al. 1997; Oghlakian et al. 2009). The present study in this model used simultaneous whole-cell recordings to demonstrate the decreased inhibition from FS-PV interneurons that contributes, at least in large part, to decreased inhibition from all different types of interneurons (Zhu and Roper 2000; Zhou et al. 2009a; Zhou and Roper 2011); although we have not examined the connections from other types of interneurons that may also be involved. Taken together, these studies have provided a compelling portrait of impaired inhibition in irradiation-induced CD. Although there is limited electrophysiological data from human CD available for comparison with animal models; we feel that our findings are applicable to some types of human FCD. This is based on reports of loss of GABAergic interneurons in some pathology studies (Ferrer et al. 1994; Spreafico et al. 1998) and reduced frequency of sIPSCs and mIPSCs in pyramidal cells in human Type II FCD (Calcagnotto et al. 2005).
Histologically, the irradiated rat model mimics certain important aspects of human Type Ib FCD that has been well described in the most recent ILAE classification system (Blümcke et al. 2011). These irradiated animals show diffuse cortical abnormalities including thinning of the cortical mantle, loss of lamination, loss of spatial orientation of the neurons, hypertrophic neurons, and periventricular and hippocampal heterotopia (Roper et al. 1995, Marin-Padilla et al. 2003). The model does not produce giant, dysmorphic neurons or balloon cells that are hallmarks of human Type II FCD (Blümcke et al. 2011). Although much has been learned about genetic abnormalities and their pathways in Type II FCD (for review: Lim and Crino 2013), the etiology and pathophysiology of Type I FCD are still relatively unknown. Studies in other models of FCD have yielded a variety of findings about interneuronal function. The freeze-lesion model of FCD has demonstrated increased excitatory synaptic input in inhibitory interneurons (Jacobs and Prince 2005), and increased strength of inhibitory synapses on layer V pyramidal cells (Brill and Huguenard 2010). In contrast, slices from human FCD specimens have shown reduced inhibitory input in pyramidal cells (Calcagnotto et al. 2005) that are similar to findings from the irradiated rat model. At this time, these findings seem hard to reconcile regarding inhibition in CD probably because mechanisms of epileptogenesis vary depending on many factors, such as types, causes and severity of CD.

Disorders of cortical development and GABAergic integrity may have implications beyond epilepsy alone. Impaired cognitive development is the most common co-morbidity in humans with CD-associated epilepsy and there is evidence that CD is an independent of predictor of impaired intelligence in these patients (Leventer et al. 1999; Korman et al. 2013). In addition, irradiated rats have impaired learning and memory functions in cognitive domains controlled by both the hippocampus and the neocortex; consistent with the diffuse distribution of CD in this model (Zhou et al. 2011; Zhou and Roper 2012) and suggesting that CD affects normal brain function beyond the epileptic circuit. Disorders of cortical development have also been implicated in autism and
schizophrenia (Arion et al. 2010; Rubenstein 2011). Of note, impaired inhibition in different brain regions has been postulated to be a prominent contributor to both of these conditions (Rubenstein and Merzenich 2003, Curley et al. 2011). In rhesus macaques, exposure to radiation during early gestation produces changes in the prefrontal cortex that mimic those found in human schizophrenia while exposure during midgestation leads to changes similar to those found in some types of human mental retardation (Selemon et al. 2013). These findings suggest that the types of changes in inhibition that we have observed in irradiated rat may have some relevance to a broad range of human neuropsychiatric disorders (Marín 2012).

In summary, this study has demonstrated that both chemical and electrical connections from FS-PV interneurons to other neurons are decreased in CD. FS-PV interneurons exert powerful inhibitory control over adjacent neurons and play a pivotal role in normal brain function by maintaining the proper balance of excitation and inhibition in the cortical circuitry. Therefore, the reduced direct inhibitory connections that we have shown in dysplastic cortex would contribute to the disrupted balance of excitation and inhibition that has already been documented in this model (Zhu and Roper 2000; Zhou et al. 2009a; Zhou and Roper 2011). These findings further elucidate the multimodal impairment in inhibition that can result from a single in utero insult. We feel that these findings may have direct implications for epileptogenesis in some types of human FCD and may have broader implications for a variety of neurocognitive disorders associated with abnormal cortical development. We are hopeful that knowledge of this type will ultimately lead to better therapies for this people with this debilitating problem.

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NOTES
Conflict of Interest: None declared.
REFERENCES


FIGURE CAPTIONS

FIG. 1. Morphological and electrophysiological characteristics of three types of neurons in control cortex. A: Representative neurons were chosen for recording in control cortex using IR-DIC microscopy. One neuron (on right), with a main apical dendritic trunk, was initially chosen as a pyramidal neuron. The other one (on left), without a main apical dendritic trunk, was initially identified as either a fast-spiking (FS) interneuron or a spiny stellate neuron. They were further characterized by electrophysiological and post hoc histological properties. After identification, it was concluded that the pair represented a pyramidal neuron and an FS interneuron. B: Representative traces illustrating the firing pattern of a representative pyramidal neuron, FS interneuron and spiny stellate neuron. Depolarizing current injection (300 ms, 300 pA) evoked high-frequency, non-adapting discharges in FS interneurons and low-frequency (regular-spiking) action potentials with adaptation in both pyramidal and spiny stellate neurons.

FIG. 2. Immunocytological properties of an FS interneuron and a pyramidal neuron in control and dysplastic cortex. A and C: Photomicrographs showing a biocytin-labeled (red) pyramidal neuron and FS interneuron in control (A) and dysplastic cortex (C). The arrows in the insets point to the double-labeled interneuron. B and D: Labeling for parvalbumin (green) showed that the FS interneuron was PV-Ir but the pyramidal neuron was not.

FIG. 3. Immunocytological properties of FS interneurons and spiny stellate neurons. Representative photomicrographs showing that the biocytin-labeled (red) FS interneurons were PV-Ir (green), but the spiny stellate neurons were not PV-Ir in both control (A) and dysplastic cortex (B). Arrowheads identify spiny stellate neurons in A and B, arrows in insets identify FS interneurons.
FIG. 4. Connections between an FS-PV interneuron and a pyramidal neuron. The neighboring FS-PV interneuron (green circle) and pyramidal neuron (red triangle) formed reciprocal synaptic connections. The presynaptic (Pre) action potential evoked by a suprathreshold current injection lead to a postsynaptic potential (PSP) in the postsynaptic neuron (post). A: Recorded uIPSPs (averaged response in green and 30 individual responses in black) in pyramidal neurons in control and dysplastic cortex when the presynaptic cells were FS-PV interneurons. The amplitude of uIPSP was decreased in the irradiated rats with CD. The two dotted lines (upper right) demonstrate the latency of the uIPSP measured from the peak of triggered AP (left line) to the onset of the uIPSP (right). The latency was similar in the two groups. B: Averaged uEPSPs in FS-PV interneurons when the presynaptic cells were pyramidal neurons. The amplitudes of the uEPSPs were similar in the two groups. The traces are expanded to better show the latency of synaptic response and half-width of action potentials (right).

FIG. 5. Connections between FS-PV interneurons and spiny stellate neurons. The neighboring FS-PV interneuron (green circle) and spiny stellate neuron (red oval) formed reciprocal synaptic connections. A: uIPSPs in spiny stellate neurons in control and dysplastic cortex; the amplitude of uIPSP was reduced in the irradiated rats. The evoked action potential from the presynaptic FS-PV interneuron is not shown. B: uEPSPs in FS-PV interneurons when the presynaptic cell was a spiny stellate neuron. The amplitude of uEPSPs was not different between the two groups.

FIG. 6. Electrical and chemical connections between two FS-PV interneurons. A: Diagram showing electrical and chemical connections. The left and/or upper cell in each pair was designated as Cell 1 and the other one was Cell 2. B: uIPSPs averaged from 30 sweeps in FS-PV Cell 2. The pair of FS-PV interneurons was reciprocally coupled by chemical synapses. The uIPSP in Cell 1 was similar to Cell 2 (not shown). The arrow points to a postsynaptic spikelet induced by electrical connections. C: The
injection of depolarizing or hyperpolarizing current in Cell 1 simultaneously affected the membrane
voltage of the non-injected Cell 2. Traces in C were single sweeps. The coupling coefficient in
irradiated rats was smaller than that in controls. D: Group data for coupling coefficient confirming that
the coupling coefficient was smaller in CD. Electrical coupling was bidirectional and coupling
coefficients from both directions were similar and were averaged from both directions. *$P < 0.01$ vs
control.

**FIG. 7.** Relationship of amplitudes of second and first uIPSP evoked by paired stimuli. A:
Representative paired uIPSPs in pyramidal neuron evoked by paired pulse stimuli in FS-PV
interneuron in control and dysplastic cortex. B: Group data for paired pulse ratio (PPR),
uIPSP2/uIPSP1 in pyramidal neurons, stellate neurons and FS-PV interneurons. *$P < 0.01$ vs control.

**FIG. 8.** Decrease of bouton density in FS-PV interneurons from dysplastic cortex. A and B:
Representative confocal images of biocytin-stained axonal segments with terminal boutons from a
control (A) and a dysplastic FS-PV interneuron (B). C: Group data for bouton density. *$P < 0.01$ vs
control.
### Table 1 Intrinsic properties

<table>
<thead>
<tr>
<th></th>
<th>FS-PV interneurons</th>
<th>Pyramidal neurons</th>
<th>Spiny stellate neurons</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (235)</td>
<td>CD (147)</td>
<td>Control (81)</td>
</tr>
<tr>
<td>$V_{\text{rest}}, \text{mV}$</td>
<td>$-65.2 \pm 0.9$</td>
<td>$-64.4 \pm 1.1$</td>
<td>$-68.5 \pm 1.1$</td>
</tr>
<tr>
<td>$R_{\text{in}}, \text{M} \Omega$</td>
<td>$171.5 \pm 9.6^*$</td>
<td>$182.2 \pm 10.1^{**}$</td>
<td>$215.7 \pm 12.3$</td>
</tr>
<tr>
<td>Time constant, ms</td>
<td>$13.9 \pm 0.9^*$</td>
<td>$14.4 \pm 1.0^{**}$</td>
<td>$19.5 \pm 1.2$</td>
</tr>
<tr>
<td>AP threshold, mV</td>
<td>$-41.6 \pm 0.8$</td>
<td>$-42.0 \pm 0.7$</td>
<td>$-39.5 \pm 0.6$</td>
</tr>
<tr>
<td>AP half-width, ms</td>
<td>$0.42 \pm 0.02^{**}$</td>
<td>$0.44 \pm 0.04^{**}$</td>
<td>$1.12 \pm 0.07$</td>
</tr>
<tr>
<td>AP amplitude, mV</td>
<td>$63.7 \pm 1.1$</td>
<td>$64.4 \pm 0.9$</td>
<td>$67.1 \pm 1.4$</td>
</tr>
<tr>
<td>F-I slope, Hz/nA</td>
<td>$344.8 \pm 15.2^{**}$</td>
<td>$355.6 \pm 21.3^{**}$</td>
<td>$63.7 \pm 6.1$</td>
</tr>
<tr>
<td>$F_{\text{RMax}}, \text{Hz}$</td>
<td>$204.5 \pm 18.3^{**}$</td>
<td>$211.2 \pm 19.1^{**}$</td>
<td>$51.2 \pm 8.2$</td>
</tr>
<tr>
<td>Adaptation</td>
<td>$1.03 \pm 0.04^{**}$</td>
<td>$1.05 \pm 0.06^{**}$</td>
<td>$0.29 \pm 0.07$</td>
</tr>
</tbody>
</table>

FS-PV interneurons, fast-spiking parvalbumin-immunoreactive interneurons; CD, cortical dysplasia; $V_{\text{rest}}$, resting membrane potential; $R_{\text{in}}$, input resistance, obtained by applying a 100 ms hyperpolarizing voltage step of 10 mV from a holding potential of $-65$ mV in whole-cell voltage-clamp mode; AP, action potential; F-I slope, slope of the relationship between injected current intensity and firing rate; $F_{\text{RMax}}$, maximal steady-state firing rate; Adaptation, spike frequency adaptation. The numbers in parentheses indicate the total number of the recorded neurons in pairs with or without connections. $^* P < 0.01$ vs Control pyramidal neurons; $^* P < 0.01$ vs Control spiny stellate neurons; $^# P < 0.01$ vs CD pyramidal neurons; $^* P < 0.01$ vs CD spiny stellate neurons.
## Table 2 Kinetic parameters of EPSPs or IPSPs in different neuronal connections

<table>
<thead>
<tr>
<th></th>
<th>PV and Pyramid</th>
<th>PV and Stellate</th>
<th>PV and PV</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PV→Pyramid</td>
<td>Pyramid→PV</td>
<td>PV→Stellate</td>
<td>Stellate→PV</td>
</tr>
<tr>
<td><strong>Connection rates</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>27/81(33.3%)</td>
<td>20/81(24.7%)</td>
<td>25/72(34.7%)</td>
<td>21/72(29.2%)</td>
</tr>
<tr>
<td>CD</td>
<td>8/52(15.4%)*</td>
<td>14/52(26.9%)</td>
<td>7/45(15.6%)</td>
<td>12/45(26.7%)</td>
</tr>
<tr>
<td><strong>Amplitude, mV</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>1.74 ± 0.19</td>
<td>2.34 ± 0.27</td>
<td>1.67 ± 0.21</td>
<td>2.27 ± 0.26</td>
</tr>
<tr>
<td>CD</td>
<td>0.94 ± 0.13*</td>
<td>2.53 ± 0.36</td>
<td>0.82 ± 0.12*</td>
<td>2.41 ± 0.34</td>
</tr>
<tr>
<td><strong>Failure rate, %</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>1.23 ± 0.47</td>
<td>1.35 ± 0.56</td>
<td>1.27 ± 0.45</td>
<td>1.63 ± 0.71</td>
</tr>
<tr>
<td>CD</td>
<td>5.42 ± 1.88*</td>
<td>1.30 ± 0.92</td>
<td>5.64 ± 1.59**</td>
<td>1.49 ± 0.94</td>
</tr>
<tr>
<td><strong>CV of amplitude</strong></td>
<td></td>
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<td></td>
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<tr>
<td>Con</td>
<td>0.31 ± 0.03</td>
<td>0.29 ± 0.03</td>
<td>0.27 ± 0.02</td>
<td>0.32 ± 0.04</td>
</tr>
<tr>
<td>CD</td>
<td>0.49 ± 0.05**</td>
<td>0.25 ± 0.08</td>
<td>0.54 ± 0.07**</td>
<td>0.30 ± 0.07</td>
</tr>
<tr>
<td><strong>Rise Time, ms</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>3.53 ± 0.44</td>
<td>2.24 ± 0.25</td>
<td>3.37 ± 0.36</td>
<td>2.13 ± 0.25</td>
</tr>
<tr>
<td>CD</td>
<td>3.65 ± 0.71</td>
<td>2.45 ± 0.41</td>
<td>3.71 ± 0.62</td>
<td>2.07 ± 0.34</td>
</tr>
<tr>
<td><strong>Half-width, ms</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Con</td>
<td>25.3 ± 4.8</td>
<td>13.9 ± 2.3</td>
<td>25.7 ± 3.9</td>
<td>12.8 ± 2.4</td>
</tr>
<tr>
<td>CD</td>
<td>25.6 ± 6.1</td>
<td>14.2 ± 2.9</td>
<td>24.7 ± 5.5</td>
<td>13.4 ± 3.6</td>
</tr>
<tr>
<td><strong>Latency, ms</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>1.25 ± 0.20</td>
<td>0.99 ± 0.22</td>
<td>1.27 ± 0.24</td>
<td>1.07 ± 0.26</td>
</tr>
<tr>
<td>CD</td>
<td>1.29 ± 0.23</td>
<td>1.04 ± 0.25</td>
<td>1.28 ± 0.19</td>
<td>1.11 ± 0.23</td>
</tr>
</tbody>
</table>

Con, control; CD, cortical dysplasia; PV, FS-PV interneuron; stellate, spiny stellate neuron; CV, coefficient variation. Connection rate was expressed as the number of connected pairs/total pairs, e.g. in the 81 recorded pairs of PV and pyramid from control, the connection rate of PV to pyramid was 27/81 (connected pairs were 27) and pyramid to PV was 20/81 (connected pairs were 20). *In reciprocal chemical coupling in pairs of PV-FS cells, the data (for amplitude, failure rate, CV, rise time, half width and latency, n = 44 in control and n = 11 in irradiated rats) included the connections from cell 1 to cell 2 and from cell 2 to cell 1. The numbers in parentheses show the connections rate as a percentage value. *P < 0.05 and **P < 0.01 vs Con.
Table 3 Electrical and chemical connectivity among pairs of FS-PV interneurons

<table>
<thead>
<tr>
<th></th>
<th>Chemical</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>–</td>
<td>→</td>
</tr>
<tr>
<td></td>
<td>Con CD</td>
<td>Con CD</td>
</tr>
<tr>
<td>Electrical –</td>
<td>9 (22.0)</td>
<td>6 (14.6)</td>
</tr>
<tr>
<td>Electrical +</td>
<td>3 (7.3)</td>
<td>8 (19.5)</td>
</tr>
<tr>
<td>Total (%)</td>
<td>12 (29.3)</td>
<td>14 (34.1)</td>
</tr>
</tbody>
</table>

Electrical –, absence of electrical connection; Electrical +, presence of electrical connection; Chemical, chemical connection; →, unidirectional chemical connection; ↔, reciprocal chemical connection. *P < 0.01 vs Con, two-tailed χ² test.
Fig. 1

A

B

Pyramidal neuron

Fast-spiking interneuron

Stellate neuron

30 mV
50 ms
Fig. 3
Fig. 5
Fig. 6
**Fig. 7**

(A) Graph showing control and CD conditions with line charts and annotations.

(B) Bar chart comparing paired pulse ratios between Control and CD conditions. The chart includes error bars and numerical data for different categories (PV→Pyramid, PV→Stellate, PV→PV).
Fig. 8