Altered Firing Rates and Patterns in Interneurons in Experimental Cortical Dysplasia

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Cortical dysplasia (CD) is associated with severe epilepsy in humans, and the in utero irradiation of fetal rats provides a model of this disorder. These animals show a selective loss of inhibitory interneurons, and the surviving interneurons have a reduced excitatory synaptic drive. The current study was undertaken to see how alterations in synaptic input would affect spontaneous firing of interneurons in dysplastic cortex. We recorded spontaneous action potentials and excitatory and inhibitory postsynaptic currents (EPSCs and IPSCs, respectively) from somatostatin (SST)-, parvalbumin (PV)-, and calretinin (CR)-immunoreactive (ir) interneurons. We found that SST- and PV-ir interneurons fired less frequently and with less regularity than controls. This corresponded to a relative imbalance in the ratio of EPSCs to IPSCs that favored inhibition. In contrast, CR-ir interneurons from CD showed no differences from controls in spontaneous firing or ratio of EPSCs to IPSCs. Additional studies demonstrated that synaptic input had a powerful effect on spontaneous firing in all interneurons. These findings demonstrate that a relative reduction in excitatory drive results in less active SST- and PV-ir interneurons in irradiated rats. This would further impair cortical inhibition in these animals and may be an important mechanism of epileptogenesis.

Keywords: epilepsy, neocortex, inhibition

Introduction

Epilepsy is one of the most common neurological disorders, afflicting an estimated 65 million people worldwide (Mbuba and Newton 2009). Many patients with epilepsy can be well treated with antiepileptic drugs, but at least 30% of patients suffer from medically intractable epilepsy (Kwan and Brodie 2000; Wheless 2006). Human cortical dysplasia (CD), characterized by a variety of abnormalities in neuronal location, orientation, and morphology (Duong et al. 1994; Mischel et al. 1995), is strongly associated with intractable epilepsy (Taylor et al. 1971; Palmini et al. 1991). Patients with intractable partial seizures and CD are generally thought to be good candidates for surgery (Hong et al. 2005). Some types of human CD demonstrate impaired GABAergic inhibition (Calcagnotto et al. 2005). Abnormalities resulting in impaired GABAergic inhibition would make the affected cortex more susceptible to seizure activity.

Irradiated rats show a reduced density of inhibitory parvalbumin (PV)- and calbindin D28k-immunoreactive (ir) interneurons (Roper et al. 1999) and reduced frequency of inhibitory synaptic currents when recorded from pyramidal cells (Zhu and Roper 2000). More recently, a reduced excitatory drive of surviving interneurons (Xiang et al. 2006) and a reduced inhibitory drive of fast-spiking (FS) interneurons (Zhou et al. 2009) have been found in this model. We have also found that the balance of excitation to inhibition ratio favors inhibition in FS interneurons (Zhou et al. 2009), which may result in an inhibitory neuron with a reduced capacity to fire when needed.

In the neocortex, 70–80% of neurons are glutamatergic excitatory neurons (pyramidal neurons), and most of the others are GABAergic inhibitory interneurons (Markram et al. 2004). Subgroups of neocortical GABAergic interneurons can be distinguished based on microanatomy, electrophysiology, and by their specific expression of various Ca$^{2+}$-binding proteins and neuropeptides (Kawaguchi and Kubota 1997). GABAergic interneurons expressing somatostatin (SST), (PV), and calretinin (CR) comprise 3 separate families of interneurons, which account for the majority of neocortical GABAergic interneurons (Gonchar and Burkhalter 1997; Kawaguchi and Kubota 1997).

The present study was undertaken to determine how alterations in synaptic input onto interneurons in CD affect their spontaneous firing in order to better understand how the altered connectivity ultimately affects cortical function in this model. We studied 3 distinct subtypes of interneurons: SST-, PV-, and CR-expressing. We recorded spontaneous firing rates in cell-attached voltage-clamp mode, without or with the blockade of synaptic currents, and quantified the relative balance of excitatory and inhibitory input by recording excitatory and inhibitory postsynaptic currents (EPSCs and IPSCs, respectively) from the same cell in whole-cell recordings. We found that SST- and PV-ir interneurons demonstrated decreased frequency and decreased regularity of spontaneous action potentials (APs) in dysplastic cortex compared with controls. In contrast, there was no difference in firing rates or regularity between CR-ir interneurons in control and dysplastic cortex. These findings correlated...
with synaptic current measurements that showed a relative imbalance of EPSC-to-IPSC frequency (in favor of inhibition) in SST- and PV-ir cells but a normal ratio in CR-ir cells. This suggests that alterations of synaptic connectivity that are found in CD have a significant impact on the function of the affected interneurons; specifically, that SST- and PV-ir cells are less active in dysplastic cortex than their counterparts in control neocortex. Further experiments with glutamatergic and GABAergic antagonists showed that synaptic excitatory input favors increased firing rates with greater regularity, and synaptic inhibitory input produces lower firing rates with greater irregularity. This was true for all subtypes of interneurons in both control and dysplastic cortex. The net overall effect of all synaptic input was accurately predicted by the ratio of frequency of EPSCs to IPSCs; that is, if the ratio favored excitation, then blockade of glutamatergic and GABAergic transmission resulted in reduced firing rates with increased irregularity and, if the ratio favored inhibition, complete synaptic blockade resulted in increased firing rates with greater regularity.

Materials and Methods

Ethical Information

All experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Florida.

Animals and Irradiation

Embryonic day 17 (E17) pregnant Sprague Dawley rats (Harlan Sprague Dawley Inc.) were either sham-irradiated or irradiated with 225 cGy of external x-rays from a linear accelerator source. Offspring were weaned at postnatal day 21 (P21). Male offspring were sacrificed for experiments at P28–P36. Rats were maintained under 12 h light/dark cycles with ad libitum access to both food and water.

Brain Slice Preparation

Rats were deeply anesthetized with 100 mg/kg (intraperitoneally) sodium pentobarbital and transcardially perfused with ice-cold cutting solution at 70 cm H2O pressure at a flow rate of 15 mL/min for 3 min. The cutting solution contained (in mM) 220 sucrose, 1.25 KCl, 1.25 NaH2PO4, 26 NaHCO3, 0.5 CaCl2, 7 MgCl2, and 10 glucose and was equilibrated with 95% O2-5% CO2 (pH 7.4 was adjusted with KOH, and osmolarity was maintained at 350–360 mOsm). After decapitation, whole brains were quickly removed and cut by a razor blade into blocks in an ice-cold and oxygenated cutting solution. Coronal slices of 300-μm thickness were cut using a Vibratome (Leica VT1000; Leica Microsystems). Sections were taken between the level of the appearance of hippocampus on the ventral side (Lehohla et al. 2006), which included somatosensory cortex (Lehohla et al. 2006), were used to record spontaneous firing rates. Recordings were performed in neurons in layer IV of somatosensory cortex in controls and in the middle region of dysplastic cortex due to absence of lamination (Schubert et al. 2001; Zhou et al. 2009). Neurons without classical morphological characteristics of pyramidal cells (triangular somata, tripolar primary dendritic tree, and a long thick apical dendrite) were randomly chosen for recording.

For recording in cell-attached voltage-clamp mode, the cell was maintained at a holding potential that was applied to achieve 0 pA injected current (Perkins 2006). Recording started when a stable baseline was established, 5–8 min after the cell-attached configuration was formed. After conclusion of the cell-attached recording, the patch of the cell membrane was broken by applying a gentle negative pressure to obtain whole-cell configuration. In this configuration, biocytin entered the cells by passive diffusion and neuronal subtypes could be further identified by electrophysiological characteristics. Synaptic currents were then recorded. EPSCs and IPSCs were recorded from the same cell in voltage-clamp mode at a holding potential of −40 mV. At that potential, EPSCs were recorded as outward currents and EPSCs were recorded as inward currents (Zhou et al. 2009). Recording for EPSCs and IPSCs started within 5–10 min to allow for stability after the whole-cell configuration was established. Cells that were exposed to blockade of synaptic currents in cell-attached voltage-clamp mode experiments were not used for recording of EPSCs and IPSCs in whole-cell recordings. Input resistance of cells was monitored by frequently applying a 100 ms hyperpolarizing voltage step of 10 mV from a holding potential that was applied to achieve 0 pA injection current to test for injection. An upright microscope (Nikon Eclipse E600M) was used to identify cortical lamina using infrared differential interference contrast optics through a 4× lens and to identify individual neurons through a 40× water-immersion lens. Recordings were made using a MultiClamp 700B amplifier (Molecular Devices). Amplifier control and data acquisition were performed using Clampex 10.1 software via 16-bit data acquisition system Digitida 1320A (Molecular Devices). Signals were digitized at 10 kHz and analyzed off-line.

Cell-attached recordings, obtained in voltage-clamp mode (Perkins 2006), were used to record spontaneous firing rates. Recordings were performed in neurons in layer IV of somatosensory cortex in controls and in the middle region of dysplastic cortex due to absence of lamination (Schubert et al. 2001; Zhou et al. 2009). Neurons without classical morphological characteristics of pyramidal cells (triangular somata, tripolar primary dendritic tree, and a long thick apical dendrite) were randomly chosen for recording.

Analysis for Electrophysiological Data

Firing activities from cell-attached or whole-cell recording mode were analyzed using the threshold of event detection module of Clampfit 10.2 (Molecular Devices). To quantitate the regularity of firing, the coefficient of variation of interspike interval (CV ISI) was calculated. CV ISI was determined by dividing the standard deviation (SD) of the interspike interval (ISI) by the mean ISI (Motulsky 1995; Bennett and Wilson 1999). To determine whether test drugs altered neuronal firing rates, analysis of firing rates was based on three 5-min continuous recordings from each cell to obtain averaged data pretreatment, treatment, and after washout of the drugs. 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 increases (Bean 2007). AP amplitude was measured from the threshold to peak. Spike width was measured at half amplitude of AP. The amplitude of fast afterhyperpolarization (fAHP) was defined as the difference in voltage between the spike threshold and the peak negativity after a spike. AP adaptation was defined as the ratio of the last ISI to the first ISI of APs.
We also obtained the relationship between injected current intensity and firing rates (f–I), which is characterized by the slope (in Hz/Na) of the linear regression. The slope for CR-ir cells was not obtained due to lack of correlation between these 2 variables.

Synaptic currents were analyzed using the Mini Analysis Program (Synaptosoft). Inward EPSCs and outward IPSCs were separately detected at the same baseline at the threshold of 6 pA. Analysis of EPSCs and IPSCs was based on 5 min of continuous recording from each cell to obtain averaged data. The automatic detection was verified post hoc by visual inspection. The instantaneous amplitude and frequency of EPSCs and IPSCs were acquired, and the instantaneous EPSC-to-IPSC ratio was obtained. The averaged instantaneous EPSC-to-IPSC ratio of each measure for each cell was calculated. The ratios were then pooled within groups and compared between groups.

### Immunohistochemistry

After recording (with 20-40 min cell-attached recording and at least 15 min whole-cell recording), slices with biocytin-injected neurons were collected for further immunolabeling of proteins with anti-SST, -PV and -CR antibodies. Slices were fixed for 72 h with gentle shaking, washed 3 times, and incubated in the primary antibody at 4 °C for 72 h with gentle shaking, washed 3 times, and incubated in the secondary antibody for 2.5 h at room temperature (−23 °C). After staining, slices were mounted on glass slides in fluoromount aqueous mounting medium (Sigma), coverslipped and sealed with clear nail polish for imaging.

Sections were examined with an Olympus IX81-DSU Spinning Disk Confocal Microscope (Olympus America). Section thickness was measured. Series images from each section were acquired with a z-step of 0.3 μm and an image size of 672 x 512 pixels. z-Axis image stacks were prepared from the series images. For cell counting, slides were rotated such that it contained all layers with immunoreactive cells. The counting number of immunoreactive cells, there were no differences between SST-, PV-, and CR-ir cells, there were no observable differences in

### Chemicals

n-2-amino-5-phosphonopentanoic acid (D-AP5), 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo quinoxaline-2,3-dione (NBQX), picrotoxin (PIC), and tetrodotoxin (TTX) were purchased from Sigma. Suppliers of primary and secondary antibodies were described in the preceding text.

### Statistical Analysis

All values are expressed as mean ± standard error of the mean. Statistical analyses were performed using one-way analysis of variance for comparing group data obtained in different groups and using paired t-test for comparing data obtained in pre- and posttreatment individual cells in the same groups. Significance level was set at P = 0.05. Computations were performed using Origin 7.5.

### Results

**Identification of SST-, PV-, and CR-immunoreactive Interneurons**

Sections with biocytin-injected neurons were subsequently incubated with primary antibodies for SST, PV, and CR and were visualized with secondary antibodies conjugated to Alexa Fluor dyes. Of successfully recovered biocytin-injected cells, 223 of 286 (78.6%) cells in control and 131 of 179 (73.2%) cells in dysplastic cortex were immunoreactive with one of 3 antibodies tested (Fig. 1A, B, C). The recovered cells with labeling of SST, PV, and CR are GABAergic interneurons (Kawaguchi and Kondo 2002). The recovered cells without labeling could be pyramidal cells or interneurons without SST-, PV-, or CR-immunoreactivity. Of immunoreactive cells, 25.6%, 54.3%, and 20.1% of 223 cells from control and 29.0%, 45.8%, and 25.2% of 131 cells from dysplastic cortex were immunoreactive for SST, PV, and CR, respectively. Colocalization of more than one protein was not observed in any of the recovered cells.

SST-ir cell bodies were predominantly fusiform or round in shape with 2 or 3 thin primary dendrites (Fig. 1A). PV-ir cells had mainly round or ovoid somata with 3-5 thin primary dendrites of similar diameter (Fig. 1B). CR-ir cells had fusiform somata with 2 primary dendrites that were antipodal in location (Fig. 1C). Although detailed morphological analyses were not performed, there were no observable differences in control and dysplastic cortex.

Intrinsic membrane properties differed between SST-, PV- and CR-ir interneurons (Table 1 and Fig. 2). For instance, SST-ir cells fired at low frequency with adaptation in response to depolarizing current pulses (Fig. 2A); PV-ir interneurons displayed continuous repetitive discharges at higher frequency with no adaptation (Fig. 2B); CR-ir interneurons discharged initial burst(s) of APs followed by irregularly spaced APs (Fig. 2C). Although intrinsic membrane properties varied between SST-, PV-, and CR-ir cells, there were no differences between controls and irradiated animals for a given cell type (Table 1).

**Cortical Dysplasia Decreases the Number of SST-, PV-, and CR-ir Interneurons**

The density of immunoreactive cells was expressed as cells per 0.1 mm². We randomly choose sections from control and dysplastic cortex (both n = 45) and determined the density of SST-, PV-, and CR-ir cells. The mean shrinkage factor was 1.157 ± 0.003 in control and 1.165 ± 0.005 in dysplastic cortex, and correction of shrinkage was performed for individual cell density. We compared the density of cells of counting field area 108 000 μm² in layer IV in control to the density of the middle region of dysplastic cortex, where we performed recordings. We found that the density of SST-, PV-, and CR-ir cells was decreased by the divided by the corrected area (measured area × shrinkage factor) to obtain cell density. Shrinkage factor is defined as the ratio of the original thickness setting of the vibratome (300 μm) to the section thickness after mounting (Price et al. 2001). We performed all image processing using Slidebook 4.2 software (Intelligent Imaging Innovations, Inc.) and ImageJ software version 1.37v (Wynne Rasband, NIH).
significantly decreased in dysplastic cortex (Fig. 1, all \( P < 0.01 \)). The density of SST-, PV-, and CR-ir cells in layer IV in control cortex was 14.0 \( \pm \) 1.2, 34.1 \( \pm \) 1.8, and 13.4 \( \pm \) 1.1 per 0.1 mm\(^2\), respectively. The density of SST-, PV-, and CR-ir cells in the middle of dysplastic cortex was 10.1 \( \pm \) 1.0, 17.5 \( \pm \) 1.4, and 10.4 \( \pm \) 0.9 per 0.1 mm\(^2\), respectively.

We also acquired the mean density of SST-, PV-, and CR-ir cells from all layers with immunoreactive cells in control (the size of middle of dysplastic cortex was 10.1 \( \pm \) 1.0, 17.5 \( \pm \) 1.4, and 10.4 \( \pm \) 0.9 per 0.1 mm\(^2\), respectively.

Table 1

|                | SST-ir cells | PV-ir cells | CR-ir cells |
|----------------|--------------|-------------|-------------|
|                | Con (57)     | CD (38)     | Con (121)   | CD (60)     | Con (47)   | CD (33)     |
| \( R_{\text{input}} \), M\( \Omega \) | 199.6 \( \pm \) 14.5 | 209.2 \( \pm \) 16.1 | 176.5 \( \pm \) 13.5 | 174.3 \( \pm \) 14.9 | 357.9 \( \pm \) 21.4 | 348.2 \( \pm \) 22.9 |
| Time constant, ms | 16.3 \( \pm \) 1.4 | 16.8 \( \pm \) 1.5 | 14.7 \( \pm \) 1.2 | 15.2 \( \pm \) 1.1 | 22.6 \( \pm \) 1.9 | 20.2 \( \pm \) 1.7 |
| Capacitance, pF  | 79.5 \( \pm \) 7.1 | 81.4 \( \pm \) 7.6 | 80.2 \( \pm \) 8.4 | 88.5 \( \pm \) 8.8 | 61.8 \( \pm \) 5.8 | 58.5 \( \pm \) 5.9 |
| AP threshold, mV | -45.4 \( \pm \) 1.3 | -44.8 \( \pm \) 1.5 | -44.7 \( \pm \) 1.1 | -45.7 \( \pm \) 1.2 | -47.8 \( \pm \) 1.4 | -47.9 \( \pm \) 1.5 |
| AP amplitude, mV | 66.2 \( \pm \) 1.7 | 67.4 \( \pm \) 1.9 | 64.5 \( \pm \) 1.2 | 66.1 \( \pm \) 1.3 | 40.5 \( \pm \) 1.4 | 42.7 \( \pm \) 1.6 |
| AP half-width, ms | 0.61 \( \pm \) 0.06 | 0.59 \( \pm \) 0.07 | 0.43 \( \pm \) 0.03 | 0.46 \( \pm \) 0.05 | 0.83 \( \pm \) 0.07 | 0.85 \( \pm \) 0.00 |
| \( I_{\text{AP}} \), mV | 12.4 \( \pm \) 0.8 | 12.2 \( \pm \) 0.9 | 21.5 \( \pm \) 1.0 | 20.4 \( \pm \) 1.2 | — | — |
| AP adaptation | 2.31 \( \pm \) 0.19 | 2.12 \( \pm \) 0.17 | 1.92 \( \pm \) 0.09 | 1.94 \( \pm \) 0.07 | 8.92 \( \pm \) 0.86 | 8.14 \( \pm \) 0.83 |
| \( f_{\text{I}} \) slope, Hz/nA | 159.2 \( \pm \) 10.9 | 163.8 \( \pm \) 11.7 | 365.5 \( \pm \) 19.2 | 383.1 \( \pm \) 21.6 | — | — |

AP, action potential; Con, control; CD, cortical dysplasia; fAHP, fast afterhyperpolarization; \( f_{\text{I}} \) slope, slope of the relationship between injected current intensity and firing rate; \( R_{\text{input}} \), input resistance.
the counting area 621 512 ± 47 311 lm²) and throughout the full extent (pia to white matter) of the dysplastic cortex (295 360 ± 21 179 lm²), and data showed that their densities were significantly decreased in dysplastic cortex (all P < 0.01). They were 13.3 ± 1.1, 23.7 ± 1.2, and 10.9 ± 0.9, respectively, in control cortex, and 8.9 ± 0.8, 12.4 ± 1.1, and 8.1 ± 0.7, respectively, in dysplastic cortex. The decreased mean density of SST-, PV-, and CR-ir cells in dysplastic cortex were significant (all P < 0.01), and the percentage decrease (compared with controls) was 32.8%, 47.3%, and 25.5%, respectively.

Change of Firing Rates and Patterns in CD

CD Demonstrates Decreased Firing Rates and Changes in Firing Pattern of SST-ir Interneurons

Spontaneous firing activity was monitored using cell-attached recordings in voltage-clamp configuration. The cell was maintained at a holding potential (–60.5 ± 3.8, n = 57 and –61.1 ± 4.3 mV, n = 38 in control and dysplastic cortex, respectively) that was applied to achieve 0 pA injected current. SST-ir interneurons in layer IV in controls and in the middle region of dysplastic cortex discharged APs spontaneously. The mean firing rate of SST-ir interneurons in irradiated rats (1.77 ± 0.15 Hz, n = 38) was significantly lower than that in control rats (2.71 ± 0.24 Hz, n = 57, P < 0.01, Fig. 3A,B). As shown in Figure 3A, the decrease in firing frequency, or increase in ISI, was accompanied by an increase in irregularity in ISI in SST-ir interneurons from dysplastic cortex. SST-ir interneurons in dysplastic cortex fired spikes less regularly (Fig. 3A) with a wider ISI distribution compared with controls (Fig. 3C). CVISI was used to quantify the irregularity of firing. The CVISI was increased in dysplastic cortex (0.122 ± 0.011, n = 38) compared with controls (0.079 ± 0.008, n = 57, P < 0.01). There was a positive correlation between the mean ISI and CVISI in SST-ir cells of both control and dysplastic cortex (Fig. 3D). These results indicate that CD is associated with increased irregularity of SST-ir interneuronal spiking.

CD Demonstrates Decreased Firing Rates and Changes in Firing Pattern of PV-ir Interneurons

PV-ir cells are generally thought to be FS interneurons. Previous studies have shown a decrease of both excitatory drive (Xiang et al., 2007). The decrease in PV-ir cell firing frequency was accompanied by an increase in CVISI in dysplastic cortex (Fig. 3D). The decrease in firing frequency was more pronounced in PV-ir interneurons than in SST-ir interneurons in dysplastic cortex (Fig. 3A,B). These results indicate that CD is associated with increased irregularity of PV-ir interneuronal spiking.

Figure 2. Electrophysiological characterization of interneurons from control and dysplastic cortex. Whole-cell current-clamp response to injection of a depolarizing current pulse of 300 ms duration and 200 pA amplitude (left and middle panels), and the analysis of the instantaneous firing frequency in representative SST- (A), PV- (B) and CR- (C) interneurons (right panel). Application of depolarizing current induced a discharge of APs at different firing frequencies and patterns in the 3 subtypes of interneuron. In response to depolarizing current (200 pA), PV-ir cells discharged APs at higher frequency without any adaptation (B) while SST-ir cell fired at lower frequency with obvious adaptation (A); CR-ir cells fired with an irregular firing pattern (C). Time bar in C also applies to A and B. Inst., instantaneous.
et al. 2006) and inhibitory drive (Zhou et al. 2009) onto FS interneurons in irradiated rats and that the balance of excitatory and inhibitory drive favors inhibition in dysplastic FS interneurons as evidenced by a reduced EPSC-to-IPSC ratio (Zhou et al. 2009). These findings suggest that firing rates in irradiated rats would be reduced. In order to test this possibility, we monitored activity of PV-ir interneurons in cell-attached voltage-clamp mode and measured spontaneous AP frequency. Holding potentials (-63.5 ± 2.4 mV, n = 121 and -64.1 ± 2.9 mV, n = 60, respectively, in control and dysplastic cortex) were applied to give 0 pA injected current. All recorded PV-ir cells in control and dysplastic cortex fired spontaneously; however, PV-ir interneurons in irradiated animals fired at lower frequencies (3.08 ± 0.24 Hz, n = 60) than controls (4.89 ± 0.31 Hz, n = 121, P < 0.01, Fig. 4A,B). As shown in Figure 4A, the decrease in firing frequency was accompanied by an increase in irregularity in ISI in PV-ir interneurons from dysplastic cortex. PV-ir interneurons in dysplastic interneurons fired spikes with greater irregularity (Fig. 4A), and the ISI distribution was wider compared with controls (Fig. 4C). The CVISI was increased from 0.055 ± 0.004 (n = 121) in controls to 0.153 ± 0.013 (n = 60, P < 0.01) in dysplastic cortex. The mean ISI was positively correlated with CVISI in PV-ir cells of both control and dysplastic cortex. Correlation coefficients were 0.715 and 0.754 in control and dysplastic cortex, respectively (both P < 0.01). These results indicate that CD is associated with increased irregularity of PV-ir interneuronal spiking.

**Cortical Dysplasia Shows No Change of Firing Rates and Pattern of CR-ir Interneurons**

In contrast to the observations in SST- and PV-ir interneurons, the firing rates and pattern of CR-ir interneurons were similar.
between control and dysplastic cortex. The firing rates were 2.45 ± 0.21 Hz (n = 47) in controls and 2.17 ± 0.26 Hz (n = 33, P > 0.05, Fig. 5A,B) in dysplastic cortex. As in the previous experiments, the holding potentials (−59.4 ± 3.4 mV, n = 47 and −60.7 ± 3.6 mV, n = 33, respectively, in control and dysplastic cortex) were adjusted to give 0 pA injected current. The firing patterns of CR-ir interneurons in both groups were irregular such that the distribution of ISI was very wide in both groups (Fig. 5C) and CVISI were also similar (0.195 ± 0.015 in control and 0.214 ± 0.019 in dysplastic cortex, P > 0.05). The mean ISI was positively correlated with CVISI in CR-ir cells of both control and dysplastic cortex. Correlation coefficients were 0.736 and 0.772 in control and dysplastic cortex, respectively (both P < 0.01).

Change of Synaptic Currents in CD

Alterations of intrinsic firing properties of interneurons could underlie the decreased firing rates of SST and PV-ir interneurons observed in cell-attached recordings in irradiated rats. To explore this possibility, we obtained whole-cell recordings after cell-attached recordings and measured spike width, amplitude of the fAHP, and input resistance. We found that the intrinsic properties of SST and PV-ir interneurons were similar between the control and dysplastic cortex (Table 1). Therefore, it seems unlikely that the altered spontaneous firing rate is caused by changes of intrinsic firing properties in dysplastic cortex. Alterations in synaptic drive could also contribute to the reduced spontaneous firing rates in dysplastic cortex. We acquired spontaneous EPSCs (sEPSCs) and IPSCs (sIPSCs) from individual cells at a holding potential of −40 mV in whole-cell voltage-clamp mode. At that potential, sIPSCs appeared as outward currents and sEPSCs as inward currents (Figs. 6A,7A), and they were confirmed as GABAergic and glutamatergic, respectively, using pharmacological blockade in PV-ir interneurons (Zhou et al. 2009). Miniature EPSCs and IPSCs (mEPSCs and mIPSCs) were acquired in the presence of TTX (1 μM). We determined the mean frequency and amplitude of s- and mEPSCs and s- and mIPSCs and the ratio of frequency and amplitude of s- and mEPSCs to s- and mIPSCs and compared between control and dysplastic cortex.

**Figure 5.** CR-ir interneurons fire irregularly and CD is not associated with change in their firing rates and pattern. (A) Representative recordings of spontaneous APs in CR-ir interneurons from control and dysplastic cortex; note that CR-ir interneurons fire irregularly in both groups. (B) Group data showing no difference of firing rates of CR-ir interneurons between control and dysplastic cortex. (C) Irregularity of spontaneous firing of APs in control was similar to dysplastic cortex.

**CD Is Associated with Decreased Frequency of EPSCs and IPSCs and Decreased Ratio of Frequency of EPSCs to IPSCs in SST-ir Interneurons**

The mean frequency of sEPSCs and sIPSCs of SST-ir interneurons (Supplementary Table 1) were 1.51 ± 0.17 and 1.89 ± 0.16 Hz, respectively, in dysplastic cortex (n = 11), and they were significantly lower than those in control cortex (3.76 ± 0.27 and 2.66 ± 0.24 Hz, respectively, n = 16, P < 0.01, Fig. 6A,B), suggesting that both glutamatergic and GABAergic drive in dysplastic interneurons was decreased. Next, we calculated the ratio of frequency of sEPSCs to sIPSCs in each recorded SST-ir cell to assess the balance of excitatory and inhibitory synaptic transmission (Zhou et al. 2009). The mean ratio of frequency of sEPSCs to sIPSCs in dysplastic cortex was 0.80 ± 0.07 and was significantly lower than that in control cortex (1.41 ± 0.11, P < 0.01, Fig. 6D), indicating that balance of frequency input was shifted to favor inhibition in dysplastic cortex. This is consistent with the decreased spontaneous firing rates in dysplastic SST-ir interneurons as described above. The amplitude of sEPSCs and sIPSCs and the ratio of frequency of sEPSCs to sIPSCs were not significantly different. They were 17.5 ± 1.5 pA and 18.9 ± 1.7 pA and 0.94 ± 0.09, respectively, in control, and 15.8 ± 1.4 pA and 16.9 ± 1.7 pA and 0.93 ± 0.11, respectively, in dysplastic cortex (P < 0.05, Fig. 6A,CD).

The synaptic events described as sEPSCs and sIPSCs could be driven by AP-dependent and -independent spontaneous transmitter release. To test whether CD alters the events driven by AP-independent transmitter release (mEPSCs and mIPSCs), TTX (1 μM) was added to the perfusate to block sodium channels and APs. As illustrated in Figure 6E, the mean frequency of mEPSCs and mIPSCs were found to decrease significantly in SST-ir interneurons from dysplastic cortex compared with controls (Supplementary Table 1). They were 1.88 ± 0.15 and 1.53 ± 0.14 Hz, respectively, in control cortex (n = 16) and 0.75 ± 0.09 and 1.01 ± 0.12 Hz, respectively, in dysplastic cortex (n = 11, P < 0.01 for both mEPSCs and mIPSCs). The ratio of frequency of mEPSCs to mIPSCs in dysplastic cortex was 0.74 ± 0.06 which was significantly lower than that in control cortex (1.23 ± 0.09, Fig. 6G), indicating that the ratio of frequency of mEPSCs to mIPSCs is also shifted toward inhibition in dysplastic cortex and AP-independent transmitter release could also...
Figure 6. The frequency of EPSCs and IPSCs and the ratio of EPSCs to IPSCs is decreased in SST-ir interneurons in dysplastic cortex. (A) Representative traces showing sEPSCs and sIPSCs recorded simultaneously at a holding potential of −40 mV in whole-cell voltage-clamp mode. (B and C) Group data showing that sEPSC and sIPSC frequency was decreased in SST-ir interneurons in dysplastic cortex (B); however, there was no difference in amplitude sEPSCs or sIPSCs between the 2 groups (C). (D) The ratio of frequency of sEPSCs to sIPSCs was decreased in SST-ir interneurons in dysplastic cortex, whereas the ratio of amplitudes was not different from controls. (E and F) Group data showing that the frequency both mEPSCs and mIPSCs was decreased in SST-ir interneurons in dysplastic cortex (E); however, there was no difference in amplitude of mEPSCs or mIPSCs between the 2 groups (F). (G) The ratio of frequency of mEPSCs to mIPSCs was decreased (*P < 0.01) in SST-ir interneurons in dysplastic cortex, whereas the ratio of amplitudes was not different from controls. Legend in G also applies to B-F.

properties of both mIPSCs and mEPSCs were also comparable between the 2 groups (data not shown).

**CD Is Associated with Decreased Frequency of EPSCs and IPSCs and Decreased Ratio of Frequency of EPSCs to IPSCs in PV-ir Interneurons**

In the present study, we also acquired the mean frequency of s- and mEPSCs and s- and mIPSCs and the ratio of frequency of s- and mEPSCs and s- and mIPSCs in PV-ir interneurons (Supplementary Table 1). Similar to SST-ir interneurons, they were all significantly decreased in dysplastic cortex. These results are consistent with our previous study (Zhou et al. 2009).

**CD Is Associated with Decreased Frequency of EPSCs and IPSCs in CR-ir Interneurons, but the Ratio of EPSCs to IPSCs Is Not Altered**

The mean frequency sEPSCs and sIPSCs were significantly decreased in CR-ir interneurons in dysplastic cortex (Supplementary Table 1). They were 2.55 ± 0.29 and 3.46 ± 0.31 Hz, respectively, in control (n = 14) and 1.67 ± 0.15 and 2.21 ± 0.27 Hz, respectively, in dysplastic cortex (n = 10, Fig. 7A,B, P < 0.01). In contrast to SST- and PV-ir interneurons, the ratio of frequency of sEPSCs to sIPSCs in CR-ir cells was similar between the 2 groups. They were 0.76 ± 0.08 in dysplastic cortex and 0.74 ± 0.07 in control cortex (P > 0.05, Fig. 7D). The amplitude of sEPSCs and sIPSCs and the ratio of amplitude of sEPSCs to sIPSCs were similar between the 2 groups. They were 17.7 ± 1.8 pA and 16.8 ± 1.5 pA and 1.05 ± 0.12, respectively, in control, and 16.3 ± 1.7 pA and 14.7 ± 1.6 pA and 1.1 ± 0.12, respectively, in dysplastic cortex (P > 0.05, Fig. 7A,C,D).

We also recorded mEPSCs and mIPSCs in the presence of 1 μM TTX (Supplementary Table 1). The mean frequency of mEPSCs and mIPSCs was significantly decreased in CR-ir interneurons from dysplastic cortex compared with controls, and they were 1.75 ± 0.16 and 2.39 ± 0.22 Hz, respectively, in control (n = 14) and 1.17 ± 0.11 and 1.51 ± 0.13 Hz, respectively, in dysplastic cortex (n = 10, P < 0.01 for both mEPSCs and mIPSCs, Fig. 7E). The ratio of frequency of mEPSCs to mIPSCs was similar between control and dysplastic cortex, and they were 0.73 ± 0.08 and 0.77 ± 0.06, respectively. The amplitude of mEPSC and mIPSC and the ratio of amplitude of mEPSC to mIPSC were similar between the 2 groups, and they were 13.3 ± 1.3 pA and 12.0 ± 1.1 pA and 1.11 ± 0.10, respectively, in control and 12.1 ± 1.3 pA and 10.8 ± 0.8 pA and 1.12 ± 0.09, respectively, in dysplastic cortex (P > 0.05, Fig. 7F,G). The kinetic properties of s- and mEPSCs and s- and mIPSCs of CR-ir interneurons, similar to that of SST- and PV-ir interneurons, showed no difference between control and dysplastic cortex (data not shown).

**Effect of Blockade of Synaptic Currents on Firing Rates and Patterns in CD**

Both excitatory and inhibitory synaptic inputs to interneurons could influence excitation of interneurons and thus the balance of excitatory and inhibitory synaptic inputs could influence firing rates and regularity (as measured by CV in this study) of interneurons. The next experiment was designed to determine how excitatory and inhibitory synaptic currents influence firing rates and patterns of interneurons. As shown in Figures 3A and 4A, the decrease in firing frequency was accompanied by an increase in irregularity in ISI in SST- and PV-ir interneurons from dysplastic cortex. We sought to determine if blockade of

![Diagram](https://academic.oup.com/cercor/article-abstract/21/7/1645/337775/1)

**Figure 6.**

- **A:** SST-ir cell from control cortex
- **B:** SST-ir cell from dysplastic cortex
- **D:** Frequency (Hz)
- **C:** Amplitude (pA)
- **F:** Amplitude (pA)
- **G:** mEPSC/mIPSC ratio

The frequency of EPSCs and IPSCs and the ratio of EPSCs to IPSCs is decreased in SST-ir interneurons in dysplastic cortex. (A) Representative traces showing sEPSCs and sIPSCs recorded simultaneously at a holding potential of −40 mV in whole-cell voltage-clamp mode. (B and C) Group data showing that sEPSC and sIPSC frequency was decreased in SST-ir interneurons in dysplastic cortex (B); however, there was no difference in amplitude sEPSCs or sIPSCs between the 2 groups (C). (D) The ratio of frequency of sEPSCs to sIPSCs was decreased in SST-ir interneurons in dysplastic cortex, whereas the ratio of amplitudes was not different from controls. (E and F) Group data showing that the frequency both mEPSCs and mIPSCs was decreased in SST-ir interneurons in dysplastic cortex (E); however, there was no difference in amplitude of mEPSCs or mIPSCs between the 2 groups (F). (G) The ratio of frequency of mEPSCs to mIPSCs was decreased (*P < 0.01) in SST-ir interneurons in dysplastic cortex, whereas the ratio of amplitudes was not different from controls. Legend in G also applies to B-F.

Contribute to the reduced firing rates. The amplitude of mEPSCs and mIPSCs were similar between the 2 groups. They were 13.2 ± 1.2 and 13.6 ± 1.4 pA, respectively, in control and 11.7 ± 1.1 and 12.3 ± 1.2 pA, respectively, in dysplastic cortex (P > 0.05, Fig. 6F). The ratio of amplitude of mEPSCs to mIPSCs also showed no difference, and they were 0.97 ± 0.12 in control and 0.95 ± 0.10 in dysplastic cortex (P > 0.05, Fig. 6G).

The kinetic properties of sEPSCs and sIPSCs in dysplastic cortex were compared with controls. For sEPSCs, the mean 10–90% rise time (control, 1.02 ± 0.09 ms, n = 16; dysplastic cortex, 1.08 ± 0.11 ms, n = 11) and the decay time constant (control, 6.31 ± 0.5 ms, n = 16; dysplastic cortex, 6.58 ± 0.7 ms, n = 11) were not different between the 2 groups. For sIPSCs, the mean 10–90% rise time (control, 2.11 ± 0.12 ms, n = 16; dysplastic cortex, 2.23 ± 0.14 ms, n = 11) and the decay time constant (control, 18.2 ± 1.2 ms, n = 16; dysplastic cortex, 18.9 ± 1.4 ms, n = 11) were not different between the 2 groups. The kinetic
excitatory and inhibitory synaptic currents would change the regularity of firing. To address those questions, we assessed the alteration of firing rates and CVISI in control and irradiated rats after inhibitory and excitatory synaptic inputs were blocked by the \(\alpha\)-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA)/kainate receptor antagonist, NBQX, and the \(N\)-methyl-D-aspartic acid (NMDA) receptor antagonist, D-AP5, and/or the GABAA receptor antagonist, PIC. After obtaining a 5 min baseline cell-attached recording, antagonists were added to the perfusate for 5 min followed by a complete washout of antagonists for 10--30 min. We calculated the percent change of firing rates and CVISI from the mean values at baseline and during the exposure of antagonists for each cell. The percent change values were then pooled within groups and compared using the paired \(t\)-test.

As shown in Figures 8 and 9 and Supplementary Table 2, application of both 10 \(\mu\)M NBQX and 50 \(\mu\)M D-AP5 significantly decreased the firing rates and increased CVISI of SST- and PV-ir interneurons while the addition of 100 \(\mu\)M PIC significantly increased the firing rates and decreased CVISI in control and dysplastic cortex, indicating that spontaneous excitatory and inhibitory input to interneurons have opposing effects on spontaneous firing; that is, spontaneous excitatory input helps maintain higher firing rates and lower irregularity (CVISI), whereas spontaneous inhibitory input maintains cell firing at lower frequency and higher irregularity.

Therefore, the net effect of synaptic input to interneurons could be dependent on the concurrent action of both excitatory and inhibitory input to interneurons or their balance (i.e., the ratio of frequency and/or amplitude of EPSCs to IPSCs). To test this hypothesis, we added both glutamatergic and GABAergic antagonists to the perfusate and examined the change of firing rates and CVISI in SST- and PV-ir interneurons, we found that the addition of 10 \(\mu\)M NBQX, 50 \(\mu\)M D-AP5, and 100 \(\mu\)M PIC significantly decreased the firing rates and
increased CV ISI in controls while they increased the firing rates and decreased CV ISI in dysplastic cortex (Figs. 8 and 9), demonstrating that the net effect of spontaneous synaptic currents are excitatory in control cortex whereas it is inhibitory in CD. This is consistent with findings from the whole-cell recordings that demonstrated a shift in the balance of excitation to inhibition toward inhibition in SST- and PV-ir interneurons in dysplastic cortex. It is also important to note that, in the presence of glutamatergic and GABAergic blockade, SST- and PV-ir interneurons in dysplastic cortex were no longer different from controls with respect to firing rate or regularity (Supplementary Table 2).

In contrast to the observations in SST and PV-ir interneurons, bath application of the 3 antagonists increased the firing rates and decreased CV ISI of CR-ir interneurons in both control and dysplastic cortex (Fig. 9, Supplementary Table 2), demonstrating that the net effect of spontaneous synaptic currents on these interneurons is inhibitory and renders the firing more irregular. This is consistent with the results from whole-cell recordings that showed that the balance of excitatory and inhibitory inputs to CR-ir cells favors inhibition in both control and dysplastic cortex.

After blocking excitatory and inhibitory inhibition with 10 μM NBQX, 50 μM D-AP5, and 100 μM PIC, the firing rates and regularity of SST-, PV-, and CR-ir interneurons were not different between the 2 groups (all \( P > 0.05 \), Supplementary Table 2), confirming that synaptic inputs to those interneurons contribute to their altered spontaneous firing rates in dysplastic cortex.

### Discussion

This study was undertaken to better understand how alterations in synaptic connectivity in the in utero irradiation model of CD affect cortical function. Specifically, we wanted to determine if the relative imbalance of EPSCs to IPSCs that had been shown in dysplastic interneurons would result in a more quiescent behavior of the affected cells. This proved to be true in that both PV- and SST-ir interneurons had reduced firing frequency that correlated with a shift in the ratio of EPSCs to IPSCs toward inhibition. In contrast, CR-ir interneurons had normal firing rates and a normal balance of EPSCs to IPSCs, even though absolute frequencies of both type of currents were reduced compared with controls. These results support the concept that the surviving PV- and SST-ir interneurons are relatively quiescent, and this would contribute to the impaired inhibition that has been demonstrated in this model. In addition, these experiments have extended our understanding of how synaptic input influences the spontaneous firing of interneurons in both control and dysplastic cortex.

There are several reasons why interneurons that fire less frequently might contribute to seizure generation. There are many examples in human pathology and epilepsy models where reduction of inhibition leads to epileptiform activity. These include loss of certain types of hippocampal interneurons in clinical and experimental temporal lobe epilepsy (de Lanerolle et al. 1989; Obenhaus et al. 1993; Cossart et al. 2001; Kobayashi and Buckmaster 2003) and loss of neocortical interneurons in clinical and experimental CD (Ferrer et al. 1999; Spreatacho et al. 1998, 2000; Roper et al. 1999; Sarkisian et al. 2001). Other studies have shown alterations in GABA receptors (Brooks-Kayal et al. 1998; DeFazio and Hablitz 1999; Redecker et al. 2000; Crino et al. 2001; Cohen et al. 2003) and reduced presynaptic release probability in GABAergic terminals (Hirsch et al. 1999). However, it is well established that interneurons play an important role in synchronization of normal and abnormal cortical activity (Whittington and Traub 2003; Bartos et al. 2007), and other studies have found increased inhibitory activity in various models of epilepsy (Cossart et al. 2001; Klaassen et al. 2006; Jones and Baraban 2007). So it is difficult at this time to make any general statements regarding levels of activity of inhibitory interneurons and propensity for seizures (Cossart et al. 2005).

The irradiated rat shows 2 major types of impaired inhibition. The first is loss of about 50% of cortical interneurons (Roper et al. 1999; Deukmedjian et al. 2004). The current study has extended these findings by demonstrating that the densities of SST- and CR-ir interneurons are reduced in irradiated rat. This had already been shown in PV-ir interneurons (Roper et al. 1999; Zhou and Roper 2010). The second finding is that the surviving PV-ir and SST-ir interneurons have an imbalance of excitatory to inhibitory synaptic inputs that favors inhibition. This had been previously shown for FS, PV-ir interneurons (Zhou et al. 2009) but not for SST-ir cells. In addition, we found that, although the frequency of both EPSCs and IPSCs are reduced in CR-ir interneurons, the overall ratio of EPSCs to IPSCs is not different from controls. Previously we hypothesized that the reason for the decreased synaptic input in interneurons in this model might be a delay in the arrival of
those neurons to the cortical plate due to a relatively long migratory pathway (compared with pyramidal cells) from the ganglionic eminence. There is evidence that migrating neurons are more susceptible to in utero radiation effects than more mature neurons (Altman et al. 1968). Therefore, the interneurons may partially miss a developmental window when synaptic contacts are being formed. In PV- and SST-ir interneurons, this seems to affect excitatory terminals more than inhibitory ones leading to an imbalance of synaptic drive. Why CR-ir interneurons would be spared this imbalance is unknown. Alternatively, alterations in synaptic function could also be responsible for these changes and our current data do not allow us to answer these questions.

The current study extends previous findings on impaired function of inhibitory neurons in irradiated rats by showing that PV-ir and SST-ir interneurons have reduced spontaneous firing rates. This had been predicted in previous studies based on altered synaptic input (Xiang et al. 2006; Zhou et al. 2009) but it had not been tested. These findings demonstrate that the surviving PV- and SST-ir neurons are relatively quiescent compared with controls. The concept of a dormant interneuron in epilepsy has been the subject of much speculation in epilepsy research but supporting evidence has been controversial (Rempe et al. 1997; Kobayashi and Buckmaster 2003; Cossart et al. 2005). The dormant basket cell hypothesis was initially proposed in limbic epilepsy models (Sloviter 1991; Sloviter et al. 2003) based on indirect measures of impaired inhibition in the absence of a detectable loss of hilar basket cells. However, no previous study has demonstrated an impairment of excitation based on quantitative comparisons of spontaneous and mEPSCs in the interneurons themselves. Using the pilocarpine model, Doherty and Dingledine (2001) demonstrated enhanced short-term depression of evoked EPSCs in hilar border interneurons, suggesting an activity-dependent impairment of excitatory drive. However, they found no alterations in the frequency of sEPSCs. Therefore, the current findings in irradiated rat are the most convincing to date in support of a class of quiescent interneurons in an epilepsy model based on quantitative analysis of synaptic input and spontaneous firing. The previous studies utilized models where epilepsy resulted from chemically induced status epilepticus in adult animals, whereas the irradiated rat involves an in utero injury that may have a larger impact on subsequent synapse formation. This may explain why quiescent interneurons are present in irradiated rat while they remain elusive in other models. The functional importance of excitatory input to interneurons was documented in one study that demonstrated that a reduction in the strength of excitatory input in CA3 FS interneurons was a critical event in the conversion of oscillatory network activity to epileptiform bursting (Traub et al. 2005). It should also be noted that reduced firing in certain classes of interneurons would also reduce inhibition in other interneurons and may have a homeostatic effect. Therefore, the ultimate effect of quiescent PV- and SST-ir interneurons on the function of the entire cortical circuit would require knowledge of all the complex synaptic connections between the various types of interneurons and pyramidal cells and is beyond the scope of the current paper.

These experiments suggest that the relative balance of excitatory and inhibitory synaptic input in a neuron is an important determinant of spontaneous firing activity, along with intrinsic membrane properties. This statement is supported through 2 lines of evidence. First, the 2 classes of interneurons that showed relatively more synaptic inhibition (PV- and SST-ir interneurons) in CD also showed reduced frequency and reduced regularity of spontaneous firing compared with their control counterparts. In contrast, CR-ir interneurons had a normal balance of EPSCs to IPSCs and their spontaneous firing parameters were not different from corresponding controls. These experiments demonstrate correlation but not causality.

The experiments with glutamatergic and GABAergic antagonists provide some evidence for a causal relationship between synaptic input and spontaneous firing. When AMPA, NMDA, and GABAA receptors were pharmacologically blocked, the firing rates and regularity of dysplastic PV- and SST-ir interneurons were no longer different from controls. We also demonstrated that the intrinsic membrane properties for each type of interneuron were not altered in CD. Therefore, when the final determinant of spontaneous firing (balance of synaptic input) was pharmacologically removed from the equation, the PV- and SST-ir cells in CD fired in the same fashion as their control counterparts. This suggests that altered synaptic input actually caused the abnormal spontaneous firing rates and patterns in dysplastic cortex.

Understanding the relative contributions of synaptic activity versus intrinsic membrane properties toward regulation of spontaneous firing is an important issue in cortical function. In control cortex, we found that firing rates of all 3 types of cortical interneurons were heavily influenced by synaptic input. In all 3 interneurons, AMPA/NMDA receptor blockade produced reduction in firing frequencies by averages of 34–58%. Blockade of GABAA receptors increased the firing rates of all 3 interneurons by averages of 35–53%. This is in contrast to cholinergic interneurons of the neostriatum where synaptic input had little effect on firing rate in vitro (Bennett and Wilson 1999). Our results are similar to recordings from CA3 pyramidal neurons where AMPA/NMDA receptor blockade reduced firing frequency (Cohen and Miles 2000). In our study, the ratio of EPSCs to IPSCs predicted the firing rate response to complete blockade of AMPA/NMDA and GABAA receptors. In control PV- and SST-ir interneurons where the ratio of EPSCs to IPSCs was >1, complete blockade resulted in a reduction of firing rates by averages of 25–27%. In control CR-ir cells where the ratio of EPSCs to IPSCs was <1, complete blockade resulted in an increased mean firing rate of 16%. In the irradiated tissue, the ratio of EPSCs to IPSCs was <1 in all 3 types of interneuron and complete blockade resulted in increased firing rates by averages of 12–18%. These results suggest that synaptic inputs exert powerful influence on spontaneous firing in cortical interneurons and that alterations in the balance of synaptic inputs in CD result in abnormal spontaneous activity in PV- and SST-ir interneurons.

Shifting the synaptic balance toward inhibition did not just reduce firing frequency it also led to more irregular firing. This was seen in dysplastic cortex where the balance was altered by in utero injury. But it was also seen in control cortex where the balance was shifted toward inhibition by blocking glutamatergic receptors. This suggests that the net overall effect of GABAergic input on a cell (presumably originating from a variety of different types of interneurons) is to make the cell fire less frequently and more irregularly. CVISI has been used as a measure of regularity of neuronal firing. Increasing CVISI is correlated with classification of neurons as regular firing,
irregular firing, and bursting (Bennett and Wilson 1999) with bursting cells having CV_{ISI} values > 1. In our study, PV- and SST-ir interneurons were regular spiking and CR-ir cells were irregular. None of the cell types in the current study fit the criteria for bursting neurons and none demonstrated a Poisson distribution (CV ≈ 1, Softky and Koch 1993). In general, CV_{ISI} tends to decrease as firing rates increase (Bennett and Wilson 1999). This observation was supported in the current study by finding a positive correlation between CV_{ISI} and ISI (inverse of frequency) in all 3 subtypes of interneuron. Therefore, the increased CV_{ISI} in PV- and SST-ir neurons from irradiated rats may simply reflect their lower firing rates. How the changes in firing rates and patterns that we have found in dysplastic interneurons would impact overall cortical function as it relates to seizures and whether the spontaneous firing of pyramidal cells would behave in the same fashion is currently unknown.

Some limitations to the study should be acknowledged. The techniques used in this paper do not measure the normal firing activity of cortical interneurons in vivo. The in vitro slice is a reductionist preparation where the cortical neurons are deprived of their distant connections, both afferent and efferent. This means that the activity of the neurons is more quiescent in the slice than it would be in vivo (Caicedo et al. 1999). In order to counteract this unavoidable effect, the experiments were performed in an environment that mildly enhanced firing of the neurons (Cohen and Miles 2000). This included raising KCl in the bath solution to 6.5 mM (normal is 3–5 mM, Hansen 1977) and maintaining the temperature of the recording chamber at body temperature, 36 °C (Chan and Yeh 2003; Shu et al. 2006). These manipulations increased the spontaneous firing of the cells to a level where alterations and differences between groups could be reliably compared. However, it did not produce epileptiform activity, even in the dysplastic cortex. This is consistent with prior studies where KCl of 8.5 mM was required to produce epileptiform bursting in the hippocampus (Traynelis and Dingledine 1988; Dhala and Staley 2004). We propose that the effects on spontaneous firing that were demonstrated in this paper would still be active in vivo and that they have relevance for cortical function in the intact animal. We cannot, however, rule out the possibility that some compensatory mechanisms from distant connections in vivo might dampen or counteract these effects.

The irradiated rat model mimics certain important aspects of human CD. These animals show diffuse cortical abnormalities including thinning of the cortical mantle, loss of lamination in the neocortex, presence of large pyramidal neurons scattered throughout the cortical mantle, loss of normal spatial orientation of neurons within the cortex, and the presence of subcortical and periventricular heterotopic gray matter (Roper et al. 1995). The model does not produce giant dysmorphic neurons or balloon cells. Therefore, it most closely replicates Type I FCD according to the Palmini classification system (Palmini et al. 2004). Although there is strong evidence that primary genetic defects may contribute to Type II FCD, the etiology of Type I FCD is less well understood and in utero injury remains a plausible cause of this disorder in humans (Marin-Padilla 1999).

Studies of interneuronal function in other models of CD have provided a variety of findings. The {\textit{Lis1}} gene is responsible for some cases of type I lissencephaly in humans (Reiner et al. 1993), and the {\textit{Lis1}} mutant mouse shows hyperexcitability in the hippocampus (Fleck et al. 2000). CA1 interneurons show increased spontaneous firing and increased frequency of sEPSCs in this model (Jones and Baraban 2007). The perinatal freeze-lesioned rat is a model of FCD (Dvorak and Feit 1977) that produces hyperexcitable cortex adjacent to a microgyrus. There is evidence for increased excitatory synaptic input in inhibitory interneurons (Jacobs and Prince 2005) as well as increased strength of inhibitory synapses on layer V pyramidal cells in this model (Brill and Huguenard 2010). In contrast, recordings from human type II FCD have shown reduced frequency of spontaneous and mIPSCs in pyramidal cells (Calcagno et al. 2005) that are similar to findings from the irradiated rat model (Zhu and Roper 2000). Although there was no overall reduction in the density of interneurons in this tissue, there were patches where interneurons were scarce. Synaptic input to interneurons was not examined in this study. At this time, it is difficult to reconcile the various findings regarding inhibition in CD and it is likely that mechanisms of epileptogenesis will vary depending on type of CD, genetic defect, and extent and timing of insult.

In summary, these findings in irradiated rat have demonstrated 2 subtypes of cortical interneurons, PV- and SST-ir, in CD that are quiescent due to an imbalance of synaptic input in favor of inhibition. This means that a single in utero insult can have profound and lasting effects on synaptic connectivity that results in a demonstrable impairment in function of interneurons. We have also shown that balance of synaptic input exerts a powerful influence on spontaneous firing properties of cortical interneurons in normal and dysplastic cortex. These findings extend our understanding of impaired inhibition in an animal model of CD and raise the possibility that similar mechanisms may be operative in some forms of human CD.

Supplementary Material
Supplementary material can be found at: http://www.cercor.oxfordjournals.org/.

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