Serotonergic Synaptic Transmission Modulates Network Dynamics in the Mouse Neocortex: Implications for Epilepsy

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Neuroscience 2012 159.23/L19

Introduction
The neocortical sheath is tightly interfaced with subcortical neureuromodulatory centers, which provide means to control the global tone of cortical excitation through a gamut of neurotransmitters and their respective receptors. Of these, serotonin (5-hydroxytryptamine; 5-HT) exerts a powerful influence over the activity state of the cortex mainly through 5-HT1, 5-HT2, 5-HT3, and 5-HT7 receptors [1]. Though the effects of 5-HT at the cellular and synaptic levels are by now well understood, its role in shaping cortical network activity is not. This study aims at elucidating the role of 5-HT in controlling the global levels of excitation within the neocortex. To this end, we used two mouse models: 1) a wild-type (WT) C57BL/6 mouse line; 2) a transgenic mouse line in which the Pet-1 transcription factor, which specifies and maintains the serotonergic phenotype of brainstem raphe neurons [2,3], has been genetically ablated (KO). The KO mice exhibit an ~80% reduction in CNS 5-HT levels [2,3] as well as a severe loss in expression of the serotonin reuptake transporter, SERT, and autoinhibitory Htr1a and Htr1b autoreceptors. Using in vitro brain slice electrophysiology, we explore the role of altered serotonergic signaling and its effect on cortical synaptic and network excitability in WT and KO mice under various pharmacological manipulations. Furthermore, we consider the implications that altered cortical 5-HT signaling may have on behavior, as is the case for depressed human patients prescribed to the selective serotonin reuptake inhibitor (SSRI), fluoxetine.

Methods
We first prepared 350 µm thalamocortical slices from somatosensory cortex of P14-P21 WT and KO mice. Whole-cell patch clamp recordings were established on layer 2/3 cortical pyramidal cells (L2/3 PCs). We recorded spontaneous excitatory post synaptic currents (sEPSCs) in current-clamp using a cesium-modified solution at the reversing potential for chloride (~40 mV) and bath applied the 5-HT3 receptor (5-HT3R) antagonist, granisetron (1 µM). To record network activity, we disinhibited the slices with bath application of 5 µm gabazine, a GABA receptor antagonist and recorded from L2/3 PCs in current-clamp with a potassium-based solution. Network activity manifested as large (~60 mV; ~500 ms) and temporally random plateau depolarizations known as paroxysmal depolarizing shifts (PDSs) [4]. WT slices were treated with 1 µM of the SSRI, fluoxetine (FLX) alone or with 10 µM ketanserin (KSN), a 5-HT2R antagonist. Disinhibited KO slices were treated with KSN alone.

Results
1.) Augmented spontaneous serotonergic signaling through 5-HT3Rs in cortical L2/3 PCs of Pet-1 KO and fluoxetine-treated WT mice in vitro

2.) Altered 5-HT signaling in KO mouse brains is associated with a fast run (15 Hz) regime of network activity in a disinhibited cortical slice

3.) Fast runs of cortical network activity in Pet-1 KO mice are reproduced in WT slices treated with fluoxetine

4.) Fast runs of network activity are dependent on activation of 5-HT2Rs in Pet-1 KO and WT fluoxetine-treated mice

Conclusions
1.) Despite global decrease in 5-HT synthesis, Pet-1 KO mouse exhibit increased serotonergic signaling through 5-HT3 receptors, a condition that can be recreated in wild-type mouse brain treated with the SSRI, fluoxetine.
2.) Pet-1 KO mouse brain slices exhibit epileptiform activity patterns (fast runs) observed previously in spontaneously seizing cats during sleep [5]. This pattern can be reproduced in WT mice treated with FLX.
3.) Fast run epileptiform discharges depend on 5-HT2 receptor activation.
4.) Increased 5-HT signaling appears to alter the cortical balance of excitation and inhibition towards epileptiform activity patterns.

Acknowledgements
We are very grateful to Dr. Evan Deneris for generously providing us with the Pet-1 knock-out mice and Katherine Lobur for maintaining the mouse colonies and performing the genotyping. This work was funded by the Mt. Sinai Health Care Foundation.

References Cited

Poster available at: http://www.case.edu/med/galanlab/publications.html