Net synaptic drive of fast-spiking interneurons is inverted towards inhibition in human FCD I epilepsy

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Abstract

Focal cortical dysplasia type I (FCD I) is the most common cause of pharmaco-resistant epilepsy with the poorest prognosis. To understand the epileptogenic mechanisms of FCD I, we obtained tissue resected from patients with FCD I epilepsy, and from tumor patients as a control. Using whole-cell patch clamp in acute human brain slices, we investigated the cellular properties of fast-spiking interneurons (FSIN) and pyramidal neurons (PN) within the ictal onset zone. In FCD I epilepsy, FSINs exhibited lower firing rates from slower repolarization and action potential broadening, while PNs had increased firing. Importantly, excitatory synaptic drive of FSINs increased progressively with the scale of cortical activation as a general property across species, but this relationship was inverted towards net inhibition in FCD I epilepsy. Further comparison with intracranial electroencephalography from the same patients revealed that the spatial extent of pathological high-frequency oscillations (pHFOs) was associated with synaptic events at FSINs.

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Abbreviations: FCD: focal cortical dysplasia; FSIN: fast-spiking interneuron; pHFO: pathological high-frequency oscillation

Introduction

Epilepsy is a disorder characterized by recurrent seizure episodes.[1,2] Approximately 50 million people suffer from epilepsy, more than a quarter of which are pharmaco-resistant.[3,4] Focal cortical dysplasia (FCD) is the most common pathology associated with pharmaco-resistant epilepsy, which requires surgery to alleviate symptoms.[5] FCD is categorized into three types: type I is characterized by abnormalities in cortical lamination, while type II further introduces neuronal dysmorphisms; when type I FCD is associated with heterogeneous brain lesions, it is classified as type III.[6-8] For FCD II pathogenesis, single causative genetic mutations in the mammalian target of rapamycin (mTOR) pathway have been identified.[9,10] However, the genetic background and epileptogenic mechanisms remain unknown for FCD I,[11,12] despite the poorer postoperative seizure-free outcome (Engel class I at 5 years: 54.5% *vs.* 67.4%).[13,14]

Excitation-inhibition (E-I) balance is a fundamental feature of the cortical network.[2,15,16] Cortical network state can remain robust to modest perturbations because of the E-I balance maintained by recurrent local activity.[15] Since the defining feature of seizure is the uncontrolled burst of electrical activity, disruption in the E-I balance from reduced inhibition has been considered the main candidate cause of epileptogenesis.[2,15,16] In support of this hypothesis, pyramidal neurons (PNs) within the ictal onset zone were found to receive less spontaneous inhibitory postsynaptic currents,[17-19] and a decrease in the number of parvalbumin-expressing interneurons (PVINs)[20-22] or $GABA_A$ receptor conductance and expression on PNs[23] have been observed. However, these studies did not focus on FCD I, even though epileptogenic mechanisms are expected to be specific to FCD type.

Intracranial electroencephalography (iEEG) from epilepsy patients revealed that pathological high-frequency oscillations (pHFOs) are increased in epileptic regions.[24,25] pHFOs are defined as ripples (80-200 Hz) or fast ripples (200-500 Hz) that are distinguished from physiological HFOs by frequent association with spikes or pathological slowing.[26] Since the resection of tissue exhibiting pHFOs is closely linked to the success of epilepsy surgery,[27-29] it is of great interest to investigate the relationship between epileptogenic mechanisms and the occurrence of pHFOs. Previous studies suggested a potential role of fast-spiking interneurons (FSINs), which represent a largely overlapping population with PVINs, in the generation of pHFOs possibly by causing out-of-phase or asynchronous firing of PNs.[30-32] Still, a comprehensive

understanding of changes in FSIN physiology and cortical E-I balance underlying pHFO generation remains elusive despite the growing clinical significance of pHFOs.

To understand the cellular mechanisms underlying FCD I epileptogenesis, we investigated the electrophysiological properties of human FSINs and PNs using whole-cell patch clamp in acute human brain slices. We prioritized neocortical layer 2/3 (L2/3) where pathological phenotypes of FCD I are the most prominent.[33,34] Then, we examined the clinical significance of these single-cell properties through correlative analysis with the spatiotemporal characteristics of pHFOs in iEEG recorded from the same patients. We found that both the intrinsic and synaptic excitability of FSINs were reduced in FCD I epilepsy. In the non-epileptic control, synaptic excitation-to-inhibition ratio at FSINs increased as more inputs were activated simultaneously, suggesting a progressively important role of FSINs in stabilizing the network under high-activity state. Such pattern of E-I balance at FSINs was preserved across species in non-epileptic conditions, but disrupted in FCD I epilepsy in the form of net inhibition of FSINs. Furthermore, we found that the spatial extent of cortical areas exhibiting ictal discharges or pHFOs was most closely related to spontaneous synaptic activity at FSINs, while pHFO occurrence could be explained by PN firing rate. We conclude that the shift in net synaptic drive at FSINs from excitation to inhibition represents the major epileptogenic mechanism of FCD I epilepsy, highlighting the pathophysiological significance of FSINs in relation to cortical E-I balance.

Results

To determine changes in the intrinsic neuronal excitability and synaptic E-I balance leading to FCD I epileptogenesis, we obtained neocortical tissue containing the ictal onset zone surgically resected from 15 patients with intractable epilepsy diagnosed with FCD I or III (FCD I accompanied by heterogenous lesions). In addition, we obtained non-pathological parts of the neocortex resected from 10 tumor patients without seizure history as a control (Supplementary Table 1). Using whole-cell patch clamp in acute human brain slices, we examined the electrophysiological properties of FSINs and PNs in neocortical L2/3.

Human L2/3 FSINs in FCD I epilepsy have decreased firing due to action potential broadening

A major contributor to cortical network hyperactivity is the decrease in the number of GABAergic interneurons or their effectiveness in inhibiting excitatory neurons,[20-23] but the cellular phenotypes of decreased inhibition have not been identified in detail. Hence, we first examined the intrinsic excitability of FSINs in control and epileptic conditions (Fig. 1a). The resting membrane potential (RMP) and input resistance (R_{in}) of FSINs were similar between control and FCD I epilepsy (Fig. 1b-c). FSINs in FCD I epilepsy, however, exhibited considerable differences in their firing properties (Fig. 1d), such that peak firing rates were lower by 40% (171.8 *vs.* 103.9 Hz; Fig. 1e). Analysis of single action potential (AP) kinetics further revealed that the AP duration of FSINs was considerably longer in FCD I epilepsy due to slower depolarization and repolarization, while AP threshold was unaffected (Supplementary Fig. S1).

The intrinsic excitability differences found in PNs between control and epileptic conditions were dissimilar to those of FSINs (Fig. 1f). For PNs, RMP was similar between groups (Fig. 1g), but R_{in} was significantly higher in FCD I epilepsy (109.4 *vs.* 156.7 MΩ; Fig. 1h). While RMP and R_{in} of human L2/3 PNs are known to be correlated with cortical depth,[35] here it was not a contributing factor as cortical depths were not significantly different between groups (Supplementary Fig. S2). In alignment with increased R_{in}, PNs in epilepsy exhibited higher firing frequency in response to smaller somatic current injection (5.6 *vs.* 10.1 Hz at 150 pA; Fig. 1i-j); however, firing rates saturated towards a similar value at larger amplitudes (Fig. 1j).

To understand the mechanisms underlying changes in FSIN and PN firing rates in FCD I epilepsy, we analyzed their correlations with single AP kinetics and found a significant relationship between FSIN firing rate and AP half-width (Fig. 1k), and in turn between AP half-width and the maximum rate of repolarization (Fig. 1l), from FSINs in both control and FCD I epilepsy. On the other hand, PN firing rate was not correlated with single AP kinetics, but instead strongly correlated with R_{in} (Fig. 1m), implying that the higher firing rates of PNs may not be attributable to changes in the dynamic properties of ion channels but to increased R_{in}. Taken together, these results suggest that the major mechanisms determining the firing output of FSINs and PNs are distinct, and differently affected by FCD I epilepsy.

Synaptic conductance from spontaneous events is biased towards inhibition in both FSINs and PNs

We hypothesized that differences in the intrinsic excitability of single neurons in FCD I epilepsy would be accompanied by changes in synaptic properties, which may have more prominent consequences towards network hyperexcitability. To this end, we first measured the spontaneous excitatory and inhibitory postsynaptic currents (sEPSC and sIPSC) at FSINs and PNs. At postsynaptic FSINs (Fig. 2a), sEPSC but not sIPSC frequency was higher (Fig. 2b), while both sEPSC and sIPSC amplitudes were larger (Fig. 2c), in FCD I epilepsy compared to control. For quantitative comparison of synaptic strengths, we converted the excitatory and inhibitory postsynaptic current amplitudes to conductances (G_E and G_I; see Methods) and found that the spontaneous inhibitory conductance ($G_{I,s}$) was higher than the spontaneous excitatory conductance ($G_{E,s}$) at FSINs, in both control and FCD I epilepsy (Fig. 2d). However, the E-I ratio of spontaneous postsynaptic conductances ($G_{E,s}/G_{I,s}$) remained at approximately 0.6 for both groups, indicating that the increase in $G_{E,s}$ in FCD I epilepsy occurred in parallel with the increase in $G_{I,s}$ (Fig. 2e). Spontaneous synaptic events at PNs followed a qualitatively similar pattern (Fig. 2f). While sEPSC and sIPSC frequencies were unchanged (Fig. 2g), sEPSC amplitude was larger in FCD I epilepsy (Fig. 2h); sIPSC amplitude was also nominally larger, but without statistical significance (23.2 *vs.* 28.0 pA, P = 0.069). $G_{I,s}$ was larger than $G_{E,s}$ by more than twofold in both control and epilepsy (Fig. 2i), resulting in a lower $G_{E,s}/G_{I,s}$ of 0.4 at PNs (Fig. 2j). These results show that spontaneous excitatory and inhibitory conductances were amplified at both FSINs and PNs in FCD I epilepsy, but to similar extents such that their ratio was preserved, with larger inhibitory component.

Synaptic excitability of FSINs increases progressively with the scale of cortical activation, but not in FCD I epilepsy

To specifically examine postsynaptic responses to presynaptic AP firing, we then measured the evoked excitatory and inhibitory postsynaptic currents (eEPSC and eIPSC) in response to local electric stimulation in L2/3 (Fig. 3a). To obtain putative single synaptic responses, we used a conventional minimal stimulation approach as has been established previously (see Methods). eEPSC and eIPSC from minimal stimulation are referred to as $eEPSC_{min}$ and $eIPSC_{min}$ (Fig. 3a, lighter traces). Additionally, we measured postsynaptic responses evoked by activation of a larger cortical volume, intended for simultaneous recruitment of the maximal number of afferents converging onto the postsynaptic neuron (Fig. 3a, darker traces). For this purpose, we

elected a stimulation protocol at which intensity the eEPSC amplitude reached a plateau (Fig. 3b; see Methods); since both the electric field from a monopolar current source as well as its effect on membrane potential are linearly proportional to current amplitude and inversely proportional to distance,[36-38] saturation of eEPSC amplitude can be expected to result from the activation of the maximal number of presynaptic connections arriving at the postsynaptic neuron. Subsequently, eEPSC and eIPSC produced by these stimulation conditions were regarded as maximally evoked responses, henceforth referred to as $eEPSC_{max}$ and $eIPSC_{max}$. Peak amplitudes of these events were determined predominantly by monosynaptic inputs, as evidenced by their short latencies that were not different from those of single synaptic events from minimal stimulation (Supplementary Fig. S3).

At FSINs in control conditions, we found that the excitatory conductance calculated from the postsynaptic current amplitude in response to minimal stimulation ($G_{E,min}$) was no longer smaller than the corresponding inhibitory conductance ($G_{I,min}$) (Fig. 3c), unlike those from spontaneous events. The trend of increasing excitatory synaptic conductance was stronger with large-scale stimulation, at which point the excitatory conductance ($G_{E,max}$) had exceeded the inhibitory postsynaptic conductance ($G_{I,max}$) (Fig. 3d). In contrast, $G_{E,min}$ and $G_{E,max}$ at FSINs in FCD I epilepsy were accompanied by larger $G_{I,min}$ and $G_{I,max}$ compared to control (Fig. 3c-d) . When the changes in G_E and G_I in response to increasing stimulation were plotted against each other, it became clear that FSINs in control conditions became progressively more excitable synaptically with larger scale cortical activation, whereas in FCD I epilepsy, inhibition was dominant over excitation regardless of the scale of stimulation (Fig. 3e, Supplementary Fig. S4). E-I ratio of postsynaptic conductances (G_E/G_I) at control FSINs therefore increased gradually from below 1 (net inhibition) with spontaneous inputs to increasingly larger values above 1 (net excitation) with synaptic activation, while G_E/G_I remained low at FSINs in FCD I epilepsy (Fig. 3f).

To further understand the mechanisms underlying this difference, we used the fold changes in synaptic conductance introduced by increasing the stimulation scale as a metric to represent functional connectivity, given that minimal or maximal stimulation respectively represents single synaptic events or the collective activation of synaptic afferents. Based on this criterion, excitatory connectivity at postsynaptic FSINs represented by $G_{E,max}/G_{E,min}$ was reduced by 55% in FCD I epilepsy, while changes in inhibitory connectivity represented by $G_{I,max}/G_{I,min}$ were nonsignificant (Fig. 3g). Excitatory connectivity at postsynaptic FSINs was substantially higher than inhibitory connectivity, consistent with known architecture of the human cortex

that connection probability between those two synapse types is similar, but the total number of excitatory connections greatly outnumber that of inhibitory connections.[39] Our findings demonstrate that synaptic E-I balance at FSINs under normal conditions is characterized by an increasing net excitatory synaptic drive in response to increased cortical activation owing to higher excitatory synaptic connectivity compared to inhibitory connectivity, but this property is inverted towards net inhibition of FSINs in FCD I epilepsy due to reduced excitatory connectivity.

Synaptic E-I relationship is conserved across species

Synaptic excitation was gradually favored over inhibition at FSINs with increasing stimulation, but only in non-epileptic conditions and not FCD I epilepsy (Fig. 3e-f). To investigate whether this E-I relationship of synaptic conductances represented a general characteristic of cortical networks, we repeated analogous experiments in the rat temporal association cortex for cross-species comparison (Fig. 3h). In rat FSINs, sEPSC was smaller than sIPSC, but $eEPSC_{min}$ was similar to $eIPSC_{min}$, and $eEPSC_{max}$ was larger than $eIPSC_{max}$ (Fig. 3i). Accordingly, G_E grew progressively larger than G_I with increasing synaptic activation (Fig. 3j), and as a result, G_E/G_I at rat FSINs evolved along a pattern strikingly similar to that from FSINs in the non-epileptic human cortex (Fig. 3k-l). These findings confirm that the relationship between excitatory and inhibitory synaptic conductances at FSINs, namely the preferential recruitment of excitatory inputs upon larger scale cortical activation to overcome the initial inhibition dominance, represents a general characteristic of cortical E-I balance conserved across species.

Network hyperactivity in FCD I epilepsy is mediated mainly by FSINs, while monosynaptic E-I relationship at PNs is unaffected

We then continued to compare the excitatory and inhibitory synaptic conductances at PNs (Fig. 4a-b). From minimal stimulation, $G_{E,min}$ was not significantly different from $G_{I,min}$, although $G_{E,min}$ was higher in FCD I epilepsy compared to control (0.3 *vs.* 0.82 nS, P = 0.0040; Fig. 4c). Similarly, $G_{E,max}$ was also comparable to $G_{I,max}$ from maximal stimulation (Fig. 4d). Nevertheless, a quantitatively similar shift towards stronger excitation in FCD I epilepsy was visible from both conditions in terms of G_E/G_I ($G_{E,min}/G_{I,min}$, 0.5 *vs.* 0.8; $G_{E,max}/G_{I,max}$, 0.5 *vs.* 0.7; Fig. 4e). In contrast to FSINs, the E-I ratio of G_E/G_I was always below 1 at PNs (Fig. 4f-g); in other words, net postsynaptic conductance at PNs always favored inhibition regardless of synaptic activation scale, in either control or FCD I epilepsy. The synaptic E-I relationship

at PNs was again not different between the non-epileptic human cortex and the rat cortex, further confirming that this represented a conserved pattern of cortical E-I balance (Supplementary Fig. S5). These results show that monosynaptic E-I relationship at PNs is relatively less affected in FCD I epilepsy, and net synaptic conductance at PNs remains biased towards inhibition regardless of cortical activation scale.

For a numerical representation of network excitability using simpler variables compatible with recurrent network models,[40] we defined the synaptic excitability variable $G = (G_{EE} \cdot G_{II})/(G_{EI} \cdot G_{IE})$, where G_{XY} denotes synaptic conductance from presynaptic population Y to postsynaptic population X, and X, Y $\in$ {E, I};[41] in other words, G is equal to G_E/G_I at PNs divided by G_E/G_I at FSINs. At the network level, FSINs were preferentially recruited during progressively higher cortical activity state in control conditions, but not in FCD I epilepsy. Specifically, G_E/G_I at PNs was lower than the G_E/G_I at FSINs in the non-epileptic control due to the progressive increase in the latter, but this relationship was inverted in FCD I epilepsy with stimulation (Fig. 4f), such that the synaptic excitability variable G decreased with larger scale cortical activation in the non-epileptic control but instead increased in FCD I epilepsy (Fig. 4g). Notably, such divergence in terms of synaptic excitability was not observed with spontaneous activity alone, suggesting that it was imparted by changes in functional connectivity. Collectively, our results indicate that the decrease in both the intrinsic and the synaptic excitability of FSINs, accompanied additionally by the increase in the R_{in} of PNs, introduces a shift in the E-I balance towards an overall elevation of cortical activity state in FCD I epilepsy.

Clinical presentation of pathological iEEG activity is associated with spontaneous synaptic activity at FSINs

pHFOs representing synchronized neuronal activity have been used as a marker for epileptic discharges.[30-32] To find potential clinical implications of our findings, we thus investigated whether the intrinsic and synaptic properties of single neurons in FCD I epilepsy that we obtained with *ex vivo* electrophysiology were associated with the characteristics of pHFOs. We estimated the extent of cortical area involved in epileptogenesis by counting the number of channels that exhibited either ictal discharges or pHFOs from each patient (Fig. 5a). Then, these single-cell and extracellular properties were compared using Pearson and Spearman correlations (Supplementary Fig. S6-S7); correlations were considered significant when both coefficients met the criteria (see Methods). The spatial extent of cortical areas presenting

pathological iEEG activity (ictal/pHFO area) varied across patients (Fig. 5b; Supplementary Fig. S8). We found that ictal/pHFO area was positively correlated with both the frequency and the amplitude of sEPSC at postsynaptic FSINs (Fig. 5c-e). Although sIPSC amplitude had no correlation with ictal/pHFO area, the strongest correlations were found between ictal/pHFO area and sIPSC frequency at either FSINs or PNs (Fig. 5f-h), both of which can be expected to reflect presynaptic FSIN activity as inhibitory inputs onto postsynaptic FSINs are known to originate primarily from other FSINs, in humans as well as rodents.[39,42] These results imply that spontaneous synaptic activity at FSINs contributes to the spatial characteristics of pHFOs at resting state, considering that these iEEG signals were obtained during a stable resting state (see Methods).

pHFO occurrence is associated with higher PN firing rate

Finally, we examined whether the propensity of pHFO occurrence was likewise correlated with single-cell properties. For this purpose, we counted ripple and fast ripple pHFOs separately from a 1-hour monitoring period (Fig. 6a-b; see Methods) and analyzed their correlations with the same single-cell properties. Similar to ictal/pHFO area, pHFO occurrence likewise varied across patients (Fig. 6c-d). We could not find an otherwise inherent tendency for pHFO occurrence aside from patient-to-patient variability, as there was no noticeable bias in the distribution of pHFO occurrences when all channels were counted together irrespective of patient (Fig. 6e-f). Unlike ictal/pHFO area which was correlated with spontaneous synaptic events, pHFO occurrence, averaged across channels displaying pHFOs per patient, showed positive correlations with PN firing frequency for both ripple and fast ripple pHFOs (Fig. 6g-i). These results suggest that, while synaptic changes in FCD I epilepsy involving FSINs contribute the most to the spatial extent of pathological activity, the generation of pHFOs is more closely related to the increased intrinsic excitability of PNs.

Discussion

Our understanding of epileptogenesis hinges on intrinsic, synaptic, and glial factors.[43] Recent advances have begun to reveal the potential link between aberrant molecular pathways and hyperexcitability in the dysplastic cortex.[44] However, these discoveries were centered on FCD

II, while epileptogenic mechanisms remain largely unknown for FCD I which is more common but less successfully treated. In a recent study using a mouse model of FCD II, hyperexcitability was found to originate not from the neurons carrying somatic mTOR mutations but instead from nearby non-mutated neurons. More importantly, synaptic E-I balance was unchanged in this model of FCD II.[45] From histological examinations, a reduction in PV immunoreactivity was observed from FCD I, but not FCD II.[22] In addition, the temporal characteristics of the interictal epileptiform discharge and the repetitive discharge in iEEG were found to be sufficiently distinct between FCD I and FCD II such that the underlying FCD type can be reliably predicted.[46] These results strongly suggest that epileptogenic mechanisms are specific to the type of associated FCD, of which FCD I is poorly understood.

In the present study, we examined the electrophysiological properties of PNs and FSINs in the adult human cortex to investigate the epileptogenic mechanisms in FCD I. Empirical knowledge from acute human brain slices regarding synaptic physiology and not only intrinsic membrane properties are scarce.[39,47-53] Moreover, these studies often used tissue outside of the ictal zone, with the specific intent to explore normal physiology unrelated to seizure activity. We instead set our focus to the pathophysiology of FCD I epilepsy by conducting experiments from the seizure onset zone. The findings from the epileptic cortex were then compared with those from the control cortex unassociated with epilepsy. Our recordings were made from L2/3; while a more comprehensive perspective incorporating the entire cortical column would be ideal, it is beyond the scope of the current study as very little is known about translaminar interactions or connectivity. Nonetheless, the choice of L2/3 is appropriate considering that the pathological phenotypes of FCD I are the most severe in supragranular layers.[33,34] Moreover, cortical expansion has evolved in such way that the relative thickness of supragranular layers that contain the majority of intracortical connections is largest in humans and other primates, unlike the rodent cortex in which infragranular layers are thicker.[54-57]

Since FSINs are the primary regulators of cortical network excitability, abnormal development or function of FSINs have been implicated with epilepsy.[58-60] It has been shown that excitatory synaptic terminals onto human FSINs have larger active zones with more functional release sites,[50] and a single AP from the presynaptic PN can produce large EPSPs at postsynaptic FSINs capable of driving APs[47,50,51,53,61] or even long-term plasticity.[51] Our findings demonstrate that both the intrinsic and synaptic excitability of human FSINs were reduced in FCD I epilepsy. Specifically, FSINs exhibited decreased firing rates, had increased single synaptic conductance from inhibitory inputs, and reduced connectivity from excitatory inputs.

Consequently, excitatory synaptic conductance at FSINs was always smaller than the inhibitory conductance in FCD I epilepsy, departing from the E-I balance preserved in the non-epileptic cortex across species. Our results also explain how intracortical electric stimulation protocols in clinical settings consisting of prolonged high-frequency, high-amplitude trains can temporarily restore cortical E-I balance, considering that specifically only FSINs but not PNs nor the non-fast-spiking interneurons (nFSIN) in the human cortex could reliably translate direct local electric stimulation to AP output with high fidelity during such trains (unpublished data, in preparation).

At the single synapse level, the higher inhibitory synaptic conductance at postsynaptic FSINs in FCD I epilepsy was accompanied by a larger increase from spontaneous inhibitory conductance. Despite involving different release machinery, spontaneous and evoked release are known to originate from the same vesicle pool.[62,63] Since G_{min} corresponds to synaptic release from AP-evoked Ca^{2+} influx whereas G_s includes those from stochastic opening of Ca^{2+} channels, G_{min}/G_s can thus be assumed to represent AP-induced enhancement of presynaptic release. In the control group, G_{min} was twice as large than G_s for excitatory inputs but equal for inhibitory inputs ($G_{E,min}/G_{E,s} = 2.0$; $G_{I,min}/G_{I,s} = 1.0$); on the other hand, G_{min} was larger than G_s for both excitatory and inhibitory inputs in FCD I epilepsy ($G_{E,min}/G_{E,s} = 2.7$; $G_{I,min}/G_{I,s} = 2.1$). We thus hypothesize that the AP broadening observed from FSINs in FCD I epilepsy may account for the increased single inhibitory synaptic conductance, considering that FSINs receive by far the most inhibitory input from other FSINs as their presynaptic partners,[42] and that $G_{I,min}/G_{I,s}$ was larger in FCD I epilepsy for both postsynaptic FSINs and PNs (1.0 *vs.* 2.1 at FSINs; 1.2 *vs.* 1.8 at PNs). While both depolarization and repolarization were slower in FSINs in epilepsy, only the rate of repolarization was correlated with AP half-width, which in turn correlated with firing rate. Since the voltage-gated potassium channels (VGKC) are primarily responsible for repolarization following AP firing, finding the specific VGKC subtypes involved with the slower repolarization at FSINs in epilepsy is of much interest in therapeutic development. The K_v3 family, including the $K_v3.1$ subunit that is predominantly expressed in FSINs, is well known to be required for the fast-spiking property of these interneurons. Relatedly, novel potassium channel openers targeting the $K_v7.2$ and $K_v7.3$ subunits that had been previously implicated with epilepsy[64,65] are undergoing clinical trial for potential treatment of focal epilepsy,[66] and overexpression of potassium channels through gene therapy has been found to produce antiepileptic effects in mouse models of FCD.[67] Given that the epileptogenic mechanisms may be distinct for different types of FCD, focusing on the

VGKC subtype that may be specifically affected by a particular type of FCD could present an effective strategy for successful clinical translation.

Oscillatory activity, characterized by the synchronized firing of PN populations, is primarily generated by post-inhibition excitation.[68,69] pHFOs, which are markers of epileptic discharges, are regulated by PVINs that are involved with the generation, propagation, and termination of ictal activity.[70-75] Previous studies using mouse models showed that the propagation of ictal activity is opposed by PVIN-mediated inhibition.[76,77] Collectively, our data support the notion that synaptic changes at FSINs in FCD I epilepsy may contribute to the generation and propagation of epileptic discharges and pHFOs. This could involve increased synchrony of post-IPSP spikes, or a larger number of PNs synchronized simultaneously by each FSIN.[69,72,73,76,78] Since the number of FSINs is also decreased in FCD I,[20-22] epileptic discharges generated at the epileptic core may not be effectively suppressed, leading to their continuous generation.[72,76] While the present study focused on the superficial layers, further studies incorporating both superficial and deeper layers together with local field potential measurements would be instrumental in exploring the epileptogenic dynamics of the broader cortical network. Given that seizure is a dynamic rather than a static state, studies with induced seizure states through pharmacological or other manipulations would also provide useful in extending our findings to seizure susceptibility.

Methods

Human surgical specimens

Human brain tissue was obtained from patients with pharmaco-resistant epilepsy associated with focal cortical dysplasia type I (FCD I), and from patients with brain tumor, who underwent surgery at Seoul National University Hospital (SNUH) Department of Neurosurgery. 15 epilepsy patients and 10 tumor patients of ages 18-76, male and female, were included in this study (Supplementary Table 1). Patients in the epilepsy group were diagnosed with FCD I or III (FCD I accompanied by heterogenous lesions) by pathological examinations following surgery. Cortical tissue originated from the temporal (n = 13), frontal (n = 9), parietal (n = 2), and occipital (n = 1) area. Tissue from epilepsy patients were obtained from the ictal onset zone. Tissue from tumor patients were obtained from a non-pathological region resected in the course of approaching the tumor during surgery. All participants gave informed consent for tissue donation, and all protocols of this study were approved by the SNUH Institutional Review Board (IRB) (2012-194-1191).

Rodent specimens

4-to-5-week-old male and female Sprague-Dawley (S-D) rats were used for this study. Animals were housed under standard environmental conditions (24-hour light/dark cycle) in a temperature and humidity-controlled room (25 ± 2 ℃) and were given food and water *ad libitum* with veterinary supervision from Seoul National University (SNU) College of Medicine Institute for Experimental Animals. A total of 30 rats were used in this study. All experimental protocols were approved by the SNU Institutional Animal Care and Use Committee (IACUC) (SNU-201119-5-1).

Acute brain slice preparation

Human brain slices

After *en bloc* surgical resection, tissue was immediately placed in an ice-cold solution at the operating theater. The solution used for transport and slicing contained (in mM): 110 choline chloride, 26 $NaHCO_3$, 2.5 KCl, 3.1 Na-pyruvate, 11.6 Na-ascorbate, 1.25 NaH_2PO_4, 10 D-glucose, 0.5 $CaCl_2$, 7 $MgCl_2$, pH adjusted to 7.4, 300-310 mOsm. The tissue container was kept

in a thermally isolated transportation box filled with ice and transported from the operating room at SNUH to the laboratory at SNU College of Medicine within 20 minutes. The tissue block was then placed orthogonal to the pia to preserve cortical layers and white matter in proper orientation. Slices of 300 μm thickness were prepared with a vibratome (VT1200S, Leica), and allowed to recover at 36 ℃ for 10 minutes before being maintained at room temperature for an additional 1 hour in artificial cerebrospinal fluid (aCSF) containing (in mM): 124 NaCl, 26 $NaHCO_3$, 2.5 KCl, 1.25 NaH_2PO_4, 10 D-glucose, 2.5 $CaCl_2$, 1.3 $MgCl_2$, pH adjusted to 7.4, 300-310 mOsm. The same aCSF was used for recordings. All solutions were continuously aerated with 95% O_2 / 5% CO_2 (pH 7.4) throughout the course of experiments, including transport and slicing. Experiments were performed within a 24-hour period after slicing.

Rat brain slices

Animals were anesthetized by inhalation of 5% (v/v) isoflurane and rapidly decapitated, after which the whole brain was removed and placed onto the vibratome. Rat brain slices containing the temporal association area (TeA) were prepared with identical solutions to those used for human brain slices.

ex vivo **electrophysiology**

Whole-cell patch clamp

All human and rat experiments were conducted under identical conditions. Slices were placed in an immersed recording chamber and perfused with the recording aCSF at 30 ± 2 ℃ using a peristaltic pump (2-4 mL/min). All recordings were made from the soma of fast-spiking interneurons (FSINs) or pyramidal neurons (PNs) in layer 2/3 (L2/3) of the neocortex, using an EPC-10 amplifier (HEKA) or a MultiClamp 700B amplifier with Digidata 1440A (Molecular Devices). Data were sampled at 10 kHz. Patch pipettes (3-7 MΩ) and monopolar stimulation pipettes (1-2 MΩ) were pulled from borosilicate glass capillaries using a PC-10 pipette puller (Narishige). Patch pipettes were filled with internal solution containing (in mM): 130 K-gluconate, 7 KCl, 2 NaCl, 1 $MgCl_2$, 0.1 EGTA, 2 Mg-ATP, 0.3 Na_2-GTP, 10 HEPES, and 0.2% (w/v) biocytin, adjusted to pH 7.3 with KOH, and 295-300 mOsm with sucrose. Cells with series resistance (R_s) below 25 MΩ were accepted, and R_s was fully monitored and compensated throughout the course of recordings. 100 μM D-2-amino-5-phosphonopentanoic

444 acid (D-APV, Tocris) was bath-applied to prevent potential long-term plasticity caused by
445 continued stimulation.

446 *Current clamp*

447 5 minutes after break-in, cells were held in current clamp at 0 pA to measure the following
448 parameters: (1) resting membrane potential (RMP), (2) input resistance (R_{in}), (3) firing
449 frequency in response to somatic current injection (f-i), (4) rate of action potential (AP)
450 depolarization (dV/dt), (5) AP threshold, and (6) AP half-width. R_{in} was calculated from
451 membrane potential responses to a hyperpolarizing current input (-50 pA, 500 ms). Step current
452 injections of 500 ms duration with amplitudes up to 500 pA in 50 pA increments were applied
453 to obtain the f-i curve. The resulting firing patterns were used to identify the cell type. The first
454 AP generated at rheobase was used to analyze the dV/dt, threshold, and half-width. AP
455 threshold was defined as the membrane potential at which dV/dt first exceeded 10 (V/s). AP
456 amplitude was calculated as the difference between the peak membrane potential and AP
457 threshold, and the width at half of this amplitude was taken as AP half-width. Liquid junction
458 potential was not corrected for in the membrane potential values reported in this study, but
459 taken into account in calculating synaptic conductances from measured postsynaptic current
460 amplitudes (see below).

461 *Voltage clamp*

462 Spontaneous and evoked postsynaptic currents were recorded under voltage clamp. Cells were
463 first held at -70 mV for excitatory inputs, then at 0 mV for inhibitory inputs. Spontaneous
464 excitatory postsynaptic currents (sEPSC) were recorded for at least 1 minute, and spontaneous
465 inhibitory postsynaptic currents (sIPSC) were recorded for at least 5 minutes, from the same
466 cell. Evoked excitatory and inhibitory postsynaptic currents (eEPSC and eIPSC, respectively)
467 were similarly recorded from the same cells. For extracellular stimulation, monopolar glass
468 stimulation electrodes filled with aCSF were positioned within L2/3, typically 40 to 150 μm
469 away from the somata of recorded cells. Stimuli were delivered via an isolator (DLS100, WPI)
470 controlled by a waveform generator (DS8000, WPI). For minimal stimulation, we took the
471 stimulation intensity at which eEPSC was produced with a synaptic failure rate of
472 approximately 50%. Under our conditions, minimal stimulation intensity was typically
473 between 2 to 6 V for both FSINs and PNs, which was not significantly different between control
474 and epilepsy groups for both FSINs (control, 4.2 ± 0.4 V; epilepsy, 4.6 ± 0.4 V) and PNs
475 (control, 3.9 ± 0.3 V; epilepsy, 4.3 ± 0.3 V), or between FSINs and PNs from the same group.

Stimulation site was selected such that large eEPSC could be reliably produced in response to broad-scale synaptic activation at a higher stimulation intensity (referred to as maximal stimulation; see Results), and was unchanged between minimal and maximal stimulation. Both eEPSC and eIPSC were evoked using the same stimulation intensity and electrode position for each cell. Excitatory and inhibitory synaptic conductances (G_E and G_I, respectively) were calculated from postsynaptic current (PSC) amplitudes, using their respective reversal potentials (E_{rev}) for nonselective cationic conductance through AMPA receptors at 0 mV or Cl^- conductance through $GABA_A$ receptors at -65 mV, and liquid junction potential of approximately 15 mV obtained from our experimental conditions.

Histology and imaging

Neurons were filled via the patch pipette with an internal solution containing 0.2% (w/v) biocytin for at least 15 minutes during electrophysiological recordings. At the end of all experiments, slices were fixed in 4% (v/v) paraformaldehyde (PFA) in phosphate-buffered saline (PBS) for less than 24 hours at 4 °C, rinsed three times with PBS, followed by subsequent immunocytochemical staining procedures. Slices were incubated in permeabilization buffer (0.3% (v/v) Triton X-100, Sigma-Aldrich) for 30 minutes (10 minutes, refreshed three times) then transferred to blocking buffer (0.3% (v/v) Triton X-100, 0.5% (w/v) Bovine Serum Albumin (BSA, Bovagen)) and kept at room temperature for a total of 3 hours (1 hour, refreshed three times). Slices were conjugated with streptavidin-Cy3 (1:500, Invitrogen) overnight at 4 °C, triple-washed with PBS, and mounted on glass coverslips with a medium containing 4',6-diamidino-2-phenylindole (DAPI, Abcam). Slices were imaged using a laser scanning confocal microscope (Leica) and analyzed with LAS X (Leica) and ImageJ (NIH). Dendritic and somatic morphology of cells were used for *post hoc* confirmation of the cell type in addition to prior electrophysiological characterization. Cells with somata located within the cortical depth of 1200 µm from pia were taken as L2/3 neurons.[35,79]

Intracranial electroencephalography

Electrode localization

Intracranial electroencephalography (iEEG) data were collected from patients with medically intractable epilepsy implanted with subdural electrode grids and depth electrodes, diagnosed with FCD I by pathology (Supplementary Table 1). Conventional and/or high-density subdural electrodes (contact diameter, 3 or 2 mm; inter-electrode distance, 10 or 5 mm, respectively;

Ad-tech Medical Instrument and PMT) as well as depth electrodes (contact diameter, 5 mm; inter-electrode distance, 10 mm; PMT) were implanted as clinically needed. The precise positioning of the electrodes for each patient were determined using the CURRY software (Compumedics Neuroscan) through co-registration of preoperative magnetic resonance (MR) images and postoperative computed tomography (CT) images (Supplementary Fig. S8).

Clinical delineation of ictal onset zone and the region exhibiting pathological high-frequency oscillations

Signals were recorded by a 128-channel amplifier system (Neuvo EEG; Compumedics Neuroscan) at a sampling rate of 2 kHz. Cortical areas corresponding to the ictal onset zone and those exhibiting high-frequency oscillations (HFOs) were identified separately. The ictal onset zone was defined as the region that initially displayed seizure activity. Pathological high-frequency oscillations (pHFOs) were identified by their characteristic high frequency and association with spikes or pathological slowing via visual inspection during both interictal and ictal phases.[28,80] We excluded putative physiological HFOs, especially ripples at the frequency range of 80 to 200 Hz, which are typically not associated with spikes or pathological slowing and mainly observed in the auditory, visual, and somatosensory areas.[26] Clinical evaluations were conducted by epileptologists at SNUH Department of Neurology.

Analysis of pathological HFOs

From a 24-hour monitoring period, data from a one-hour segment between 9 to 11 AM were selected for the detection of pHFOs during a stable, awake resting state. Signals were notch-filtered to remove line noise at 60 Hz and its related harmonics. Given that pHFOs are defined as ripples (80-200 Hz) and fast ripples (200-500 Hz),[30,81] signals were band-pass filtered for this frequency range using a 4^{th}-order Butterworth filter. The short-time energy (STE) method was used to automatically detect HFOs from preprocessed signals.[82] STE from signal x(t) was defined by:

$$\mathrm{STE(t)} = \sqrt{\frac{1}{\mathrm{N}} \sum_{i=t-N+1}^{t} x(i)^2}$$

where N represents a window size of 3 ms. To correct against capacitive artifacts, we applied a winsorization strategy by replacing STE values exceeding 10 standard deviations (SD) above the median, by median + 10 SD. pHFOs were then detected using RIPPLELAB.[81,83]

Specifically, the winsorized data were segmented into 10-minute epochs, after which the median and SD of STEs within each epoch were recalculated. We defined windows as those representing pHFOs when two or more consecutive windows were associated with STE values above median + 5 SD, and accompanied by more than 6 peaks per window with amplitudes larger than mean + 3 SD of the rectified band-pass filtered signal. Multiple windows meeting these criteria which were separated by less than 10 ms were taken together to be corresponding to a single pHFO event.[83,84]

Data analysis

Data were analyzed and visualized using custom codes in MATLAB (MathWorks) including PVBS (https://github.com/flosfor/pvbs), ClampFit (Molecular Devices), Igor Pro (Wavemetrics), and Prism (GraphPad). Statistical information is expressed as mean ± standard error of the mean (SEM), where *n* indicates the number of cells. The level of statistical significance was accepted when $P < 0.05$ (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$) using Mann-Whitney U test, two-sample or paired-sample t-test, or repeated measures ANOVA followed by Tukey's range post-hoc test, where appropriate. Pearson correlation coefficient was obtained to determine the goodness of fit for linear regressions, and Spearman correlation coefficient was additionally used for comparison between *ex vivo* electrophysiology and iEEG data. Correlations were considered positive when the coefficient was greater than +0.2, or negative when the coefficient was smaller than -0.2, with $P < 0.05$.

Data availability

The data that support the findings of this study are available from the corresponding authors, upon reasonable request.

Author contributions

Conceptualization, E.C., C.K.C., J.Y., W.-K.H.; Validation, E.C., H.L., S.-H.L., C.K.C., J.Y., W.-K.H.; Investigation, E.C., J.K., G.L., J.S.; Resources, E.C., S.-H.L., C.K.C., J.Y., W.-K.H.; Writing - original draft, E.C., J.Y., W.-K.H.; Writing - review & editing, E.C., C.K.C., J.Y., W.-K.H.; Visualization, E.C., J.K., G.L., J.Y.; Supervision, C.K.C., J.Y., W.-K.H.; Funding acquisition, S.-H.L., C.K.C., W.-K.H.

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Competing interests

The authors report no competing interests.

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Figure legends

Figure 1. Intrinsic membrane properties of FSINs and PNs in L2/3 of the human neocortex. (a) Confocal image of representative FSINs, labeled with biocytin. Scale bar, 50 μm. **(b)** FSIN resting membrane potential (RMP). Control, -64.0 ± 0.7 (mV); FCD I, -63.2 ± 0.7 (mV); P = 0.4237. **(c)** FSIN input resistance (R_{in}). Control, 237.4 ± 12.6 (MΩ); FCD I, 287.0 ± 17.1 (MΩ); P = 0.0578. **(d)** Representative examples of FSIN firing in response to somatic current injection. **(e)** FSIN firing rate was lower in FCD I epilepsy. Control, 171.8 ± 5.6 (Hz); FCD I, 103.9 ± 5.3 (Hz); P = 1.06e-10; at +500 pA. **(f)** Confocal image of a representative PN labeled with biocytin. Scale bar, 100 μm. **(g)** PN RMP. Control, -67.1 ± 0.6 (mV); FCD I, -68.4 ± 0.6 (mV); P = 0.1992. **(h)** PN R_{in} was higher in FCD I epilepsy. Control, 109.4 ± 7.0 (MΩ); FCD I, 156.7 ± 8.7 (MΩ); P = 1.21e-05. **(i)** Representative examples of PN firing in response to somatic current injection. **(j)** PN firing rate was modestly higher in FCD I epilepsy for smaller current injection amplitudes, but saturated towards a similar maximum. Control, 5.6 ± 1.1 (Hz); FCD I, 10.1 ± 1.2 (Hz); P = 0.0054; at +150 pA. **(k)** FSIN firing rate was negatively correlated with AP half-width (control, r = -0.5044, P = 3.41e-05; FCD I, r = -0.2781, P = 0.0362). Firing rates at +500 pA injection were used for correlative analyses. **(l)** FSIN AP half-width was negatively correlated with the maximal rate of repolarization (control, r = -0.7285, P = 2.85e-11; FCD I, r = -0.8119, P = 1.31e-11). **(m)** PN firing rate was positively correlated with R_{in}. Left, control (r = +0.9055, P = 2.85e-26); right, FCD I (r = +0.8801, P = 5.69e-26). Firing rates at +150 pA injection were used for correlative analyses.

Figure 2. Spontaneous synaptic inputs onto FSINs and PNs. (a) Representative traces of spontaneous excitatory postsynaptic currents (sEPSC) and spontaneous inhibitory postsynaptic currents (sIPSC), measured at FSINs from control and FCD I epilepsy. Scale bar, 25 pA (EPSC) or 100 pA (IPSC), 100 ms. **(b)** sEPSC, but not sIPSC, frequency at FSINs was higher in FCD I epilepsy. sEPSC: control, 18.9 ± 1.4 (Hz); FCD I, 24.4 ± 2.4 (Hz); P = 0.0441. sIPSC: control, 25.0 ± 1.4 (Hz); FCD I, 26.4 ± 2.3 (Hz); P = 0.5970. **(c)** sEPSC and sIPSC amplitudes at FSINs were larger in FCD I epilepsy. sEPSC: control, 19.4 ± 1.3 (pA); FCD I, 25.7 ± 1.8 (pA); P = 0.0055. sIPSC: control, 19.3 ± 0.8 (pA); FCD I, 26.3 ± 2.4 (pA); P = 0.0049. **(d)** spontaneous excitatory postsynaptic conductance ($G_{E,s}$) was smaller than spontaneous inhibitory postsynaptic conductance ($G_{I,s}$) at FSINs from both control and FCD I epilepsy.

Control: $G_{E,s}$ = 0.23 ± 0.02 (nS), $G_{I,s}$ = 0.39 ± 0.02 (nS), P = 3.37e-10. FCD I: $G_{E,s}$ = 0.30 ± 0.02 (nS), $G_{I,s}$ = 0.53 ± 0.05 (nS), P = 5.25e-05. **(e)** $G_{E,s}/G_{I,s}$ at FSINs favored inhibition, and was similar between control (0.59) and FCD I epilepsy (0.57). **(f)** Representative sEPSC and sIPSC traces at PNs. Scale bar, 25 pA (EPSC) or 100 pA (IPSC), 100 ms. **(g)** sEPSC and sIPSC frequency at PNs was similar between control and FCD I epilepsy. sEPSC: control, 17.1 ± 1.4 (Hz); FCD I, 20.5 ± 1.7 (Hz); P = 0.1807. sIPSC: control, 20.4 ± 1.5 (Hz); FCD I, 23.0 ± 1.5 (Hz); P = 0.2668. **(h)** sEPSC, but not sIPSC, amplitude at PNs was larger in FCD I epilepsy. sEPSC: control, 16.5 ± 0.7 (pA); FCD I, 21.0 ± 0.9 (pA); P = 0.0012. sIPSC: control, 23.2 ± 1.8 (pA); FCD I, 28.0 ± 1.7 (pA); P = 0.0689. **(i)** $G_{E,s}$ was smaller than $G_{I,s}$ at PNs from both control and FCD I epilepsy. Control: $G_{E,s}$ = 0.19 ± 0.01 (nS), $G_{I,s}$ = 0.46 ± 0.04 (nS), P = 4.16e-09. FCD I: $G_{E,s}$ = 0.25 ± 0.01 (nS), $G_{I,s}$ = 0.56 ± 0.03 (nS), P = 1.15e-14. **(j)** $G_{E,s}/G_{I,s}$ at PNs further favored inhibition with a ratio lower than those at FSINs, and similar between control (0.42) and FCD I epilepsy (0.44).

Figure 3. Net excitatory synaptic drive at FSINs scales with cortical activation in control, but is inverted towards inhibition in FCD I epilepsy. (a) Representative EPSC and IPSC traces at FSINs, evoked by minimal ($eEPSC_{min}$ and $eIPSC_{min}$; lighter traces) or maximal ($eEPSC_{max}$ and $eIPSC_{max}$; darker traces) stimulation. **(b)** eEPSC amplitude at FSINs saturated with increasing stimulation intensity. eEPSC amplitudes are presented as normalized values respective to $eEPSC_{min}$ for each cell. **(c)** Single excitatory synaptic conductance from minimal stimulation ($G_{E,min}$) was not significantly different from single inhibitory synaptic conductance ($G_{I,min}$) at FSINs in both control and FCD I epilepsy. Control: $G_{E,min}$ = 0.52 ± 0.15 (nS), $G_{I,min}$ = 0.45 ± 0.16 (nS), P = 0.8626. FCD I: $G_{E,min}$ = 0.76 ± 0.13 (nS), $G_{I,min}$ = 1.48 ± 0.48 (nS), P = 0.8853.**(d)** Total excitatory synaptic conductance from maximal stimulation ($G_{E,max}$) was larger than total inhibitory synaptic conductance ($G_{I,min}$) at FSINs in control, but not FCD I epilepsy. Control: $G_{E,max}$ = 2.26 ± 0.33 (nS), $G_{I,max}$ = 0.95 ± 0.28 (nS), P = 0.0010. FCD I: $G_{E,max}$ = 1.69 ± 0.20 (nS), $G_{I,max}$ = 3.89 ± 1.30 (nS), P = 0.5831. **(e)** Excitatory (filled circles) and inhibitory (empty circles) synaptic conductances at FSINs, with different scales of cortical activation. Net synaptic drive at FSINs progressively favored excitation in control, but this relationship was inverted towards inhibition in FCD I epilepsy. **(f)** G_E/G_I at FSINs progressively favored excitation in control ($G_{E,min}/G_{I,min}$ = 0.59, $G_{E,min}/G_{I,min}$ = 1.15, $G_{E,max}/G_{I,max}$ = 2.37), but not in FCD I epilepsy ($G_{E,min}/G_{I,min}$ = 0.57, $G_{E,min}/G_{I,min}$ = 0.51, $G_{E,max}/G_{I,max}$ = 0.44). **(g)** Fold differences between synaptic conductances from total (G_{max}) or single (G_{min}) synaptic

activation at FSINs, as a measure of connectivity. G_{max}/G_{min} from excitatory inputs was larger than that from inhibitory inputs in control ($G_{E,max}/G_{E,min}$ = 7.5 ± 1.2; $G_{I,max}/G_{I,min}$ = 2.3 ± 0.5; P = 9.25e-05), but not in FCD I where $G_{E,max}/G_{E,min}$ was substantially smaller compared to control (P = 0.0062) and similar to $G_{I,max}/G_{I,min}$ ($G_{E,max}/G_{E,min}$ = 3.4 ± 0.6; $G_{I,max}/G_{I,min}$ = 3.3 ± 1.0; P = 0.3579). **(h)** Representative example of a rat FSIN from TeA L2/3. **(i)** Representative traces of spontaneous (left) and evoked (right) EPSC and IPSC at rat FSINs. **(j)** Excitatory and inhibitory synaptic conductance at rat FSINs, from spontaneous events or evoked by minimal or maximal stimulation. **(k)** Synaptic excitability of FSINs in the rat cortex also scaled with cortical activation towards net excitation. **(l)** G_E/G_I at rat FSINs increased progressively with increased cortical activation, similar to human FSINs in the non-epileptic control.

Figure 4. Monosynaptic conductances at PNs are unaffected in FCD I epilepsy. (a) Representative EPSC and IPSC traces at PNs. **(b)** eEPSC amplitude at PNs saturated with increasing stimulation intensity. **(c)** $G_{E,min}$ was not significantly different from $G_{I,min}$ at PNs in both control and FCD I epilepsy. Control: $G_{E,min}$ = 0.31 ± 0.06 (nS), $G_{I,min}$ = 0.63 ± 0.19 (nS), P = 0.0757. FCD I: $G_{E,min}$ = 0.82 ± 0.14 (nS), $G_{I,min}$ = 1.07 ± 0.18 (nS), P = 0.4773. **(d)** $G_{E,max}$ was not significantly different from $G_{I,max}$ at PNs in both control and FCD I epilepsy. Control: $G_{E,max}$ = 2.52 ± 0.42 (nS), $G_{I,max}$ = 4.90 ± 1.49 (nS), P = 0.6866. FCD I: $G_{E,max}$ = 2.75 ± 0.48 (nS), $G_{I,max}$ = 3.78 ± 0.78 (nS), P = 0.6489. **(e)** Excitatory (filled circles) and inhibitory (empty circles) synaptic conductances at PNs, with different scales of cortical activation. Inhibition was always dominant over excitation at PNs in both control and FCD I epilepsy. **(f)** G_E/G_I at FSINs and PNs; left, from the human non-epileptic control or the rat cortex; right, from human FCD I epilepsy. **(g)** Synaptic excitability variable $G = (G_{EE} \cdot G_{II})/(G_{EI} \cdot G_{IE})$, in human non-epileptic control, human FCD I epilepsy, and the rat cortex. **(h)** Summary diagram illustrating changes in the intrinsic and synaptic excitability of neuronal populations that lead to network hyperactivity in FCD I epilepsy. Synaptic weights represent respective conductances normalized to that of excitatory synapses onto the excitatory population (G_{EE}).

Figure 5. Cortical area exhibiting pathological activity *in vivo* is associated with spontaneous synaptic events at FSINs recorded *ex vivo*. (a) Representative examples of electrode locations from iEEG implants in two patients. The spatial extent of cortical areas displaying pathological iEEG patterns (ictal/pHFO area) is represented by the number of

channels displaying either ictal activity or pHFOs. **(b)** Distribution of the number of channels corresponding to ictal/pHFO area across patients. **(c)** Representative traces of sEPSC at postsynaptic FSINs, recorded from slices obtained from the same patients shown in panel **a**. **(d)** Ictal/pHFO area in FCD I epilepsy was positively correlated with sEPSC frequency at postsynaptic FSINs ($r = +0.5535$, $P = 0.0400$). **(e)** Ictal/pHFO area was positively correlated with sEPSC amplitude at FSINs ($r = +0.5882$, $P = 0.0269$). **(f)** Representative traces of sIPSC at FSINs and PNs, from the same patients. **(g)** Ictal/pHFO area was positively correlated with sIPSC frequency at FSINs ($r = +0.6533$, $P = 0.0155$). **(i)** Ictal/pHFO area was positively correlated with sIPSC frequency at PNs ($r = +0.7263$, $P = 0.0049$).

Figure 6. pHFO occurrence is associated with higher PN firing rate. (a) Representative examples of iEEG recordings displaying pHFOs at the ripple frequency range. **(b)** Representative iEEG recordings displaying pHFOs at the fast ripple frequency range, from the same patients shown in panel **a**. **(c)** Distribution of the number of ripple pHFO events observed within a 1-hour monitoring period, averaged across all pHFO-displaying channels, by each patient. **(d)** Distribution of the number of fast ripple (FR) pHFO events per hour, averaged across pHFO-displaying channels, by each patient. **(e)** Distribution of the number of ripple pHFO events per hour, from all channels irrespective of patient. **(f)** Distribution of the number of FR pHFOs per hour, from all channels irrespective of patient. **(g)** Representative traces of PN firing, recorded from slices obtained from the same patients shown in panels **a** and **b**. **(h)** The average number of ripple pHFO events observed per pHFO-displaying channel from the 1-hour monitoring period was positively correlated with PN firing rate ($r = +0.6217$, $P = 0.0176$). **(i)** Mean FR pHFOs per channel per hour was positively correlated with PN firing rate ($r = +0.5348$, $P = 0.0488$).