General Anesthesia is Associated with a Reduction of Cortical Inhibitory Interneuron Activity *In Vivo*

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Background

- Cognitive functions including sensory processing, consciousness, and memory are disrupted by anesthetics [1, 2, 3].
- General anesthesia is thought to be a state of suppressed net cortical activity characterized by decreased activity of excitatory pyramidal neurons and enhanced GABAergic inhibitory activity [4, 5].
- In cortical circuits, pyramidal neuron activity is precisely sculpted by inhibitory interneurons, which in the cortex express three, non-overlapping markers: 5HT3a-receptor (5HT3aR), parvalbumin (PV), and somatostatin (SOM) [6].
- Interneurons play an important role in regulation of cortical function, but the effect of general anesthetics on interneuron activity remains unknown [5, 6].
- In this study, we aimed to characterize cortical interneuron activity in the living mouse brain under general anesthesia.

Methods

- A genetically encoded calcium indicator, GCaMP6, was expressed in layer 2/3 and layer 5 pyramidal cells and interneurons in layer 2/3 using transgenic (*Thy1*-GCaMP6) and knock-in Cre (PV-Cre, SOM-Cre, 5HT3a-Cre) mice with adeno-associated virus encoding GCaMP6 [7, 8].
- We used 2-photon calcium imaging to record the neuronal activities of pyramidal neurons and interneurons in the primary somatosensory and motor cortices during awake, anesthetized, and recovery states. Mice were anesthetized with either 1% sevoflurane or ketamine-xylazine (KX).
- We measured the fluorescent signals of each soma over time, calculating the $\Delta F/F_0$, where F_0 is the baseline fluorescent signal. Image analysis was performed in NIH ImageJ and data was evaluated with paired t-test in GraphPad Prism.

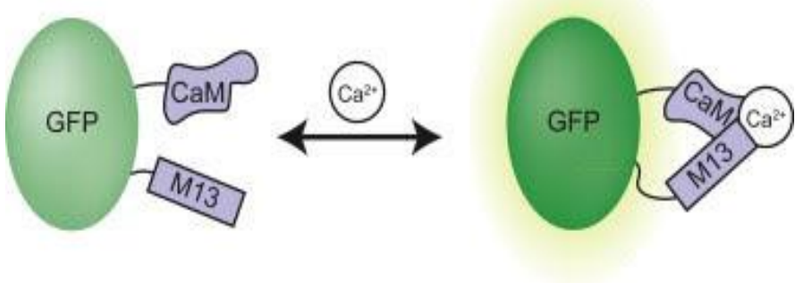

Fig 1 – GCaMP6, an indicator for calcium, was expressed in layer 2/3 pyramidal cells using transgenic (*Thy1*-GCaMP6) and interneurons with knock-in Cre (PV-Cre, SOM-Cre, 5HT3a-Cre) mice and adeno-associated virus. Calcium influx during neuronal activity results in fluorescence changes detected at the somata. *Image Source: Whitaker M. Methods in Cell Biology 99: 153.*

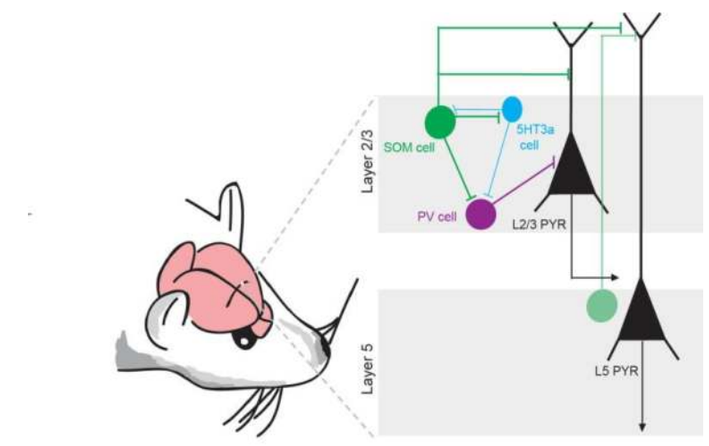

Fig 2 – *in vivo* two-photon microscopy was used to visualize layer 2/3 pyramidal neurons and the three subclasses of interneurons (5HT3a, SOM, PV). 5HT3a target SOM, SOM target pyramidal cell (PYR) dendrites, and PV target PYR soma. The same cells were observed before, during, and after anesthetic exposure with sevoflurane or ketamine-xylazine.

Results

- Sevoflurane and KX anesthesia significantly decreased the average F/F_0 of both pyramidal cells and all interneurons classes (5HT3aR, PV, and SOM) as compared to awake animals.
- Decreased neuronal activities under anesthesia occurred across all observed cortical areas.
- Suppressed neuronal activities continue into the recovery period as compared to awake state.

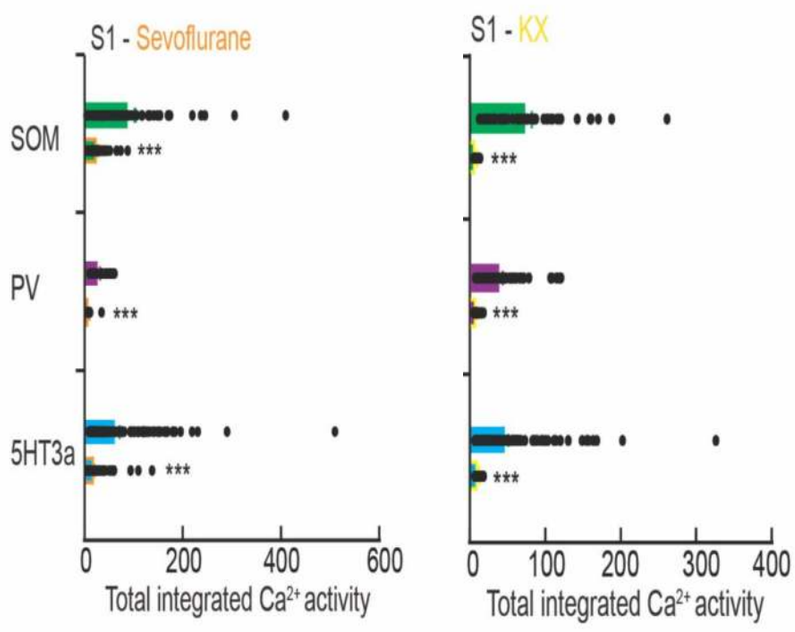

Fig 3 - Neuronal activity of all three classes of inhibitory interneurons (SOM, PV, 5HT3a) were decreased under general anesthesia compared with both sevoflurane and kx-anesthesia.

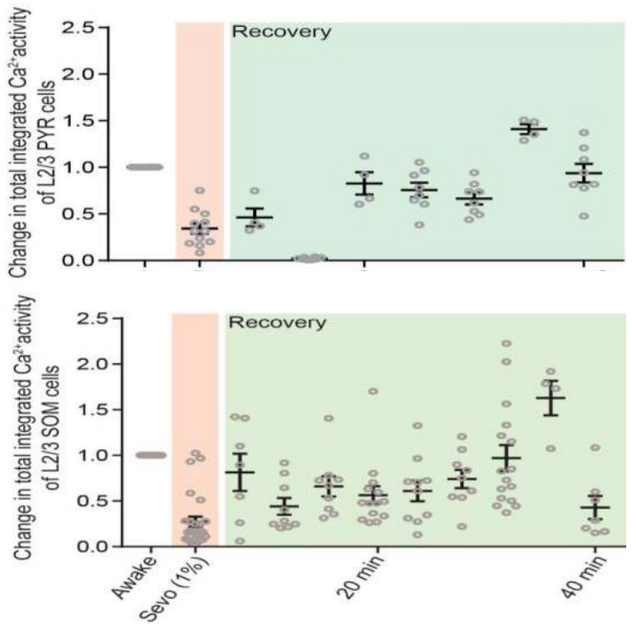

Fig 4 – Decreased activity of pyramidal cells and interneurons persisted into the recovery period.

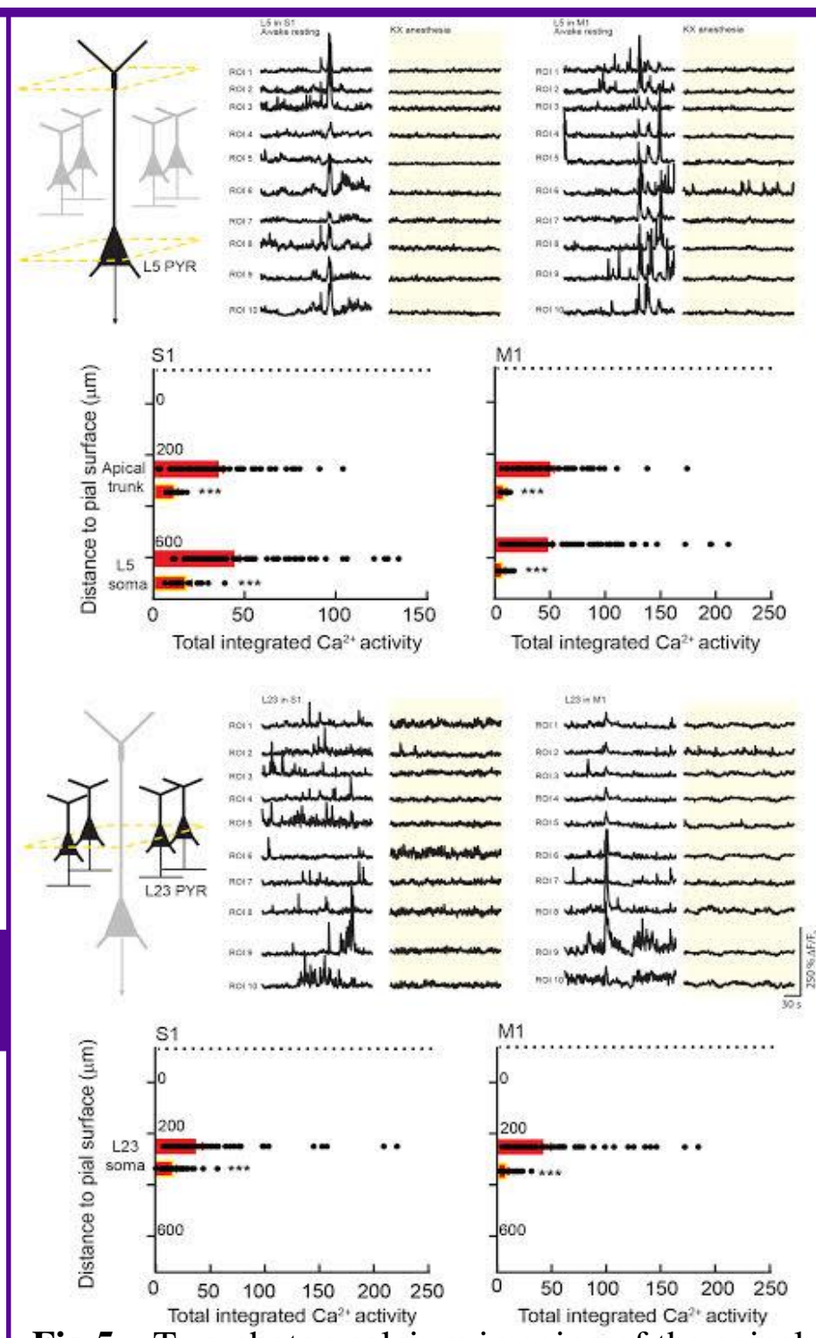

Fig 5 – Two photon calcium imaging of the apical trunk and somata of layer 5 as well as layer 2/3 pyramidal soma under both awake state and GA (1% sevoflurane and KX).

Conclusion

- Our results suggest that general anesthesia results in not only the suppression of excitatory pyramidal cell activity, but also decreased GABAergic inhibition from interneurons.
- Neuronal activities continue to be suppressed into the recovery period, suggesting a persistent effect on cortical dynamics in the perioperative period.

References

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