Modular propagation of epileptiform activity: evidence for an inhibitory veto in neocortex.

Andrew J. Trevelyan, David Sussillo, Brendon O. Watson, Rafael M. Yuste.

Supplementary information

Supplementary movie. Imaging of intracellular Ca2+ fluxes for propagating epileptiform activity. The movie shows the Ca^{2+} signal during a 19s period (plays at double speed) as the 1st ictal event experienced by this slice propagates across the field of view. It was imaged at 60Hz (for visualization, the movie was downsampled 1 in 6 frames) using a 20x objective (horizontal field of view = 330µm). It shows layers 4 and 5; the pia is to the top, white matter to the bottom. The slice was bulk loaded with the Ca^{2+} indicator OGB1. The signal is shown as ∆F/F₀ to allow a better visualisation of the change of Ca^{2+} fluorescence. Individual cells are bright spots, but the general increase in neuropil signal is also readily apparent. The movie clearly shows an early recruitment of a horizontal band of cells in layer 5, and after some delay, a progressive, episodic recruitment of the rest of the visualised network.

Supplementary figure 1. The astrocyte specific marker, sulforhodamine 101 (SR101) allows neuronal and glial cells to be distinguished easily. Both neurons and glia take up the Ca^{2+} indicator, OGB1 (Ai), but only glial cells take up SR101 (Aii.) There are 6 labelled glia labelled in this view (marked a-f). Aiii. shows the Ca^{2+} fluorescence for 3 of the glia (a-c) and for 3 representative neurons (1-3 – also labelled in photomicrograph Ai). Glia have slower and delayed Ca^{2+} rises compared to the neuronal signal.

Supplementary figure 2. Calculation of the inhibitory index. The left panels show the V_{clamp} and I_{clamp} recordings from a pair of adjacent layer 5 pyramidal cells, with the lower left panel showing the derivative of the V_{clamp} recording (dI/dt), with 3 and 5 standard deviations above the mean marked. The inhibitory index calculations for points
a, b and c are shown in the right panels. These plots show the histogram of the values for the dI/dt plots greater than 5 standard deviations above the mean (200ms bins digitized at 5kHz giving 1000 data points). The integral of these plots (equivalent to the integral of the dI/dt trace above 5stds) give the inhibitory indices. The values given are normalized to the peak index during the event.

**Supplementary figure 3. Rapid propagation of ictal events when network is partially disinhibited.** Paired recording, from two layer 5 pyramidal cells separated by 770µm, showing an ictal event soon after switching ACSF bathing solution to one containing 5µM picrotoxin and lacking Mg\(^{2+}\) ions. The delay (calculated from the midpoints of the initial upslope of the depolarizing shifts in the two cells as shown) was 53ms, indicating a velocity of \(~14\)mm/s. The propagation speeded up over the first 5-10 events (range 8-18mm/s), and thereafter was relatively constant at about 25-30mm/s. A second paired recording (405µm separation) gave similar propagation velocities. These values are similar to those reported by other groups in the partial disinhibition model of epilepsy, and about 2 orders of magnitude faster propagation than the 0 Mg\(^{2+}\) model with intact inhibition.

**Supplementary figure 4. Stepwise recruitment of neurons to an ictal event.** We show other examples of the intermittent recruitment pattern in the 0 Mg model of epilepsy. The traces are all aligned to the single biggest step. Both panels show the same data; in the lower panel, the traces are offset to show the individual traces better.

**Supplementary figure 5. Different patterns of activation in layers 4 and 5.** In some slices, the build up of activity in layer 5 occurs over a very protracted period. Here we show a slice clustered using the same method as shown in Figure 4, but showing the mean ΔCS/CS\(_{\text{min}}\) plots for each cluster (instead of the dCS / dt plots). The blue and green clusters ratchet up their activity over the time course of many barrages. The recruitment of the three groups of layer 4 cells though is in each case quite abrupt. Notably, the layer 4 clusters have almost identical patterns of recruitment with a single step in the Ca\(^{2+}\) signal much larger than the other steps.
Supplementary figure 6. Ictal events in adult and juvenile neocortex share the same key features. All the imaging experiments in this study were performed on slices taken from postnatal day 13-16 mice. Unfortunately, bulk-loading does not work well in older slices, so we are forced to make comparisons based on the electrophysiological recordings. We recorded from animals aged P30, P31 and P49, and ictal events in all share the same key features as the juvenile mice slices. Voltage clamped pyramidal cells experienced repeated synaptic barrages that were initially inhibitory and progressively switched to being overwhelmingly excitatory. Further supporting evidence that these same principles apply to adult cortex comes from the original description of propagation in Mg$^{2+}$-free media (Wong, B. Y. & Prince, D. A. The lateral spread of ictal discharges in neocortical brain slices. Epilepsy Res 7, 29-39 (1990)). They used extracellular electrodes to record propagating epileptiform activity in slices taken from adult guinea pig, and recorded very slow propagation (160µm/s – in our study, the initial events propagated at 120µm/s) initially in about half the slices. As in our study, slow propagation was only recorded in the earliest events, and subsequent events propagated at much greater speeds.