Abnormal cell-intrinsic excitability and cortical network activity in serotonin-deficient mice

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Introduction
The neocortical sheath is tightly interfaced with subcortical neuromodulatory 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. 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 aimed at elucidating the role of serotonin on neural network excitability and activity 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 ETS gene plays a critical role in 5-HT neuron development and is required for normal serotonin phenotype of brainstem raphe neurons [1], has been genetically ablated (KO). The KO mice exhibit an ~80% reduction in CNS 5-HT levels [1] as well as a severe loss in expression of the serotonin reuptake transporter, SERT, and inhibitory 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.

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 postsynaptic currents (sEPSCs) in voltage-clamp using a cesium-modified solution at the reversal potential for chloride (~80 mV) and bath applied the 5-HT3 receptor (5-HT3R) antagonist, granisetron (GSN; 1 µM). To record network activity, we disinhibited the slices with bath application of 1-5 µM gabazine, a GABAA receptor antagonist and recorded from L2/3 PCs in current-clamp with a potassium-based solution. Network activity manifested as large (~60 mV; ~500ms) and temporally random plateau depolarizations known as paroxysmal depolarizing shifts (PDS). WT slices were treated with 3 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. Emergence and propagation of network activity were investigated with a high-density multielectrode array as displayed in the figure above.

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Results: Patch-Clamp Experiments

Pyramidal cells from KO mice receive larger and more frequent excitatory barrages of synaptic activity. These synaptic currents are mostly mediated by 5-HT3 receptors and can be blocked with granisetron (GSN).

Pyramidal cells from KO mice are more excitable in brain slices that are partially disinhibited with gabazine. This increased excitability is mediated by 5-HT2 receptors and can be suppressed with 5-HT2 receptor blocker Ketanserin.

Results: Seizure Susceptibility

WT and KO mice have the same susceptibility to epileptic seizures. In response to convulsant pentetrazol, the seizure latency and duration are the same in both groups.

Results: Network Activity and its Propagation

Neural activity propagates less and more slowly through cortical networks in the KO mice. This is accounted for by less complex dendritic arbors in pyramidal cells, and therefore reduced network connectivity.

Conclusions
1) Despite global decrease in 5-HT synthesis, Pet-1 KO mice 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].
2) Pyramidal cells from Pet-1 KO mice are overall more excitable than from WT.
3) Pet-1 KO brain slices occasionally exhibit spontaneous epileptiform activity patterns (fast runs). These patterns can be reproduced in WT mice treated with gabazine and fluoxetine [2].
4) Neural activity propagates less and more slowly through cortical networks in Pet-1 KO than in WT controls.
5) This is explained by reduced dendritic complexity in pyramidal cells, leading to reduced cortical network connectivity.
6) As a result, despite increased single-cell excitability, the brain of Pet-1 KO mice is not more susceptible to seizures than WT brains.

References