Recurrent Excitation Facilitates High-Conductance Network Events in the Mouse Neocortex

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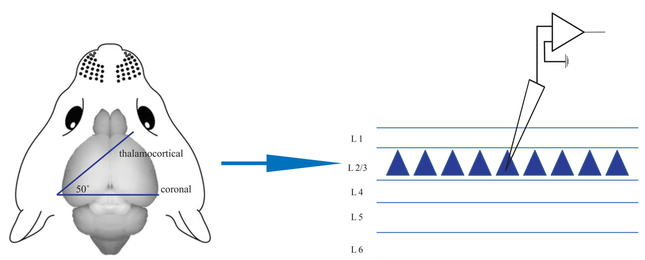
Introduction

Most synapses impinging onto an individual pyramidal cell (PC) in the neocortex originate from nearby excitatory cells [2,4], signifying the importance of recurrent excitatory connections in cortical circuits. This connectivity scheme is exemplified in the barrel cortex of rodents, in which feedforward activation of a single barrel results in reverberant feedback activity in the superficial cortical layers that is spatially confined to a single cortical column [3]. We hypothesized that the amount of recurrent excitation directly correlates with the amount of network activity. To test this hypothesis, we used a mouse model in which the genetic ablation of the mitogen-activated protein kinase Erk2 results in reduced density of mature PCs within cortical layers 2/3 [in preparation]. The decrease in PC density should cause a reduction in the number of local synaptic contacts onto neighboring PCs, provided that the number of projections from the spared PCs is unaltered. **We, thus, compared global network excitability in brain slices from wild-type and mutant mice in the form of high-conductance events**. A simple computer model exhibiting bistability can account for the role of recurrent excitation in modulating network excitability observed in experiments.



Methods

We used 350 µm thalamocortical slices [1] of somatosensory cortex from juvenile (P14-P21) C57BL/6 wild-types (**WT**) and mutant mice (**cKO**) in which expression of Erk2 was conditionally abolished within the dorsal telencephalon using an Emx1-cre during early embryonic development (E9). Voltage traces were obtained from layer 2/3 pyramidal cells (PCs) using single-cell patch clamp experiments in the current-clamp configuration. Network activity was increased with 5 µM gabazine, a $GABA_A$ receptor antagonist, to induce spontaneous synaptic **high-conductance events** (**HCEs**; ~10nS).Voltage recordings were performed for 10 minutes to quantify the frequency of disinhibition-induced HCEs. Non-parametric statistical analysis was performed using bootstrap methods to test for differences in HCEs between WTs and cKOs. In computer simulations, we employed Matlab to generate a non-linear two-dimensional model of reciprocally connected excitatory and inhibitory populations with weak and strong recurrent excitation to examine its effect on the frequency of HCEs.

Results

1. Deletion of Erk2 during early embryonic development results in decreased network excitability in layer 2/3 of somatosensory cortex *in vitro*

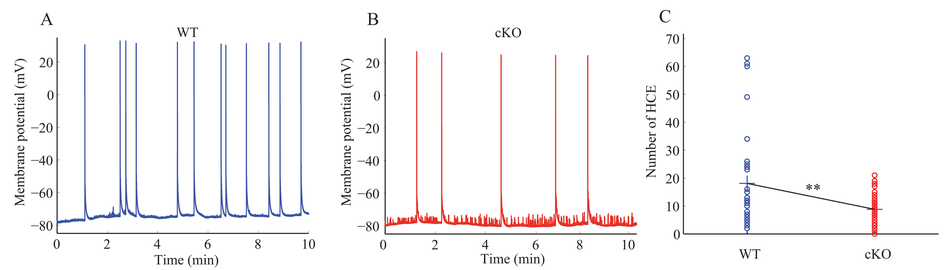


Figure 1: Cortical network activity recorded from single L2/3 PCs from somatosensory cortex or WT and cKO mice. **A**,**B** show raw voltage traces of HCEs as a proxy for network activity in WT and cKO animals, respectively. An individual HCE is characterized by a plateau depolarization of ~60 mV, lasting ~400 ms, followed by a decay (tail) of the membrane potential lasting ~500 ms. A single action potential typically rides on top of the plateau, after which depolarization block prevents further spiking (see Figure 3A). **C**. Quantification of number of HCEs over a 10 minute period. Crosses connected by a line demarcate the means of the distributions (WT: n = 33; cKO: n = 32; $p < 0.05$, non-parametric bootstrap analysis).

2. HCE decay is altered in Erk2 cKO mice, while plateau remains unchanged

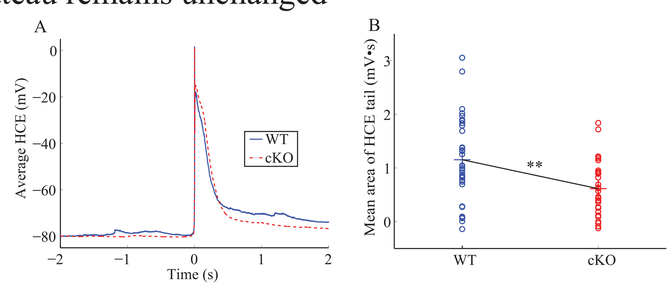


Figure 2. A. Averaged HCEs from WT and cKO mice. Individual HCEs traces were aligned relative to event onset and then averaged for the WT and cKO groups. Note the difference during the decay (tail) phase of the HCE. **B.** Quantification of the mean area of the HCE tail taken as the the integral of the membrane voltage during the HCE tail (WT: n = 33; cKO: n = 32; $p < 0.05$, non-parametric bootstrap analysis)

3. Recurrent network excitation is reduced in layer 2/3 PCs of Erk2 mutants

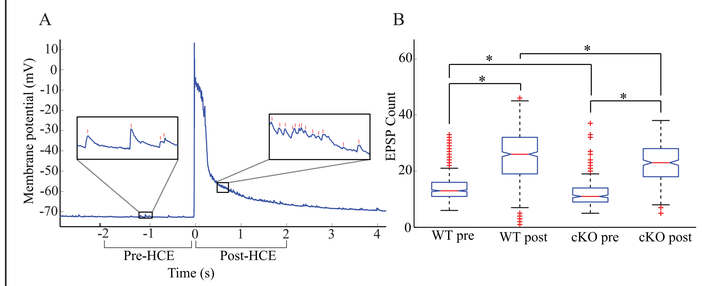
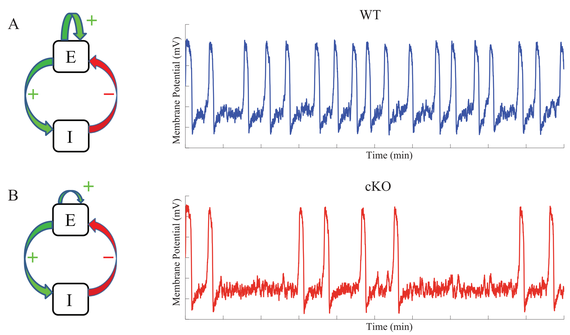


Figure 3. Quantification of recurrent excitation before and after HCE onset. **A.** Example trace of a single HCE from a layer 2/3 PC. Insets show excitatory postsynaptic potentials (EPSPs) demarcated by rasters during a 2 second period before HCE onset (Pre-HCE) and during a 2 second period after HCE onset (Post-HCE). **B.** Boxplot of the EPSP count during the Pre- and Post-HCE periods in WT and cKO mice. Red crosses represent outliers. In both WTs and cKOs, a significant increase in the EPSP count is present after HCE onset, suggesting that increase in activity is due to activation of the local cortical network (WT: n = 33; cKO: n = 32; $p < 0.05$, non-parametric bootstrap analysis). WTs have significantly higher EPSP counts during the Pre- and Post-HCE period than cKOs, suggesting that cKOs have a reduced recurrent excitation.

4. Reduced recurrent excitation decreases frequency of HCEs in a model of excitatory and inhibitory neuronal populations

Figure 4. A simple model of excitatory and inhibitory populations accounts for the experiemental observations. We generated a two-dimensional model exhibiting bistable dynamics. One variable describes the activity of the excitatory population and the other of the inhibitory population. Activity is reported as a generic membrane potential of a neuron in each population. Uncorrelated noise added to both poulations facilitates transitions from the low to the high state in a fashion similar to the experimentally observed HCEs. Duration of the high state is determined by the onset of feedback inhibition, which scales supralinearly with excitation. Activity was simulated for two levels (high and low) of recurrent excitation.



Acknowledgements

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Conclusions

- Erk2 conditional knockout mice serve as an appropriate model to probe cortical excitability owing to their phenotype of having reduced cell density in cortical layers 2/3.
- The frequency of high-conductance events recorded in current clamp from cortical pyramidal cells in a disinhibited brain slice serves as a proxy for cortical network excitability.
- Erk2 cKO exhibit decreased network excitability and diminished excitatory feedback.
- A simple computational model of excitatory and inhibitory neuronal populations reproduces the decreased excitability phenotype observed experimentally when recurrent excitation is reduced.