Seizures as imbalanced up states: excitatory and inhibitory conductances during seizure-like events

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Žiburkus J, Cressman JR, Schiff SJ. Seizures as imbalanced up states: excitatory and inhibitory conductances during seizure-like events. J Neurophysiol 109: 1296–1306, 2013. First published December 5, 2012; doi:10.1152/jn.00232.2012. Precisely timed and dynamically balanced excitatory (E) and inhibitory (I) conductances underlie the basis of neuronal network activity. Normal E/I balance is often shifted in epilepsy, resulting in neuronal network hyperexcitability and recurrent seizures. However, dynamics of the actual excitatory and inhibitory synaptic conductances (g_e and g_i, respectively) during seizures remain unknown. To study the dynamics of E and I network balance, we calculated g_e and g_i during the initiation, body, and termination of seizure-like events (SLEs) in the rat hippocampus in vitro. Repetitive emergent SLEs in 4-aminopyridine (100 µM) and reduced extracellular magnesium (0.6 mM) were recorded in the identified CA1 pyramidal cells (PC) and oriens-lacunosum molecular (O-LM) interneurons. Calculated g_e/g_i ratio dynamics showed that the initiation stage of the SLEs was dominated by inhibition in the PCs and was more balanced in the O-LM cells. During the body of the SLEs, the balance shifted toward excitation, with g_e and g_i peaking in both cell types at nearly the same time. In the termination phase, PCs were again dominated by inhibition, whereas O-LM cells experienced persistent excitatory synaptic barrage. In this way, increased excitability of interneurons may play roles in both seizure initiation (Žiburkus J, Cressman JR, Barreto E, Schiff SJ. J Neurophysiol 95: 3948–3954, 2006) and in their termination. Overall, SLE stages can be characterized in PC and O-LM cells by dynamically distinct changes in the balance of g_e and g_i, where a temporal sequence of imbalance shifts with the changing firing patterns of the cellular subtypes comprising the hyperexcitable microcircuits.

excitatory/inhibitory balance; synaptic conductance; seizure; interneurons; microcircuits

ANIMAL BEHAVIOR EMERGES FROM precise and finely tuned inhibitory and excitatory neuronal activation patterns (Buzsaki 2006; Klausberger et al. 2003; Klausberger and Somogyi 2008; Shen et al. 2011; Turrigiano and Nelson 2000; Yizhar et al. 2011). In normal neuronal activity in vitro and in vivo, such as recurrent cortical “up” states, excitatory (g_e) and inhibitory synaptic conductances (g_i) may be quite balanced (Borg-Graham et al. 1998; Haider et al. 2006; Shu et al. 2003). During initiation of scratch-like network behavior in turtle spinal motor neurons and interneurons, g_e and g_i peak in phase with the total membrane conductance (Berg et al. 2007). During the transition between awake and sleep states, the g_e and g_i in neocortical cells in vivo show complex interactions, and inhibition can transiently dominate during these cycles (Rudolph et al. 2007). Given that normal network states depend on appropriately balanced excitatory (E) and inhibitory (I) cell interplay, it is important to characterize the nature of the g_e/g_i balance in pathological network activity as a function of identified E and I cell subtypes.

In hyperexcitable epileptic tissue, seizures are historically thought to stem from decreased g_i and increased g_e. The growing body of experimental and theoretical evidence suggests that interneuron activity is preserved in human (Cohen et al. 2002; Huberfeld et al. 2011; Truccolo et al. 2011) and animal epileptic tissue (Avoli et al. 1996a; Higashima et al. 1996; Lopantsev and Avoli 1998; Velazquez and Carlen 1999). Furthermore, seizure synchronization patterns in vitro and in vivo are complex and, similarly to other behavioral states (Buzsaki 2006), appear related to precise E and I cell subtype-specific interactions (Fujiwara-Tsukamoto et al. 2010; Gutkin et al. 2001; Netoff and Schiff 2002; Truccolo et al. 2011; Ullah et al. 2009; Žiburkus et al. 2006). E-I cell interplay during seizure-like events (SLEs) and seizure electroencephalography (EEG) data from human patients (Schiff et al. 2005) support a natural partitioning of seizures into initiation, body, and termination stages. Despite the importance of g_e and g_i dynamics in network disorders such as epilepsy, ratios of g_e to g_i during seizures have not been studied. Furthermore, to date, all of the studies on g_e and g_i dynamics, with the exception of putative spinal interneurons in turtles (Berg et al. 2007), have been limited to explorations of putative neocortical pyramidal cells. This leaves a void of information on spontaneous g_e/g_i balance and dynamics during seizures in distinct cell subtypes.

To investigate dynamics of g_e and g_i, before, during, and after the SLEs in identified excitatory and inhibitory hippocampal cell subtypes, we used a pharmacological seizure model and recorded synaptic conductances in PC and O-LM cells in transverse hippocampal slices, in area CA1. g_e and g_i calculations were derived from multiple recurrent SLEs recorded in the same cell voltage-clamped at different holding potentials. Our data suggest divergence in network input dynamics between E and I cells, whereby different dynamical ratios g_e/g_i were observed during distinct SLE stages. Collectively, these findings demonstrate a novel dynamical pattern of excitatory and inhibitory conductances underlying the temporal and spatial evolution of SLEs.
METHODS

Animals. Experiments were performed on Sprague-Dawley rats [postnatal days 18–30 (P18–P30)] with approval from the Institutional Animal Care and Use Committees of George Mason University, the Pennsylvania State University, and the University of Houston.

Electrophysiology. P18–P30 rats were anesthetized with diethyl ether and decapitated, and their brains were removed, the hippocampi isolated, and transverse 350-μm sections cut in cold dissection buffer (in mM: 2.6 KCl, 1.23 NaH₂PO₄, 24 NaHCO₃, 0.1 CaCl₂, 2 MgCl₂, 205 sucrose, and 20 glucose) using a vibratome.

Slices were incubated for 1 h in artificial cerebrospinal fluid (ACSF; pH 7.3, 30°C, in mM: 130 NaCl, 0.6 MgSO₄, 3.5 KCl, 1.2 CaCl₂, 10 glucose, 2.5 NaH₂PO₄, and 24 NaHCO₃) aerated with 95% O₂-5% CO₂, transferred to a submersion recording chamber (Warner Instruments), and perfused (1 ml/min, 34°C). Borosilicate glass microelectrodes (3–6 MΩ) were made for voltage-clamp recordings (in mM) 120 cesium gluconate, 5 N-(2,6-dimethylphenyl carbamoylmethyl)triethylammonium bromide (QX-314), 2 KCl, 0.5 EGTA, 20 HEPES, 10 phosphocreatine, 0.3 NaGTP, 2 NaCl, 4 MgATP, and 0.3% neurobiotin (pH 7.25, 295 mOsm). A few recordings were also performed in current clamp, using similar intracellular solution, except cesium gluconate was replaced by potassium gluconate and QX-314 was excluded from the pipette (Hazra et al. 2012; Žiburkus et al. 2006). Extracellular recordings were performed with glass micropipettes (1–3 MΩ) filled with 0.9% NaCl.

Alignment of the seizure start times was based on an identifiable feature of the extracellular recording: the fast positive extracellular field (FPES; 1–5 mV; see Fig. 1 in Žiburkus et al. 2006). O-LM interneurons were targeted because of their importance in normal (Gillies et al. 2002; Klausberger and Somogyi 2008; Pike et al. 2000; Somogyi and Klausberger 2005) and pathological hippocampal rhythms (Aradi and Maccasferri 2004; Žiburkus et al. 2006) and their vulnerability in epilepsy (Oliva et al. 2002; Sanon et al. 2005; Santhakumar and Soltesz 2004). E cells in pyramidal and I cells in oriens layers were visually preselected using a differential infrared contrast microscope (Zeiss) with a mounted zoom tube. Extracellular electrodes were placed in the upper stratum (st.) pyramidale near the patched cells. Recordings were performed using Axon MCC 700A and 700B amplifiers (Molecular Devices), filtered (4 kHz whole cell, 1 kHz extracellular), and digitized at 10 kHz (Digidata and pClamp7; Molecular Devices).

SLEs were induced pharmacologically by applying 100 μM 4-aminopyridine (4-AP; Sigma) in decrease-magnesium (0.6 mM [Mg²⁺]ₙ) ACSF. 4-AP increases inhibitory and excitatory neuron signaling by partial blockade of repolarizing A-type potassium channels, causing broadening of action potentials and membrane depolarization (Avoli et al. 1996b, 1988; Somjen 2004; Žiburkus et al. 2006). The decreased [Mg²⁺] in the ACSF partially removes N-methyl-D-aspartate (NMDA) receptor blockade and reduces divalent charge screening (Huberfeld et al. 2011), increasing the duration and intensity of 4-AP in vitro SLEs. Both pharmacological models alone or in conjunction have been used to mimic temporal lobe epilepsy and pharmacoresistance, often observed in the intractable forms of epilepsy (Albus et al. 2008; D’Antuono et al. 2010; Loscher 2011; Wakah et al. 2010).

To determine excitatory synaptic reversal potential (Eₑ) in this convulsant solution, we blocked inhibition using picrotoxin (PTX; 50 μM). In a few instances, a bipolar stimulating electrode (200-μm outer diameter; WP1) was placed either on the Schaefer collateral pathway (n = 5) in the st. radiatum or on the alveus/orniens excitatory projections to CA1 pyramidal cells (n = 4) and interneurons (n = 5), respectively. In all cases, while in the presence of PTX, spontaneous burst firing occurred and electrical stimulation was unnecessary. Likewise, a stimulation-evoked inhibitory synaptic reversal potential (Eᵢ) was measured in the presence of NMDA [R-2-amino-5-phosphonopentanoate (APV); 25 μM] and α-amino-3-hydroxy-5-methyl-4-isozxazolopropionic acid (AMPA)/kainate receptor [6-cyano-7-nitroquinoxaline-2,3-dione (CNQX); 10 μM] antagonists. Eᵢ for pyramidal cells was measured by stimulating st. pyramidale/orniens (n = 5) or pyramidal/radiatum (n = 5) borders. Eₑ for the horizontal oris/orniens was measured by stimulating oris (n = 6) or oris/radiatum border (n = 5). These varied sites of stimulation were chosen on the basis of known anatomic locations of the bulk inhibitory and excitatory projections to these cell subtypes (Freund and Buzsaki 1996; Pouille and Scanziani 2004). All of the drugs were bath applied.

To track the temporal evolution of inhibitory and excitatory conductances during the seizures, E and I cells were voltage-clamped at a range of holding potentials (−80 to +40 mV). Cells used for final analysis had to meet the criteria of undergoing at least six seizures at different positive and negative holding potentials while maintaining a stable recording and an input resistance higher than 100 MΩ. To test for stability of the patch and cell, after each holding potential (−80 to +40 mV), we checked that when the cells were held at −60 to −80 mV during interseizure intervals, the change of bias current did not exceed 150 pA of the original holding current value. We randomly alternated between positive and negative holding potentials for each subsequent ictal event. The preictal SLE state in 4-AP is commonly associated with increases in the frequency of firing and bursting in the individual cells and networks, monitored by whole cell and extracellular DC recordings. Increases in frequency of firing and bursting monitored electrically and through the audio speaker were used as the cues by the experimenter to anticipate the impending SLE and change the holding potential with compensated capacitance. If a seizure did not occur, the cells were returned to the negative holding potential (−60 to −80 mV) and changes in bias holding current were monitored. Liquid junction potential value (LJPV) using this whole cell solution was measured as +13 mV. All of the holding potentials and reversals were reported with the adjusted LJPV value.

Data analysis and dynamic conductance measures. Data were analyzed using Clampfit and Matlab. To analyze interictal burst (IB) reversals, the minimal amplitude (root mean square, RMS) of spontaneous activity observed at a given holding potential was considered as a synaptic potential reversal value. To determine the IB reversal values, we used two voltage-clamp protocols: continuous holding square wave-like current injections and slowly depolarizing (15 s: −80 to +40 mV) ramp current injections. To find the reversal potential within the ramps, we calculated the smallest RMS value of cell current deflections within 1,000-ms bins and matched these nominal current measurements to the corresponding membrane voltage value. Since the two experimental methods using square wave and ramp current injections did not show significantly different results, we report IB reversal values grouped from both types of measures and according to their respective cell subtypes.

To record synaptic current reversal during prolonged SLEs, we held the cells using continuous voltage clamp at a range of membrane potentials (−80 to +40 mV). To calculate excitatory and inhibitory conductances, we used an analysis method similar to that previously used in the in vivo (Borg-Graham et al. 1998; Rudolph et al. 2007) and in vitro experiments (Haider et al. 2006; Shu et al. 2003). To calculate the average total reversal potential (Eᵢ) over the course of a seizure, we binned the data every 100 ms. Within the time bins, all of the seizure episode currents were measured across all of the holding potentials for each cell. To isolate the synaptic currents, we first calculated the portion of the current produced by the inherent leakiness of the cells. To account for this leak current, we calculated the total amount of current (at a given membrane potential) during the 1-s period before the ictal discharges. We visually selected 1 s of data that did not contain large bursts. We then subtracted this ambient leak value from the subsequent bursts and ictal event currents. The current values at each holding potential were fit to a line. The intercept with the abscissa reveals the total synaptic reversal potential (Eᵢ), and the slope gives the total synaptic membrane conductance (gₑ).
value is the sum of the excitatory and inhibitory conductances, $g_e$ and $g_i$. The $E_T$ is a potential value that would balance the total synaptic drive. That is, it is the potential at which excitation and inhibition are balanced. Depolarized $E_T$ values indicate dominance by the excitatory, inward current, and hyperpolarized $E_T$ values indicate a shift toward outward inhibitory currents. $E_T$ and $g_T$ values are required to calculate the individual excitatory and inhibitory conductances by using time-dependent Ohm’s law. By inserting the measured excitatory and inhibitory reversal potentials, $E_e$ and $E_i$, we then calculated the individual conductances, $g_e$ and $g_i$. Our calculations were done using the following set of Eqs. 1–4, where $V$ is the voltage-clamp holding potential and $I_T$ is the total synaptic current:

$$V = I_T(t)/g_T(t) + E_T(t) \quad (1)$$

$$g_T(t) = g_i(t) + g_e(t) \quad (2)$$

$$g_e(t) = g_T(t)[E_e - E_T(t)] / (E_e - E_i) \quad (3)$$

$$g_i(t) = g_T(t)[E_i - E_T(t)] / (E_i - E_e) \quad (4)$$

Conductance values for all of the pyramidal cells and the interneurons were averaged across the E and I cells, respectively, and are presented in this article as means ± SE.

**Statistical analysis.** Running ANOVA analysis was performed on the individual $g_e$ and $g_i$ values and on $g_e/g_i$ ratios throughout the evolution of the SLEs (see Fig. 6). Values at a confidence level of 95% or higher were considered as significant. To determine whether time course of the $g_e/g_i$ ratio differed significantly during the process of SLE, we normalized $g_e$ and $g_i$ individually (see Fig. 6, C and D).

**Immunohistochemistry and neuronal reconstructions.** The cells that exhibited morphological features of pyramidal cells and were immunohistochemically negative for parvalbumin were identified as pyramidal (Fig. 1). The cells that contained horizontally projecting dendrites and axonal terminals extending toward the apical pyramidal dendrites to the radiatum-lacunosum-molecular border and that were positive for somatostatin were identified as O-LM cells (see summary table in Fig. 1).

During the recordings, cells were filled with neurobiotin. Neurobiotin-filled cells were fixed for 24–76 h in 4% paraformaldehyde in 0.1 M phosphate buffer (PB). The neurobiotin-filled cells were visualized using 7-amino-4-methylcoumarin-3-acetic acid (AMCA excitation/emission)-conjugated streptavidin (1:1,000; Vector Laboratories, Burlingame, CA). Somatostatin and parvalbumin immunoreactivity was detected using a mixture of two primary antibodies raised in rabbit or mouse (Maccafferri et al. 2000). For somatostatin, we used mouse monoclonal anti-somatostatin (anti-Som) at 1:1,000. For parvalbumin, we used polyclonal rabbit anti-parvalbumin (anti-PV) at 1:1,000. Parvalbumin staining was used to make sure that recordings were indeed done from pyramidal CA1 cells. Somatostatin stain in conjunction with subsequent neuronal reconstructions was used to identify O-LM interneurons. Secondary antibodies were conjugated with fluorophores [anti-PV was conjugated with fluorescein isothiocyanate (FITC) and anti-SOM with tetramethylrhodamine isothiocyanate (TRITC)]. After the staining, slices were wet mounted using aqueous medium and were analyzed using fluorescent (Zeiss 2FS) and confocal (Olympus BX) microscopes equipped with sensitive cameras (Cooke Sensicam or Hamamatsu Orca). Using a ×40 objective lens, we identified neurobiotin-positive cells that were also positive for somatostatin and parvalbumin. After fluorescent antibody staining and analysis, the slices were demounted, washed, and re stained with streptavidin. Briefly, using the Vector stain ABC Elite kit (Vector Laboratories), we incubated the slices with 0.1% Triton X overnight and then used standard 3’,3’-diaminobenzidine histochemical methods. Axonal visualization was improved using a PB solution containing nickel ammonium sulfate. The recorded cell morphologies were then reconstructed using Neurolucida (Microbrightfield, Fig. 1).

**RESULTS**

**Epileptiform activity recordings in the CA1 pyramidal cells and OLM interneurons.** Single and dual whole cell and simultaneous extracellular recordings were performed in the identified hippocampus CA1 area O-LM and PCs. O-LM cells were targeted due to their high susceptibility to damage by seizures (Oliva et al. 2002; Sanon et al. 2005; Santhakumar and Soltesz 2004) and their involvement in seizure initiation in the in vitro 4-AP model (Žiburkus et al. 2006). O-LM cells were first selected visually on the basis of their somatic location in the oriens and bipolar horizontally extending dendritic trees, occasionally containing detectable axon branching toward radiatum and lacunosum molecular layers. Exact subtypes of the recorded cells were confirmed by post hoc morphological reconstructions and dual immunohistochemical staining using distinct cellular markers (Fig. 1). Some cells were only par-
tially filled with neurobiotin, thus allowing us to trace only portions of the axons. To confirm that basket cells were not recorded when pyramidal layer neurons were targeted, we used antibody directed against parvalbumin-positive cells. Post hoc, all of the pyramidal layer cells used in the analysis were identified as PCs. Somatostatin positively stained 85% of all of the putative O-LM cells.

To analyze inward and outward synaptic currents and their reversals during short interictal bursts and prolonged seizures, we employed a well-studied in vitro seizure model of 4-AP and lowered [Mg\(^{2+}\)]\(_o\) (0.6 mM; Isaev et al. 2005; Khosravani et al. 2003, 2005; Žiburkus et al. 2006). Whole cell recordings were performed in current- and voltage-clamp modes. The internal recording solution for voltage-clamp contained blockers for potassium and fast sodium conductances (Cs\(^{2+}\) and QX-314) and neurobiotin. This experimental paradigm allowed us to isolate inhibitory and excitatory synaptic currents (Fig. 2).

Since the active membrane properties of the cells were rapidly blocked by QX-314, we could not reliably employ electrical characteristics to aid in distinguishing between the recorded cell subtypes (Gupta et al. 2000). In addition to blockade of the voltage-gated sodium channels, QX-314 also blocks G protein-gated potassium conductances, such as GABA\(_h\) (Alreja and Aghajanian 1994; Otis et al. 1993; Slesinger 2001). Thus potassium conductances in these experiments were blocked by both cesium and QX-314 in the pipette solution.

**Interictal burst reversals in the CA1 pyramidal cells and OLM interneurons.** We first studied the reversal potentials of both cesium and QX-314 in the pipette solution. Potassium conductances in these experiments were blocked by both cesium and QX-314 in the pipette solution.

After determining the reversal potential values, we needed to determine the total synaptic current reversal potential values, \(E_T\). During the repetitive spontaneous SLEs, PC and O-LM cells were temporarily voltage-clamped at a range of negative to positive holding potentials.

**Measuring \(E_e\) and \(E_i\) in hyperexcitable media.** To calculate the dominant conductances during the prolonged duration SLEs, we first needed to determine \(E_e\) and \(E_i\) in the presence of 4-AP and lowered [Mg\(^{2+}\)]\(_o\) (Fig. 3). Before application of 4-AP, equivalent stimulation of the Schaffer collaterals in normal ACSF solution in the presence of either the inhibitory or the excitatory blockers showed the \(E_e\) reversed at \(0 \text{ mV} (±6.7 \text{ mV})\) and \(E_i\) at \(-73 \text{ mV} (±5.9 \text{ mV})\) (corrected for LJPV, \(n = 5\) in each condition). To measure \(E_e\) in the presence of 4-AP, the inhibition was blocked by addition of PTX to the bath. In 4-AP and PTX, we observed spontaneous long-duration excitatory bursts that reversed at highly positive membrane potentials (\(E_e = +23.5 \text{ mV}\) ; Fig. 3). In turn, to measure \(E_i\) in the presence of 4-AP, all of the excitatory synaptic transmission was blocked by APV and CNQX and inhibitory currents were electrically evoked. Inhibition reversed at the value of \(E_i = -60 \text{ mV}\). This showed that in the absence of intrinsic voltage-gated properties of the cells and in 4-AP, excitatory and inhibitory synaptic currents reversal values are shifted toward more positive membrane potentials.

**When excitation equals inhibition: \(E_T\).** After determining the \(E_e\) and \(E_i\) values, we needed to determine the total synaptic current reversal potential values, \(E_T\). During the repetitive spontaneous SLEs, PC and O-LM cells were temporarily voltage-clamped at a range of negative to positive holding potentials.

**Excitation/inhibition balance during seizures.** The equations for \(E_T\) and the reversal potentials of the excitatory and inhibitory currents, \(E_e\) and \(E_i\) respectively, are as follows:

\[
E_T = \frac{E_e - E_i}{\ln(z_1 - z_2)}
\]

where \(z_1\) and \(z_2\) are the total conductances of the excitatory and inhibitory channels, respectively.

**Fig. 2. Interictal burst reversals in O-LM and pyramidal cells.** A: concurrent whole cell (EPSP) and extracellular (EC) recording traces of spontaneous excitatory potentials in a pyramidal cell held at \(-70 \text{ mV}\) in current-clamp mode. B: excitatory postsynaptic currents (EPSC) recorded in the pyramidal cell held at \(-70 \text{ mV}\) using voltage clamp. C: inhibitory currents (IPSC) recorded in the pyramidal cells at \(+20 \text{ mV}\). D: interictal burst reversal potential values (\(E_T\)) were significantly different between the 2 types of cells: pyramidal (\(-27.6 ± 7.2 \text{ mV}, n = 13\)) and O-LM cells (\(-7.7 ± 9.6 \text{ mV}, n = 21\) \((P < 0.0001, \text{unpaired} t\text{-test})\). Pyr, pyramidal cells; Int, O-LM interneurons.
potentials (−80 to +30 mV; METHODS). Figure 4, A and B, illustrates simultaneous dual whole cell recordings in current- and voltage-clamp modes and the extracellular recording of the SLEs. The start of the body of SLEs was always determined by the FPES in the extracellular signal (Fig. 4, A and B). Qualitative observations of seizures in interneurons and PCs indicated that there is a dynamic conductance interplay associated with the initiation, body, and termination phases of the seizures.

An O-LM interneuron example with 14 seizures recorded at different holding potentials is shown in Fig. 4C. It illustrates that outward currents corresponding to inhibition, revealed at positive holding potentials, were more prominent before and after the ictal discharges; inward currents, corresponding to excitation revealed at negative holding potentials, dominated during the body of the ictal discharges (Fig. 4, A–C). When several seizures were acquired in the same cell at different holding potentials, it allowed us to measure dominant synaptic conductances and produce linear fits of the average current-voltage (I-V) dynamics at every second before, during, and after the body of the SLEs. To isolate the synaptic inputs, leak currents were subtracted throughout the entire extent of the seizures as shown in Fig. 4D.

To calculate $g_{e}$ and $g_{i}$, we needed to determine the total synaptic conductance values $g_{T}$ and the total synaptic reversal values $E_{T}$ (Eqs. 1 and 2). Holding cells at an array of potentials allowed us to calculate the average I-V relationships in the PC and O-LM cells throughout the entire extent of the seizure period. Average I-V plots for PC and OLM cells are shown in Fig. 5, A and B. Each inset in the plots shows the average SLE I-V plots for all of the O-LM and PCs at 1-s intervals (calculated at an array of holding potentials). The average SLE I-V plots were fitted linearly, and the $g_{T}$ values were obtained from the slope of these curves. The calculated slope values were steeper in O-LM cells compared with those in the PCs.

Individual representative and average $E_{T}$ values are shown in Fig. 5, C–E. Similarly to the interictal bursts (Fig. 2B), total synaptic currents during ictal-like events reversed at more positive potentials in O-LM interneurons (Fig. 5, D and F) compared with those in the PCs (Fig. 5, C and E).

To calculate $g_{e}$ and $g_{i}$, we also needed to determine the driving force, defined by the difference in the $E_{e}$ and $E_{i}$ values (Fig. 3) and $E_{T}$ (Fig. 5; Eqs. 3 and 4). In both cell subtypes, the $E_{T}$ values shifted to more positive values during the body of the seizures. In O-LM interneurons, this excitatory shift persisted into the termination part of the seizures. In PCs, $E_{T}$ returned to preseizure levels 15 s after the start of the ictal event (FPES). $g_{e}$ and $g_{i}$ interplay during SLEs. Calculated average conductance values showed that before the initiation of the SLEs, inhibitory conductances dominated PCs (Fig. 6, A and C). In
O-LM cells, inhibitory synaptic currents were more balanced in amplitude with the excitatory ones. During the start of the ictal events, \( g_e \) and \( g_i \) peaked during the FPES with \( g_e \) dominating both cell types (Fig. 6, A–D). Excitation remained dominant in OLM cells (Fig. 6, B and D), whereas inhibition dominated PCs during the termination stage (Fig. 6, A and C). This strikingly increased excitation in the O-LM cells during the ictal discharge (Fig. 6, B and D) could be linked to the failure of the “inhibitory veto” (Trevelyan et al. 2006), causing interneurons to enter a temporary depolarization block reported earlier (Žiburkus et al. 2006). The prolonged, yet gradually equalizing \( g_e/g_i \) ratios during the termination phase in the O-LM cells could also be the only remaining mechanism of excitation-driven inhibitory termination of the SLEs (Fujiwara-Tsukamoto et al. 2004).

Statistical comparison of the \( g_e \) and \( g_i \) changes in the two cell subtypes highlighted the differences in the synaptic dynamics (Fig. 6, E and F). Conductance balance in the PCs during SLE was significantly different at the initiation and termination of the SLEs, because the neurons were dominated sequentially by excitation and then back to inhibition (Fig. 6E). In comparison, in O-LM cells, conductance balance showed a significant increase in excitation that lasted well into the body and termination stage of the SLEs (Fig. 6F). This suggests that during epileptogenic-like hyperexcitability, the nearby located interneurons and PCs tune into distinct network processes, some dominated by inhibition, others by excitation.

**DISCUSSION**

To our knowledge we report the first comparative whole cell voltage-clamp study in excitatory and inhibitory cells during SLEs. The E/I balance in pyramidal and O-LM cell subtypes differed dynamically during the progression of the SLEs. At the onset of the SLEs, PCs were dominated by inhibitory inputs, but O-LM received relatively balanced E and I inputs. Inhibition again dominated during the termination of SLEs in the PCs. In O-LM cells, excitation dominated the termination phase of the SLEs, suggesting that continuous excitation of the inhibitory circuits persists and may be an important mechanism to terminate the seizures. These dynamic changes in E and I conductances also support the notion of earlier work that suggested a tripartite division of seizures into distinct initiation, body, and termination stages (Schiff et al. 2005; Timofeev and Steriade 2004; Žiburkus et al. 2006). Present measurements provide a new perspective into the underpinnings of these sequential dynamical SLE stages.

Conductance increases during pathological activity. Large conductance increases during epileptiform events were previously observed only in the model of “paroxysmal depolarizing shifts” (PDS) (Rutecki et al. 1987). When shorter duration PDSs were voltage-clamped in the presence of \( K^+ \) channel blockade (tetraethylammonium, TEA) in putative PCs, the authors found that initiating and terminating synaptic currents reversed at more negative potentials compared with the peak of the PDS. This suggested that the time points preceding and following the PDSs contained a larger component of inhibitory

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**Fig. 4.** In vitro seizure recordings in current- and voltage-clamp modes. A: traces of repetitive seizures recorded simultaneously in current clamp (pyramidal cell, top trace), voltage clamp (O-LM interneuron, middle trace), and extracellular recordings (bottom trace). Pyramidal cell is in current clamp at \(-63 \text{ mV}\), and O-LM interneuron is held at \(-70 \text{ mV}\) in voltage clamp. Cesium gluconate was substituted with potassium gluconate, and QX-314 was omitted from pyramidal cell recording solution. B: same cell pair as in A, but O-LM interneuron is voltage-clamped at \(+40 \text{ mV}\) to record outward currents. All of the seizure ictal event start times were aligned to the fast positive extracellular potential shift (FPES; Žiburkus et al. 2006a). C: example of 14 repetitive seizure traces recorded at different holding potentials (\(-30 \text{ to } +35 \text{ mV}\)) in the same O-LM interneuron as in A and B. Note the qualitative differences in the dominant currents before, during, and after the termination of the ictal events. D: calculated synaptic \( g_{syn} \) and leak \( g_{leak} \) for the traces in C shown at a single time window for 1 s preceding the FPES.
synaptic input, although in TEA, $E_e$ was measured, $E_i$ was not, and synaptic conductance calculations were omitted (Rutecki et al. 1987).

E/I imbalance is often assumed to underlie seizures in epilepsy (McCormick and Contreras 2001); nonetheless, the dynamics of $g_e$ and $g_i$ balance during more prolonged seizures have not been studied until now. Recently, the E/I balance theory also extended into the studies of the social behavior dysfunctions, such as autism and schizophrenia (Gogolla et al. 2009; Hashimoto et al. 2003; Kehrer et al. 2008; Markram and Markram 2010; Rubenstein and Merzenich 2003; Vattikuti and Chow 2010; Yizhar et al. 2011). As dynamical instabilities, seizure and SLE initiation may represent near-maximal E and I conductances. It is likely that the relation between the effects of altered E/I balance in seizures and that of cognitive disorders and social dysfunction will likely rely on different patterns of microcircuit activity.

**Up state vs. SLE.** Perhaps the best dynamically characterized normal recurrent neuronal network events are up states in neocortical PCs recorded in vitro and in vivo (Borg-Graham et al. 1998; Haider et al. 2006; Hasenstaub et al. 2005; Rubenstein and Merzenich 2003; Vattikuti and Chow 2010; Yizhar et al. 2011). As dynamical instabilities, seizure and SLE initiation may represent near-maximal E and I conductances. It is likely that the relation between the effects of altered E/I balance in seizures and that of cognitive disorders and social dysfunction will likely rely on different patterns of microcircuit activity.

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In the current study, we used similar techniques that were previously employed in vivo and in vitro to study up state activity (Berg et al. 2007; Borg-Graham et al. 1998; Haider et al. 2006; Hasenstaub et al. 2005; Rubenstein et al. 2007; Shu et al. 2003). To date, our understanding of the dynamical changes in the E/I conductance balance is limited to the analysis of up states. We used a common seizure model and calculated conductances in two subtypes of cells using whole cell patch clamp during SLEs. We showed that there is a considerable disparity in the way CA1 O-LM interneurons and PCs receive network inputs as a function of distinct seizure stages. Our findings may be related to diverse activity patterns in local neuronal subsets observed in vivo in cats and in epileptic human patients (Rubenstein et al. 2007; Truccolo et al. 2011).
Using dynamical total reversal potential values, we have shown that during SLEs, similarly to up states, $g_E$ and $g_i$ peak at the start of the seizures. Nevertheless, the SLEs studied here demonstrate a unique temporal interplay of $g_E$ and $g_i$, consistent with the temporal spiking activity interplay previously found in the same neuronal subtypes (Žiburkus et al. 2006). How up states, or seizures, might dynamically differ in disorders of E/I balance such as autism and schizophrenia or in models of other diseases remains to be investigated. Furthermore, additional studies of the excitatory and inhibitory conductances in other seizure and epilepsy models will be necessary to determine if the dynamics observed in 4-AP are present in other chronic or posttraumatic epilepsy models. In general, the complex interactions between and among the excitatory and inhibitory networks make it difficult to map the results of this experiment to other models. However, the temporal interplay and the maximal excitatory synaptic input barrage during the ictal-like events observed here are likely to be a shared feature in many types of seizures in which E and I cell activity is preserved. Remarkably, the E/I interplay and heterogeneity in epileptiform cell signaling observed in 4-AP has now been demonstrated in several seizure models, and even in epileptic human brain recordings; although we cannot be sure that the conductance measurements are the same, we do know that the interplay pattern is found in many differing models and may have broad applicability in helping to explain network instabilities in seizures (Cymerblit-Sabba and Schiller 2010; Derschansky et al. 2008; Fujiwara-Tsukamoto et al. 2010; Huberfeld et al. 2011; Lasztoczi et al. 2009; Trevelyan and Schevon 2012; Truccolo et al. 2011).

From the methodology perspective, dynamic total reversal potential provides a good approximation of the conductance changes (Hasenstaub et al. 2005). Nevertheless, we are cautious when interpreting the data based on dynamic total but static individual $E_i$ and $E_E$ potential reversal values. In our experiments, seizures arise under the global influence of partial potassium conductance blockade of 4-AP. This compound elevates the resting membrane potentials towards depolarization, in both excitatory and inhibitory cells and generates patterns of activity that share many similarities with in vivo
experimental and clinical seizures. Furthermore, blockade of glutamatergic or GABAergic receptors may influence $E_E$ and $E_I$ reversal potentials through changes in steady-state levels of ionic concentrations. Although such pharmacological manipulations are used as a standard in such studies, we are cautious when assuming that the $E_E$ and $E_I$ values determined using synaptic receptor blockers are stable during heightened states of network excitability.

Nevertheless, beyond this pharmacological manipulation of normal hippocampus, changes in ionic driving forces as a function of ongoing activity have been noted in a variety of in vivo models of epilepsy. In the pilocarpine model of epilepsy, $E_I$ can be shifted upward by up to 9 mV (Benini et al. 2011). In neonatal excitatory and inhibitory neurons of the hippocampus, chloride reversal was recently reported to be at an average value of −50 mV, about 20 mV above the value reported and calculated in mature neurons in vitro (Dzhala et al. 2010). On the other hand, Khirug et al. (2010) report that even a single seizure can redistribute chloride transporters and lower the $E_{\text{Cl}}$ into more negative values in the CA1 neurons. These findings all suggest that increasing excitability through partial depolarization of neurons may generate seizures, and perhaps the precise level of depolarization required to observe seizures may be less important than the functional balance between the excitation and inhibition achieved.

Up states, IBs, and especially prolonged duration SLEs allow for significant changes in the extra- and intracellular ionic compositions (Cressman et al. 2009; Ullah et al. 2009), which must alter the individual $E_E$ and $E_I$ values. Apart from the intracellular chloride loading, increases in the intracellular sodium concentration and extracellular potassium concentration ([K+]o) often take place during epileptogenic or heightened neuronal activity (Somjen 2004). During seizures, [K+]o can change by three to four times its basal concentration, yet even small fluctuations in [K+]o raise the K+ reversal potential, exerting significant effects on neuronal excitability. For example, our ongoing computational investigations suggest that increases in [K+]o from 4 to 12 mM cause over a 10-mV shift in the reversal potential of glutamatergic AMPA receptors (Cressman et al. 2009). Nonetheless, the ultimate and comprehensive representations of the E/I dynamics will require individual ionic driving forces underlying $E_E$ and $E_I$ to be simultaneously and dynamically monitored, a challenge presently beyond our technical capabilities.

Furthermore, extracellular calcium concentration during the ictal events in 4-AP can also drop dramatically. In previous studies, extracellular calcium concentration in brain slices was shown to drop from 1.8 to ~1.3 mM (Avoli et al. 1996b) during ictal activity. In cultured cell preparations, a similar reduction in calcium resulted in nearly fourfold reduction in the amplitude of excitatory postsynaptic potentials (Rettig et al. 1997). Our experiments were performed in 1.2 mM calcium concentration, a low baseline level, and show that the excitatory synaptic activity increases during the interictal bursts and ictal-like events; with a selective and temporal loss of O-LM interneuron spiking activity (Hazra et al. 2012; Žiburkus et al. 2006). In the absence of sufficient extracellular calcium, synaptic transmission may be reduced and electrical coupling may play a role in the dynamics we see. However, if the cell were attached ohmically to a set of voltage sources that changed in a stereotypical way during a seizure, then the currents at lower holding potentials would be affected in patterns similar to ones held at higher potentials. This is not seen in these experiments because the time course of excitatory and inhibitory currents do not reflect such common patterns. Finally, one recent report suggested that gap junctions may not play as significant a role as was previously thought in generating epileptiform activity in the 4-AP model (Connors 2012).

$E_E$ and $I_I$ cells “tuning into” different network inputs. The increased variability and depolarized values of the total reversal potential during IB and SLEs in the O-LM cells suggest that interneurons of the same subtype may receive or tune into different inhibitory and excitatory synaptic inputs. Increased excitation of the O-LM cells is also consistent with our previous reports describing the use of current-clamp recordings in the same seizure model, which showed O-LM cells discharging spikes at higher rates before seizure initiation, followed by the prolonged depolarization block during the body of the SLEs, during which PCs fired at high rates (Schiff et al. 2008; Žiburkus et al. 2006).

Under conditions in which voltage-gated conductances are not blocked, the balance of E and I inputs depends on their relative somatodendritic location (Pouille and Scanziani 2004). Computational modeling and experiments show that space clamp nonuniformity is substantial for voltage-gated conductances even in the proximal branching dendrites of nonspherical cells (Armstrong and Gilly 1992). Inclusion of voltage-gated conductance blockers (QX-314 and Cs2+) in our experimental paradigm allowed us to use our recorded cells as passive antennas tuned into the hyperexcitable network (Bar-Yehuda and Kornreich 2008; Feidervish and Gutnick 1996). In this way, both the inhibitory and the excitatory conductances in the two cell subtypes were unaffected by the active voltage-gated conductances (Bar-Yehuda and Kornreich 2008). Attenuation in the strength of the synaptic inputs would then depend only on the passive membrane properties and the overall axial resistance. Given that O-LM cells are electrotonically more compact than PCs, this could cause overestimation of the total reversal potential values in PCs. Previous in vivo and in vitro measurements of conductances employed sharp electrode recording techniques in putative PCs (Berg et al. 2007; Borg-Graham et al. 1998; Haider et al. 2006; Hasenstaub et al. 2005; McCormick et al. 2003). Although this type of recording does not affect the intracellular environment to the same extent as does the patch pipette, it is at a disadvantage in its space-clamping ability. Nevertheless, it remains to be seen whether epileptic conductance dynamics observed here in distinct inhibitory and excitatory cell subtypes follow the same trends in vivo and in other in vitro seizure models.

The role of early inhibitory network recruitment and its clinical significance. During SLEs, inhibition dominated PCs except for a restricted period of runaway excitation at seizure initiation. In many normal brain processes, such as up states or other neuronal oscillations (Buzsaki 2006), inhibition plays a precise role in either balancing or shaping the excitatory responses. For example, during both the conscious and unconscious states, neocortical neurons can be dominated by inhibition (Rudolph et al. 2007). Other experiments in neocortical slices using cell-attached recordings and calcium imaging showed that an inhibitory “veto” precedes the breakthrough excitation of epileptiform discharges (Trevelyan et al. 2006, 2007), and such patterns have recently been observed in human
seizures (Schov et al. 2012). These experiments suggest that inhibitory inputs play important roles in the early defense and potential forecasting of epileptic activity.

When interpreting EEG recordings of seizures clinically, one has to consider that underlying variations in the signal may stem from interplay of inhibitory and excitatory networks and their conductances (Schiff et al. 2005; Truccolo et al. 2011). In an experimental setting, focally generated theta rhythms can be synchronized by local inhibitory inputs (Glasgow and Chapman 2007). In epilepsy, potentially overcompensating increases in inhibition may also signal the start of the seizure (Truccolo et al. 2011). The pronounced early inhibitory component in PCs (Fig. 6A) in the current study suggest that network inputs may be controlling these cells at first but then failing and allowing runaway excitation to peak at the start of the SLEs. Likewise, increased excitation of the inhibitory cells following the peak of the conductances sustains the overall inhibitory network activity and promotes the inhibitory seizure “terminating veto.” It seems likely that these novel network mechanisms act concurrently with others, such as changes in ionic conductances (Cressman et al. 2009; Ullah et al. 2009), contributing to a transient pathological failure in neuronal activity control. We are hesitant to oversimplify seizure dynamics and instead suggest a multifaceted mechanism of pathology that spans failures in individual ion concentrations, intrinsic cellular properties, and network dynamics. Greater understanding of intrinsic cellular and network failures during seizures will help us construct more accurate dynamical models to be applied for improved seizure understanding, forecasting, and cell subtype-specific network control strategies.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

J.Z. and S.S. conception and design of research; J.Z. performed experiments; J.Z. and J.R.C. analyzed data; J.Z., J.R.C., and S.S. interpreted results of experiments; J.Z. and J.R.C. prepared figures; J.Z. drafted manuscript; J.Z., J.R.C., and S.S. edited and revised manuscript; J.Z. approved final version of manuscript.

REFERENCES


EXCITATION/INHIBITION BALANCE DURING SEIZURES


