Background and significance

For over fifty years, seizures have been thought of as "hypersynchronous" events (W. Penfield and H. Jasper, 1954; Wyler, A.R., et al, 1982; Kandel E.R. and 1991), and the existing computational models of seizures have almost uniformly assumed that seizures represent a straightforward increase in synchronization. However, recent experimental data indicates that in fact individual neuronal units may alternately desynchronize and synchronize (Netoff T. and Schiff S., 2002). The latter study examined synchrony levels in *subthreshold* (synaptic activity) between two CA1 pyramidal cells during seizure-like events in *in vitro* rat hippocampal slice preparations. The goal of the present study is to extend previous observations to describe synchrony in both the *subthreshold* (synaptic) and *suprathreshold* (spiking) events among pyramidal-pyramidal, pyramidal-interneuron, and interneuron-interneuron cell pairs before, during, and after *in vitro* seizures.

Dynamical evolution of interactions between individual neurons during seizures were quantified using three forms of cross-correlations: the full voltage trace, the discrete spiking events alone, and the time trace with the spikes removed. We find that the synchronization between neurons can be qualitatively different when characterized as either synaptic currents or spike correlations. Our results further show that the interactions between different types of neurons (pyramidal-pyramidal, interneuron-pyramidal, and interneuron-interneuron) in seizures are complex and cannot, e.g., be simply described or modeled by an increase in "synchrony".

Materials and Methods:

Electrophysiology: Simultaneous dual and triple whole-cell patch clamp recordings were performed in the CA1 area of hippocampus in Sprague Dawley rats (P 18-30). Animals were anesthetized with Diethyl-Ether and the brains were immediately removed and placed in the slurry of dissecting buffer (in mM: 2.6 KCl, 1.23 NaH_2PO_4, 24 $NaHCO_3$, 0.1 $CaCl_2$, 2 $MgCl_2$, 205 Sucrose and 20 Glucose) for 3 minutes. Hippocampi were carefully isolated and transverse 350 um sections were made in the cold dissection buffer using a vibratome. Slices were incubated for an hour in normal ACSF (pH 7.3, 30ºC) containing (in mM) 130 NaCl, 1.2 $MgSO_4$, 3.5 KCl, 1.2 $CaCl_2$, 10 Glucose, 2.5 NaH_2PO_4, 24 $NaHCO_3$ and aerated with 95%O_2 5%CO_2. Following incubation, slices were transferred to a submersion recording chamber (Warner Instruments) and continuously perfused (1 ml/min, 34^oC) with oxygenated ACSF. Dual and triple simultaneous whole cell recordings in CA1 were performed using differential infrared contrast optics of a Zeiss Axioskop (40x water immersion lens). Borrosillicate glass micropipettes for current clamp were pulled to a final tip resistance of 3-7 MOhm and filled with (in mM): 116 Potassium guconate, 6 KCl, 0.5 EGTA, 20 Hepes, 10 Phosphocreatine, 0.3 Na GTP, 2 NaCl, 4 MgATP, and 0.3% neurobiotin (pH 7.25, 295mOsm). Extracellular recording was performed with borosilicate glass micropipettes pulled to a final tip resistance of 1-3 MOhm and filled with 0.9% NaCl. Figure 1 shows schematics demonstrating our general experimental approach. Cells selected for patching were generally between 30-200 um apart, either in stratum pyramidale, stratum oriens, or stratum radiatum. One or two extracellular recording electrodes were placed in the cell body layer close to the patched cells. Recordings were performed using MCC 700, Axoclamp 2B and 2A amplifiers (Axon Instruments); data were low pass filtered at

5KHz and digitized at 10KHz using National Instruments Data acquisition board. Seizures were evoked by bath applying 50-200 uM concentration of 4-Amino Pyridine (4-AP; Sigma). Seizures here are defined as sustained long-lasting (>10s) paroxysmal extracellular network events which are accompanied by a substantial extracellular negative DC shift, and which contain clear phases of initiation, body, and termination (Traynelis and Dingledine, 1988).

Histochemistry: All of the recorded cells were filled with 0.3% neurobiotin. Slices with filled neurons were processed using standard biocytin histological methods (Horikawa and Amstrong, 1988). Stained cells were examined under a light microscope and morphological reconstructions were performed using Camera Lucida.

Data analysis: The degree of synchrony between cell pairs was investigated in three ways to determine the relative degree of correlation between synaptic responses (subthreshold) and spiking events (suprathreshold). The first method uses the entire signal to measure the cross correlation between the two cells. The second method removes the spikes using only the subthreshold data between spikes. The final method treats the spikes as point processes and uses only these discrete points to measure the correlation. In order to ascertain temporal information, the data was sectioned into nonoverlapping fixed length windows (100 msec). In each case the correlation was measured over lag times up to half of the window length.

Results: A total of 41 neuronal pairs were recorded in three experimental permutations: 11 Pyramidal-Pyramidal, 26 Interneuron-Pyramidal, and 5 Interneuron-interneuron cell pairs. Seizures in recorded cells were observed 46% of the time. A total of 192 seizures were recorded. The cell types were identified by their anatomic location, electrophysiological membrane properties (Figure 2), and histological reconstructions (Figure 3). Interictal burst firing (sub-second duration, lack of negative extracellular DC potential shift, Figure 4) is clearly distinguished from seizures (longer than 10 second duration, accompanying DC negative extracellular potential shift, Figure 5).

Figure 6 shows the analysis of interactions between an interneuron and a pyramidal cell. The top panel A shows the raw data from these two neurons before, during, and after a seizure. Panel B shows the spike point process correlations which increase during this seizure. The crosscorrelation on the complete raw data set is shown in Panel C, and below this, the crosscorrelation on the tracing with the spikes removed. The ratio of the spike removed to the complete data crosscorrelation shows the relative contribution of the synaptic currents to the total correlation, plotted in Panel D. The time course of this ratio in Panel D illustrates the relative increase in synaptic correlations between the inhibitory and excitatory networks at the beginning of the seizure, and the gradual decrease towards the end, where the output spike correlations between these networks become more intense (Panel B).

We found that synchronization during seizures can be qualitatively different when calculated from synaptic currents (cell inputs) versus spike correlations (cell outputs). Furthermore, these patterns of increasing and decreasing synaptic versus spike correlations appear dependent upon the specific network (inhibitory versus excitatory), as well as whether the individual cells impaled from the network(s) are directly connected

synaptically or via gap junctions. Seizure initiation and termination interactions appear discriminable from the body of the seizure.

These findings are critically important to the computational modeling of epileptic seizures. The majority of extant seizure models are based upon the rather unsupported speculation that seizures are a straightforward manifestation of an increase in synchronization within 'the network' of neurons under study. As our knowledge that persistent neuronal network activity in many settings reflects a rough 'balance' between inhibitory and excitatory interactions (e.g., working memory, orientation tuning, etc.), our findings here show that seizures also reflect a complex interplay between inhibition and excitation. The ability to model such dynamics accurately is critically dependent upon knowledge of the biological interactions which generate seizures.

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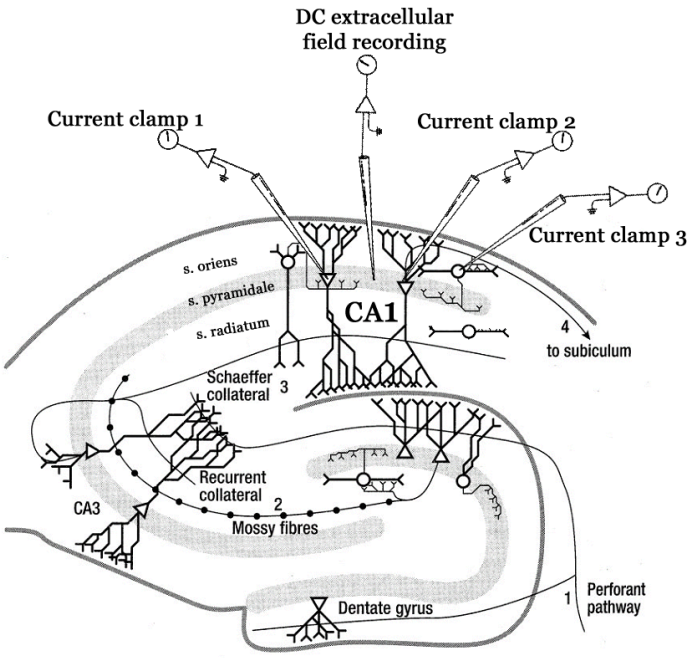

Figure 1. Experimental approach. Simultaneous dual or triple whole cell and extracellular recording in the CA1 area of rat hippocampus. Diagram adopted from McLeod P., Plunkett, K. and Rolls, E.T (1998). Also see Wayne Stewart's "Metaphysics by Default" (http://mbdefault.org)

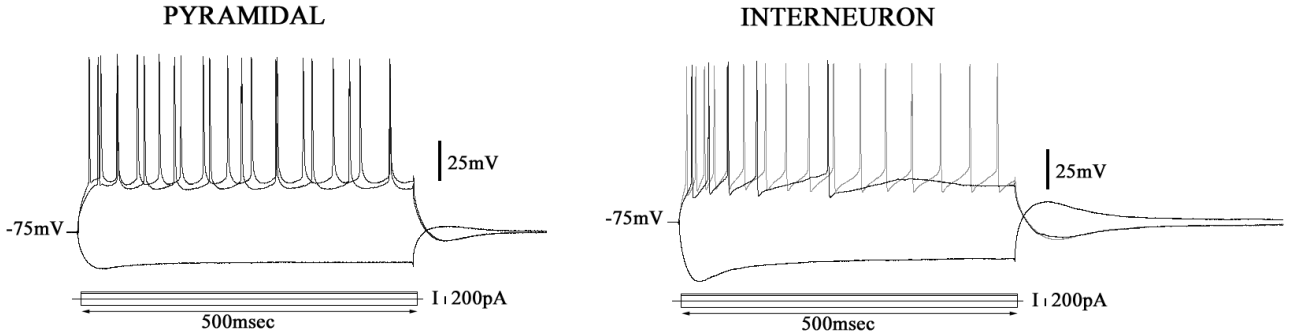

Figure 2. Physiological characterization of neuronal subtypes. Representative examples of pyramidal (left) and interneuron (right) membrane firing properties following incremental current injections.

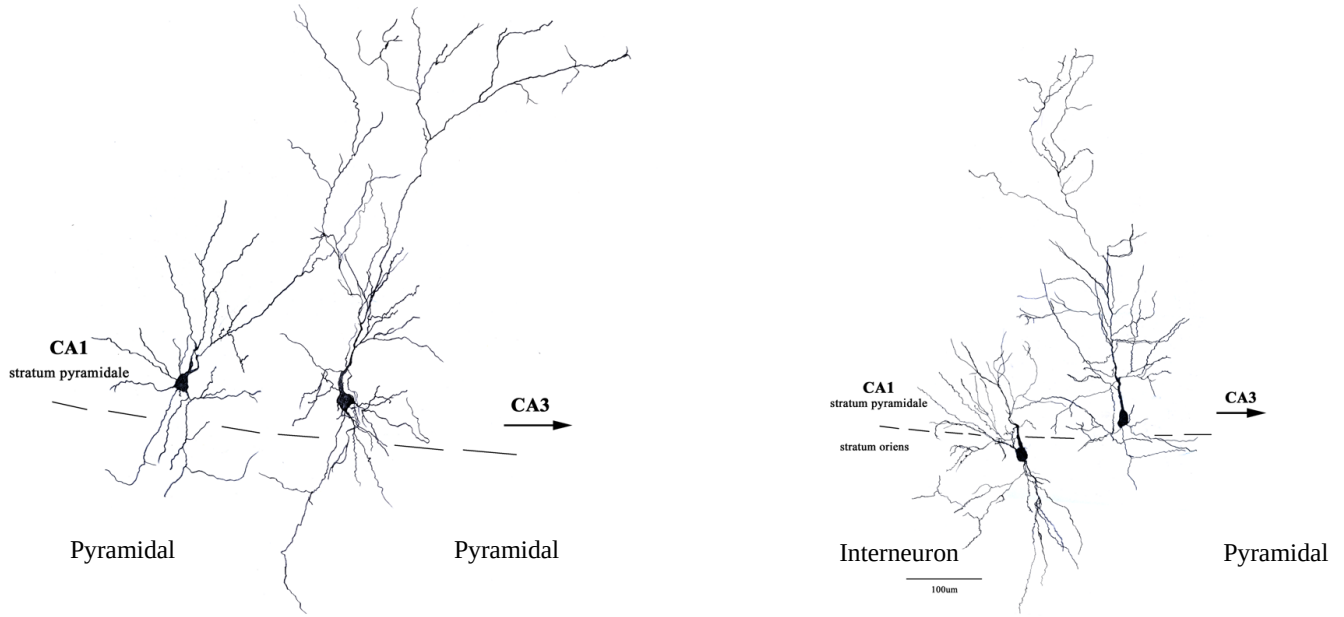

Figure 3. Morphological characterization of neuronal subtypes. *Camera lucida* drawings of pyramidal-pyramidal (left) and interneuron-pyramidal (right) cell pairs.

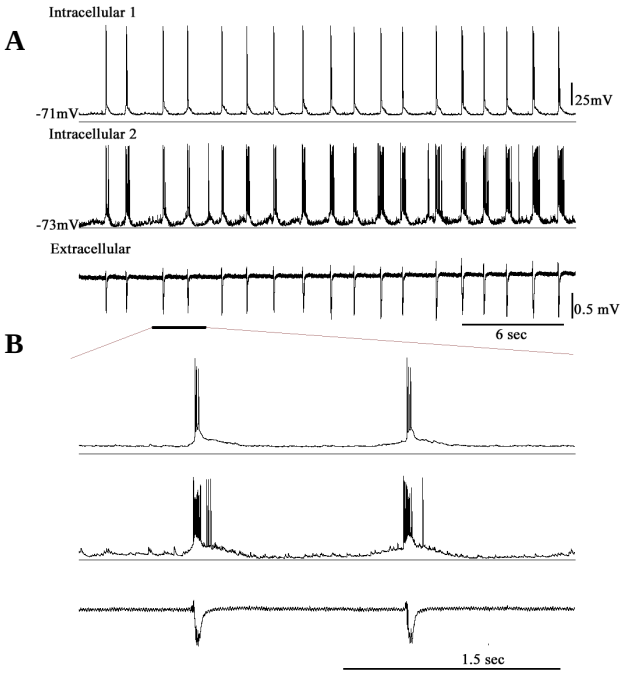

Figure 4. Interictal bursts in the presence of 4-AP. A. Simultaneously measured in intracellular and extracellular recordings. **B.** Detail of traces in A at expanded time scale.

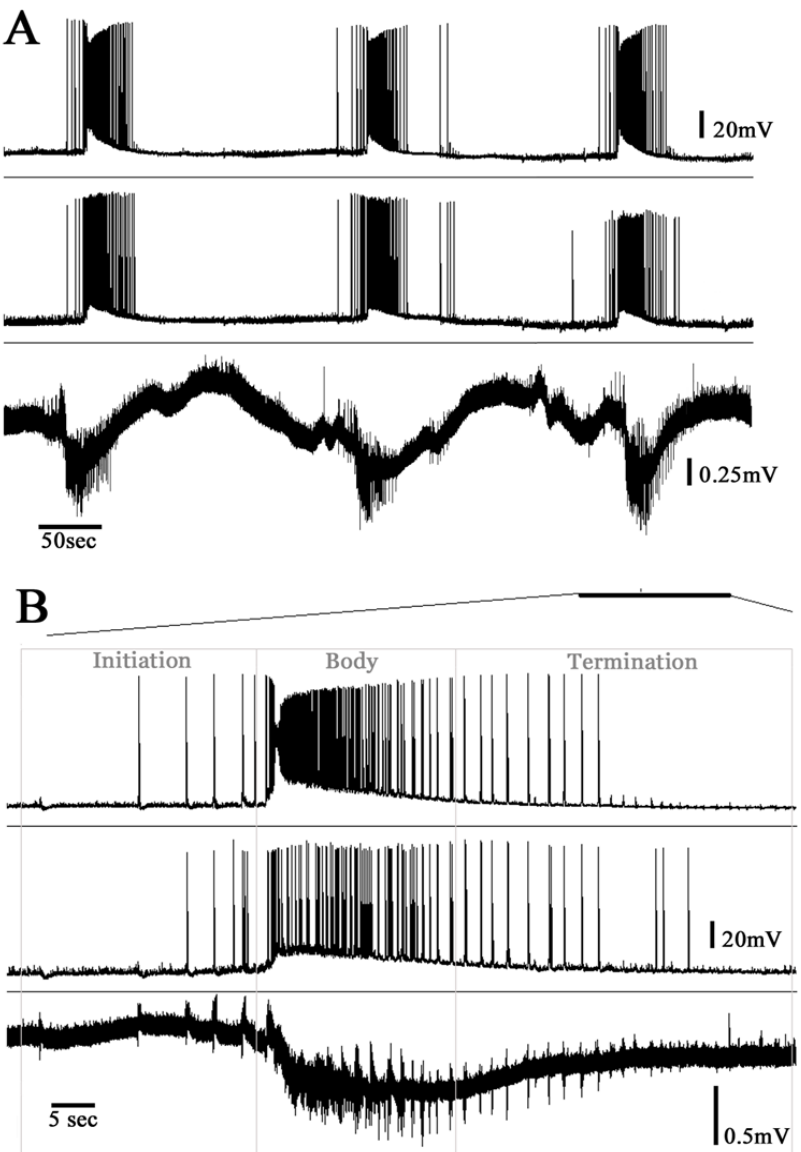

Figure 5. Seizures in 4-AP. A. Example of recurrent seizures during simultaneous dual whole cell (interneuron-top trace; pyramidal-middle trace) and extracellular (bottom trace) recording. Intracellular seizures are accompanied by a negative DC shift in extracellular recording. **B.** Detail of traces in A in an expanded time scale.

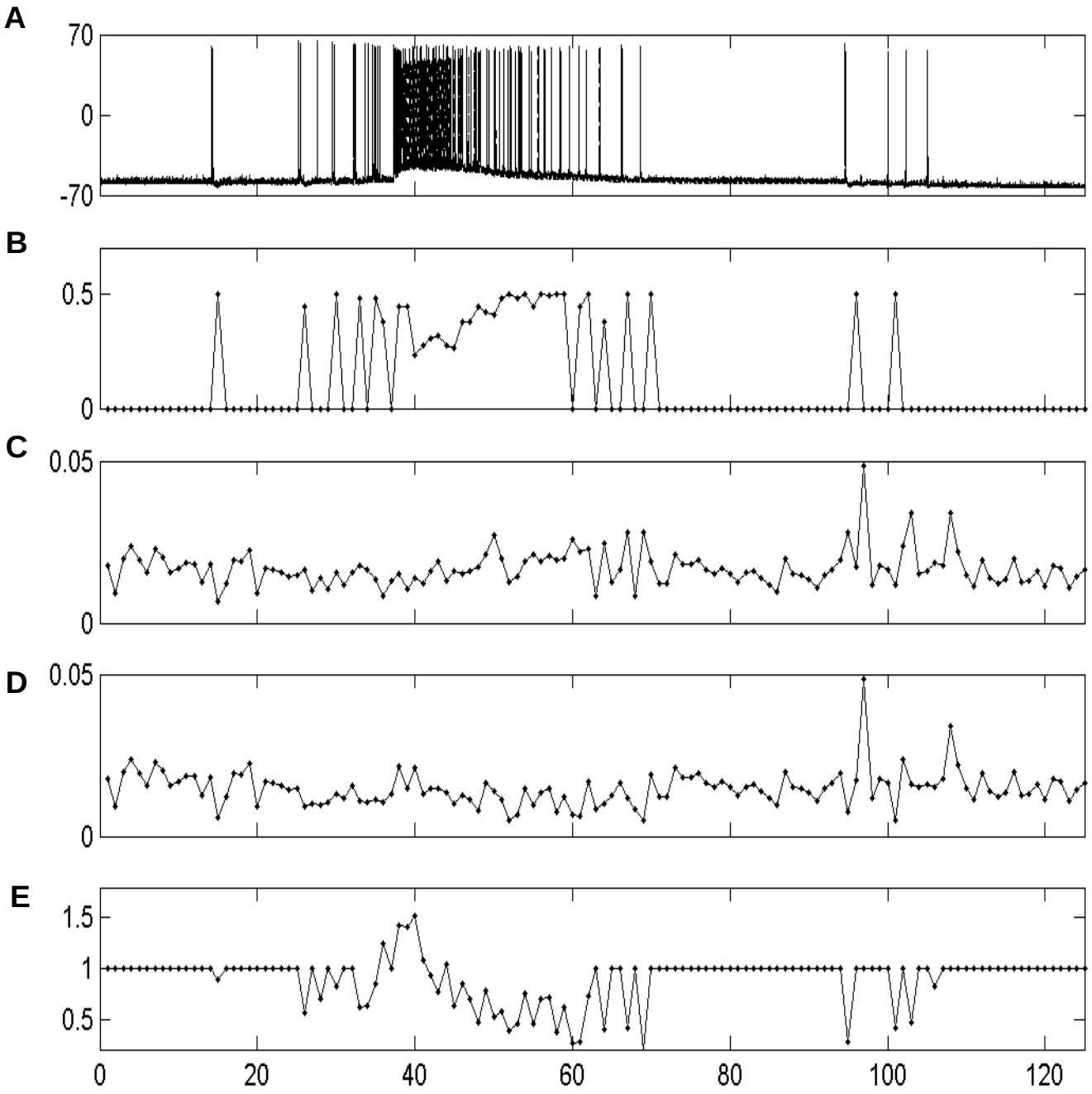

Figure 6. Correlation analysis. **A.** Simultaneous recordings from an inhibitory and excititory cell during seizure. **B.** Spike correlation throughout seizure. **C.** Correlation function using the full signal and **D**, with the spikes removed. **E.** The ratio of correlations measured without spikes (**D**) to that with spikes (**E**).