Recovery of Function After Brain Injury: The Secret Life of Axons

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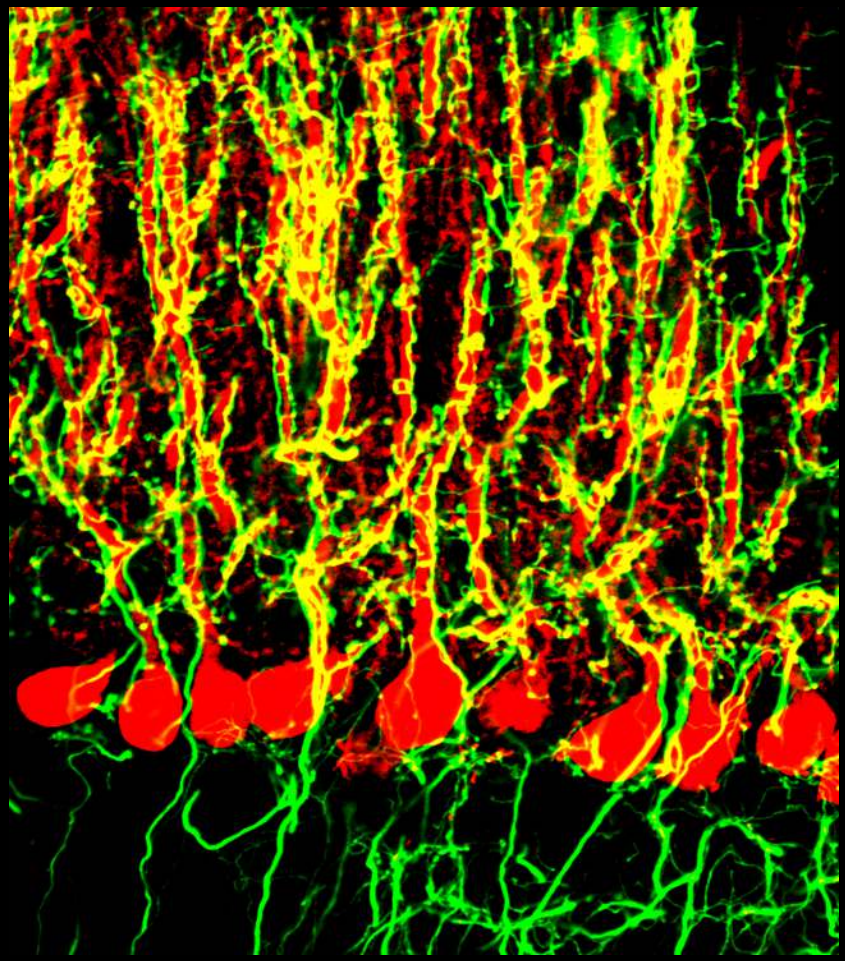
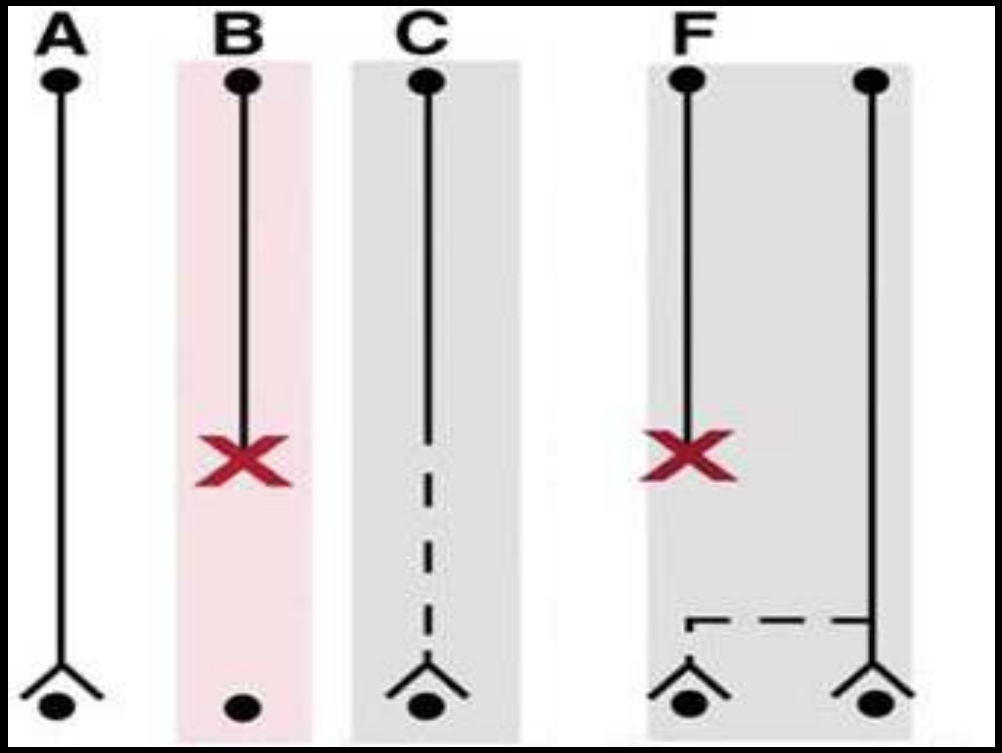


IBBS Science Writers' Boot Camp *May 28, 2014*

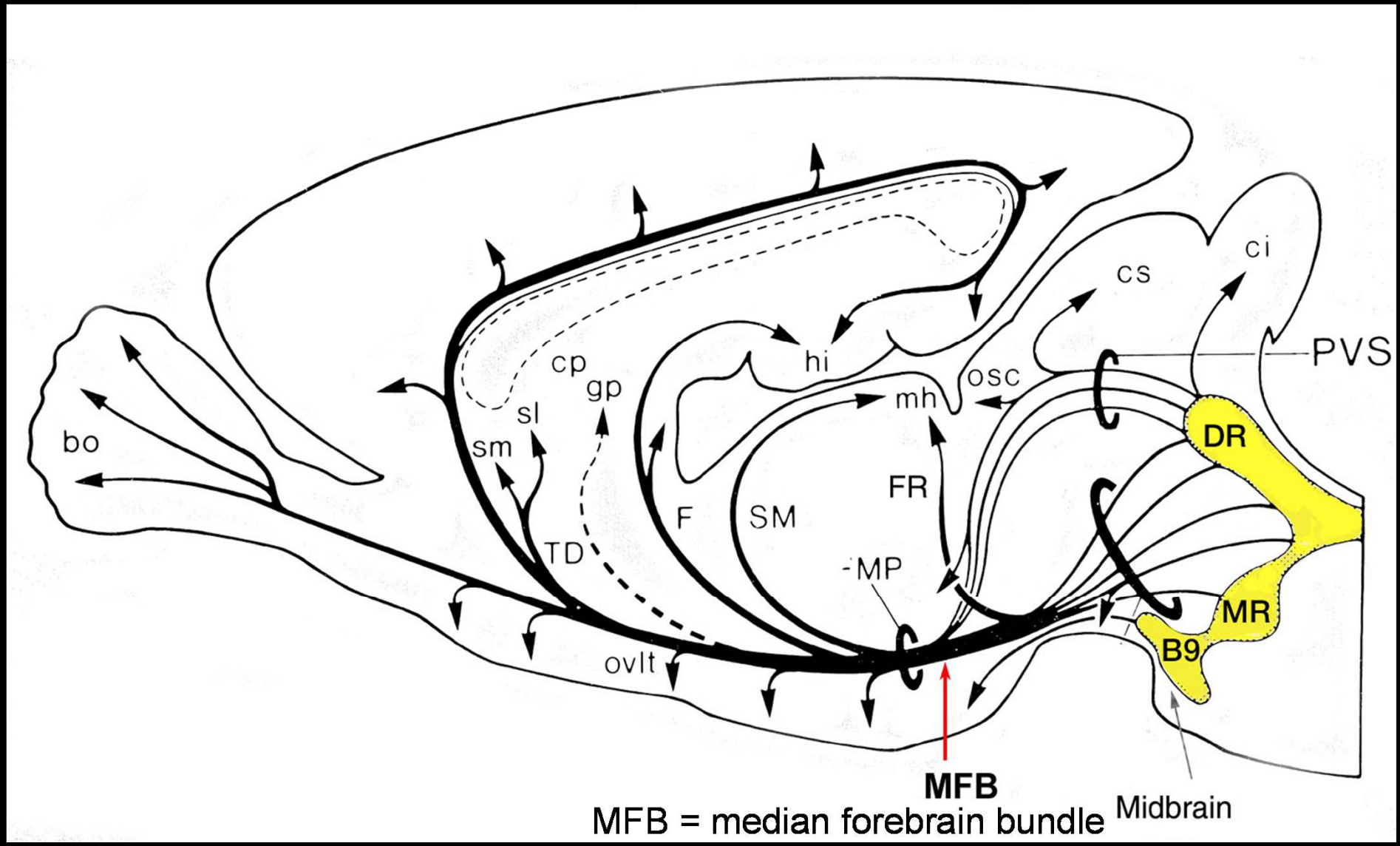
Forms of Axonal Plasticity Following Injury in the Adult Brain: Regeneration and Sprouting



Modified from Tuszynski & Steward Neuron 74:777 (2012)

Serotonin axons originate from the raphe complex

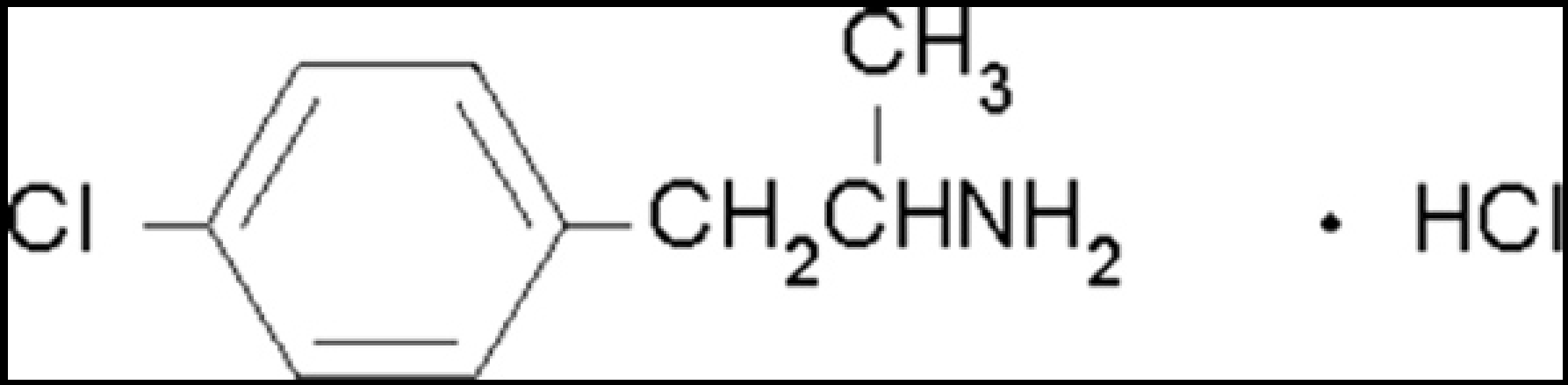
(rat brain - sagittal)



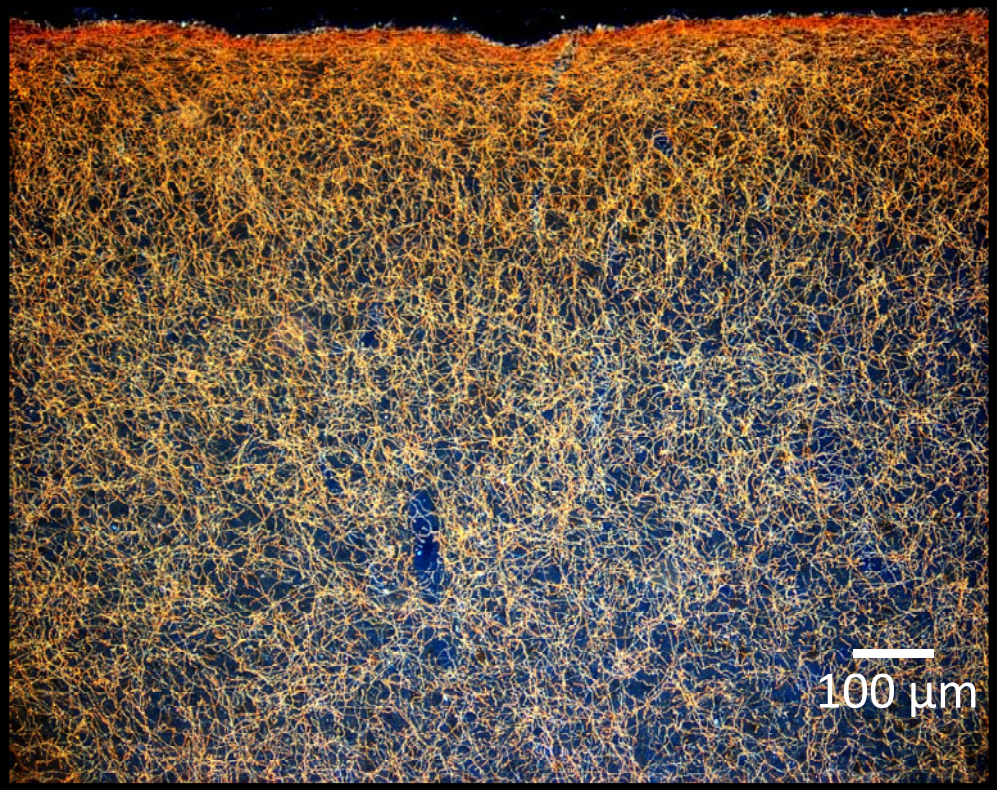
Parent et al., Neuroscience 6:115 (1981)

Amphetamine toxicity to the serotonin system

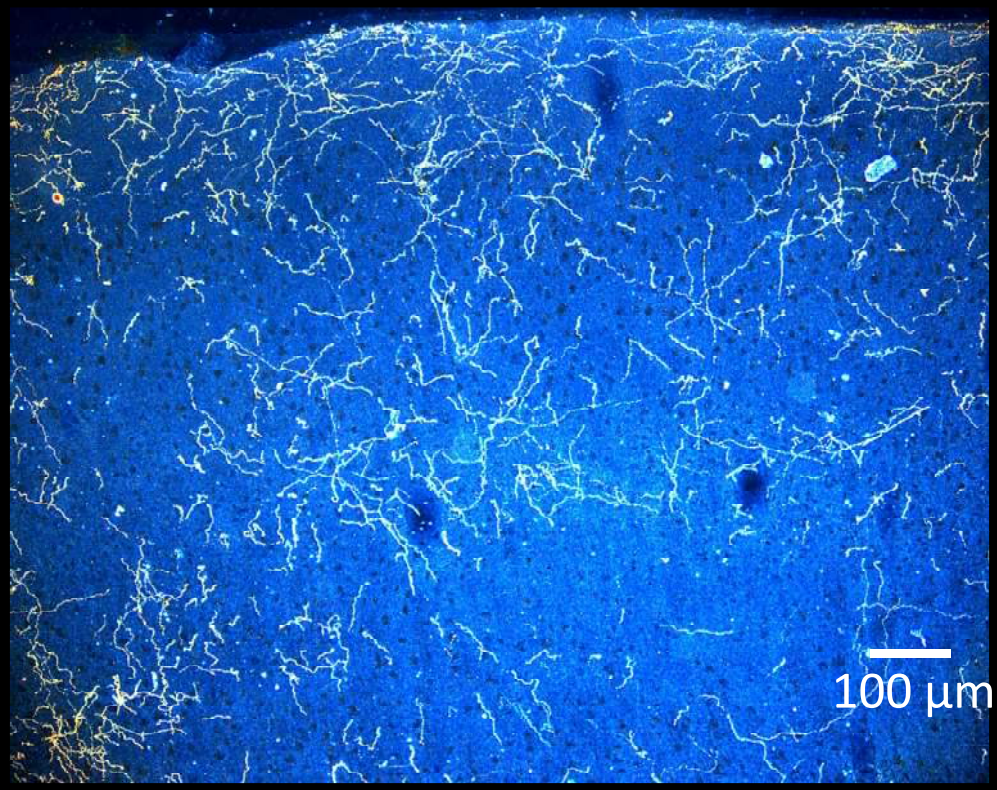
- *para*-chloro-amphetamine (PCA) >> methamphetamine > amphetamine > fenfluramine>> MDMA



PCA treatment reduces serotonin axon staining in fixed tissue from the neocortex of rats



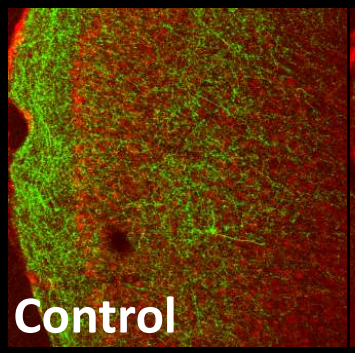
Frontal Cortex, Saline Control

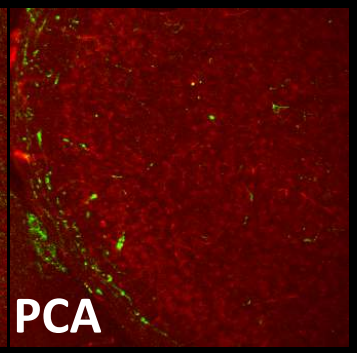


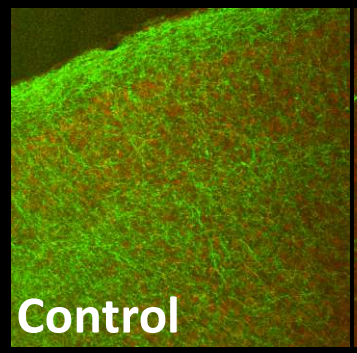
Frontal Cortex, 2 weeks after PCA

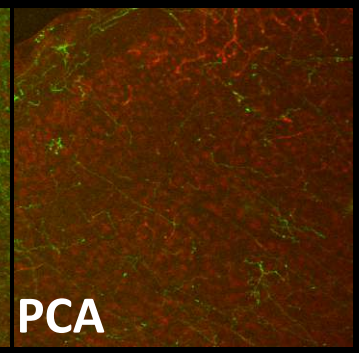
- *para*-chloro-amphetamine (PCA, 10mg/kg), 2 doses, 24 hr apart
- Similar depletion of 5HT axons was seen throughout the brain.

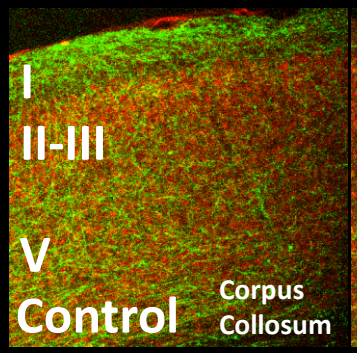
PCA treatment ablates serotonin axons in the neocortex and the anterior portion of the medial forebrain bundle

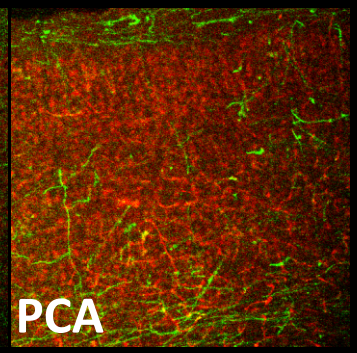


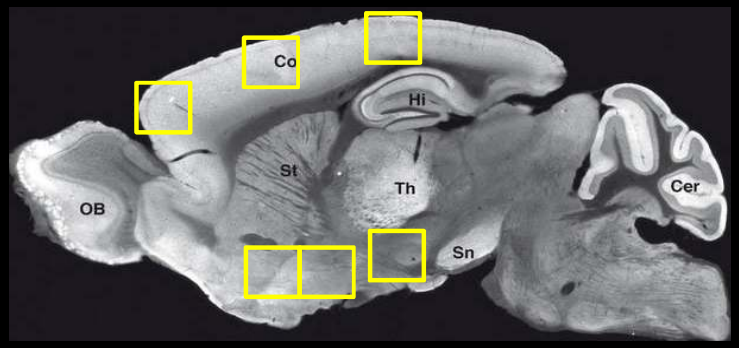


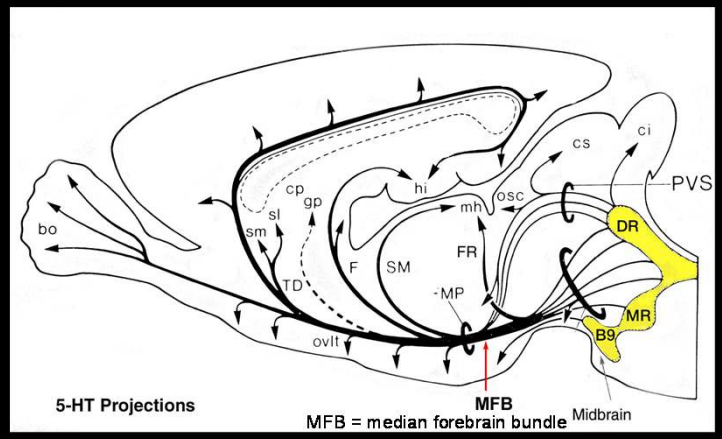


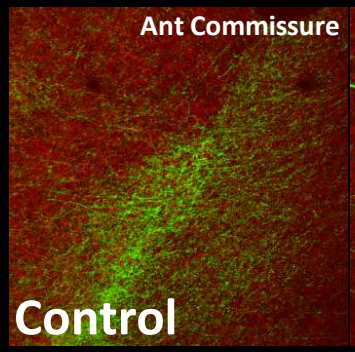


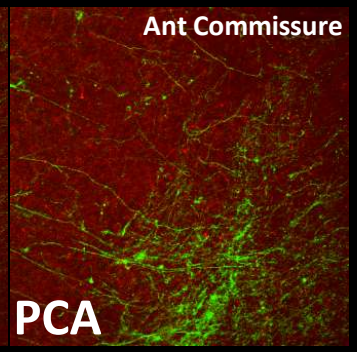


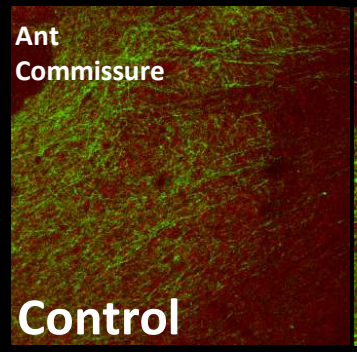


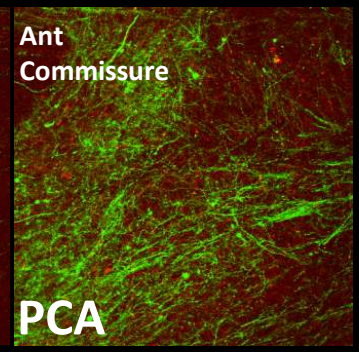


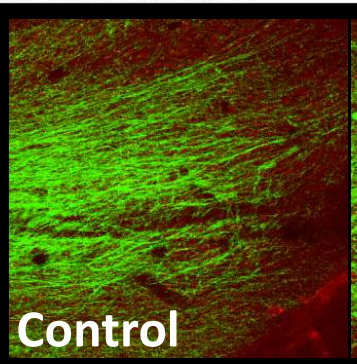


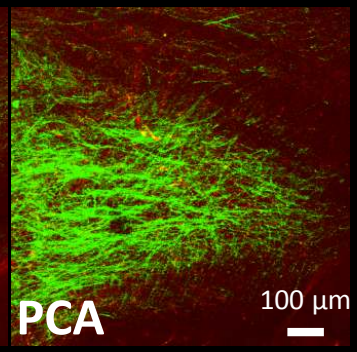






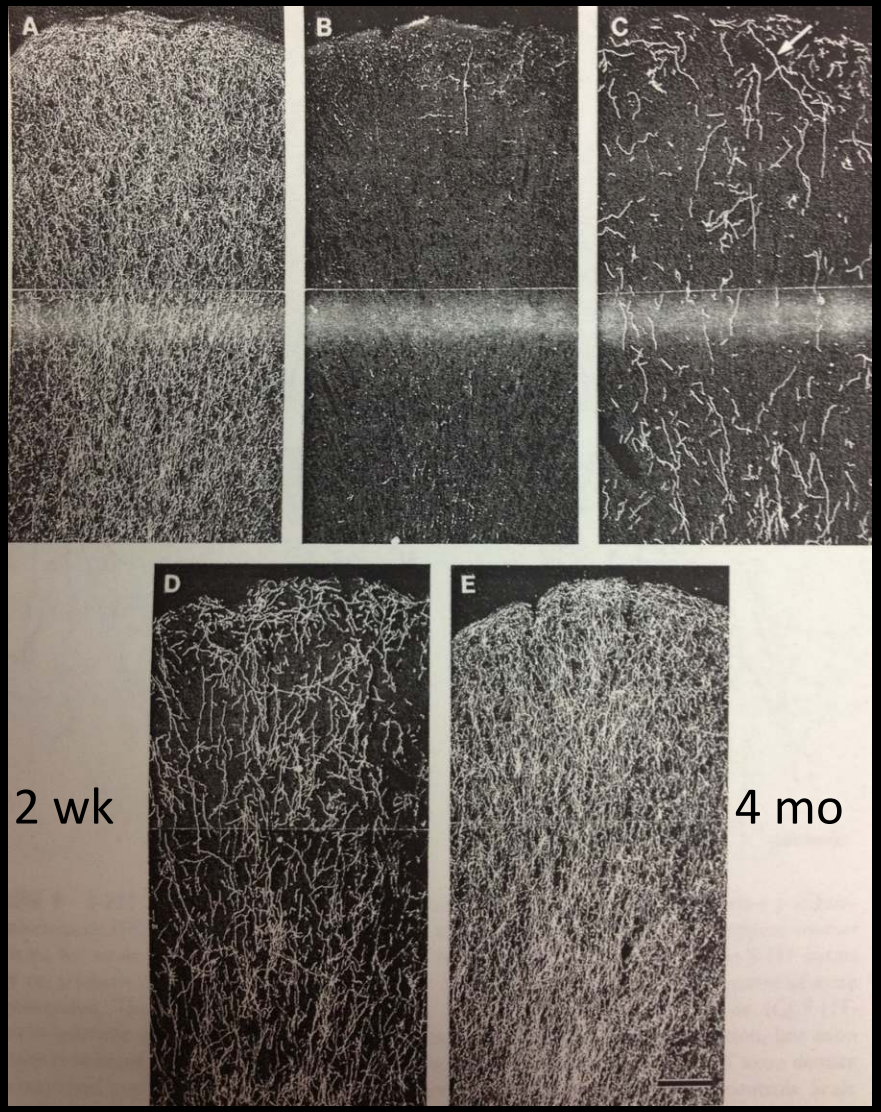


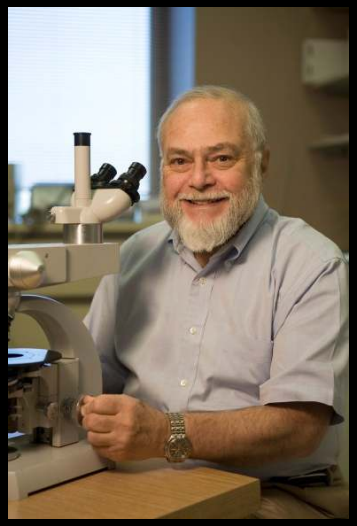




Slow recovery of 5-HT immunoreactivity following PCA in rats

Control 4 hr 3 day

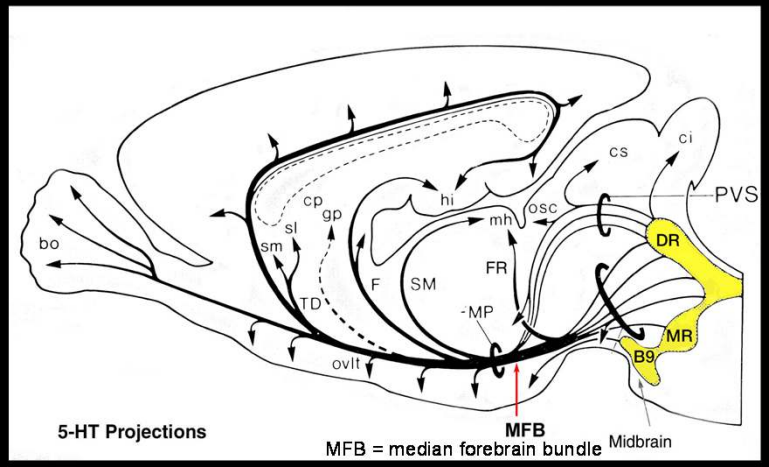




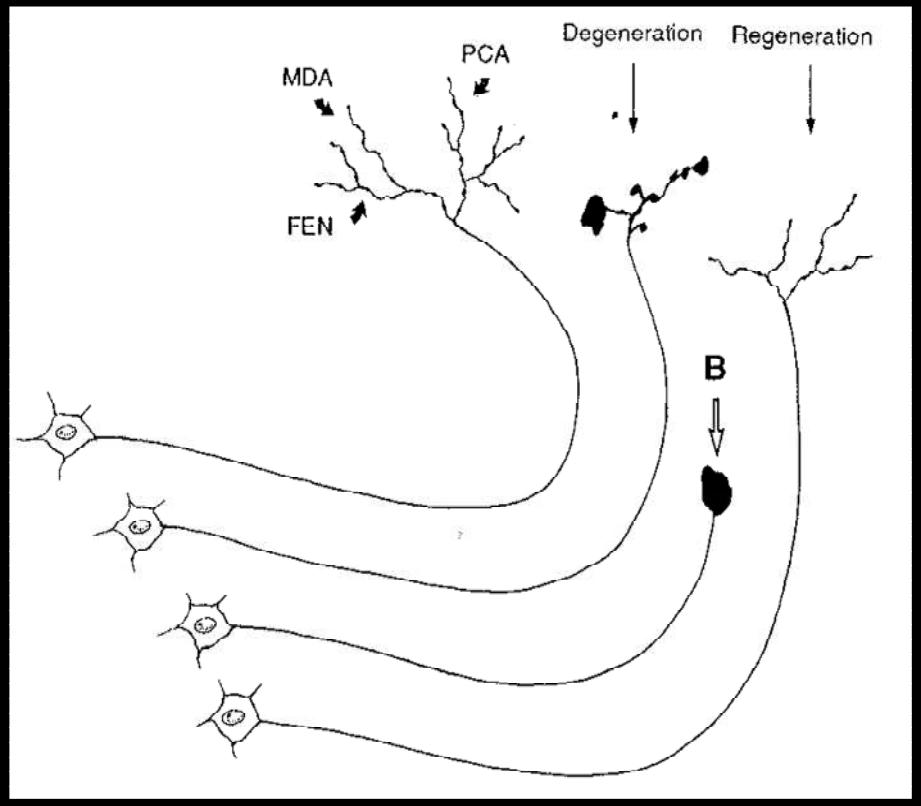
Mark Molliver

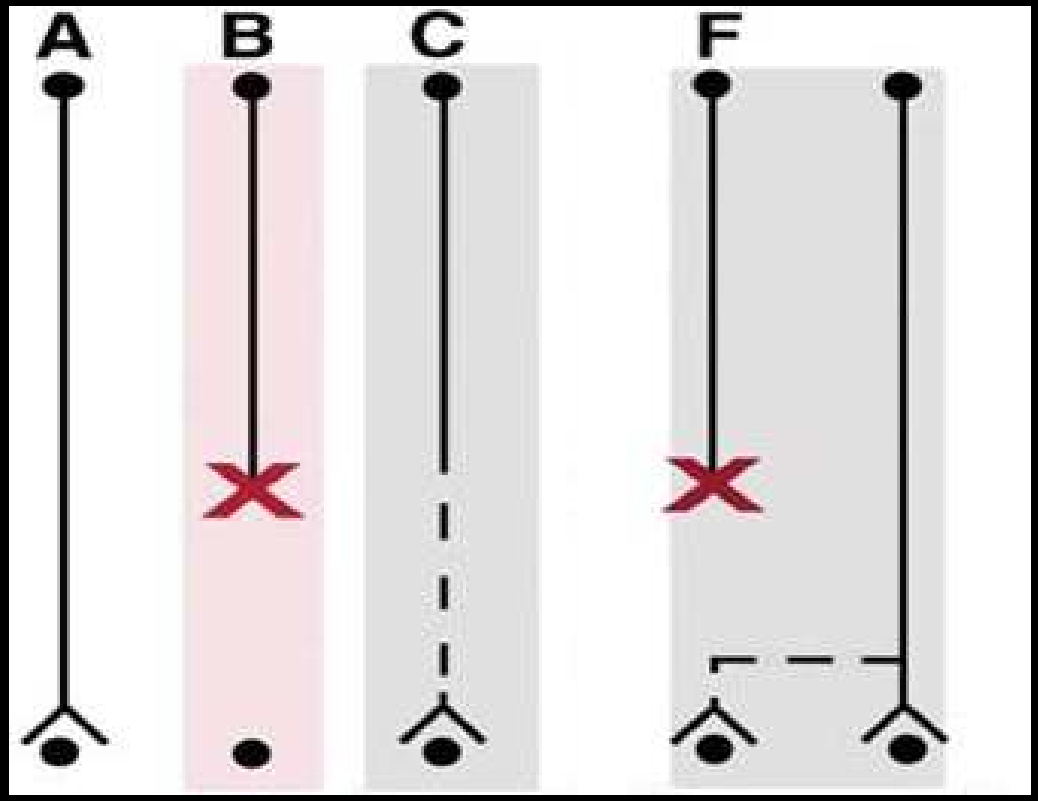
5- HT PROJECTIONS TO FOREBRAIN

(rat brain - sagittal)



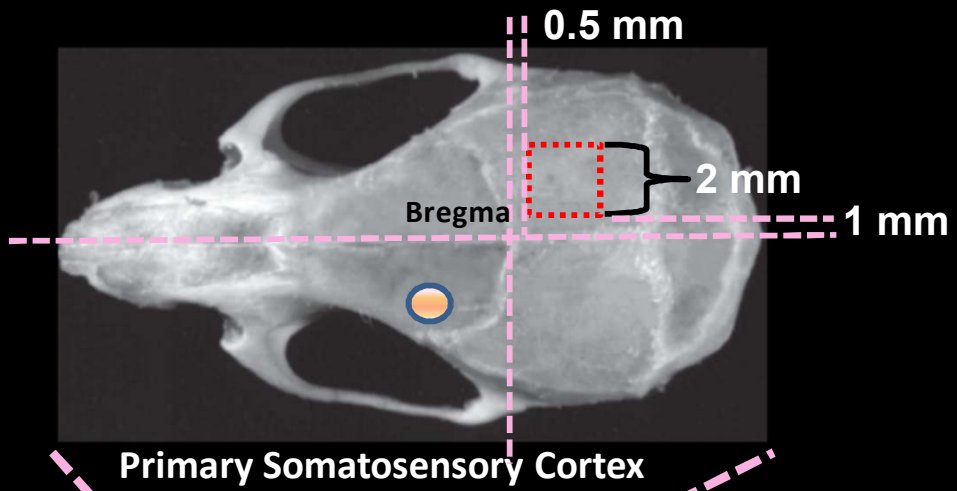
Fixed tissue experiments cannot easily distinguish axonal regeneration from sprouting, nor can they assess the dynamic aspects of recovery



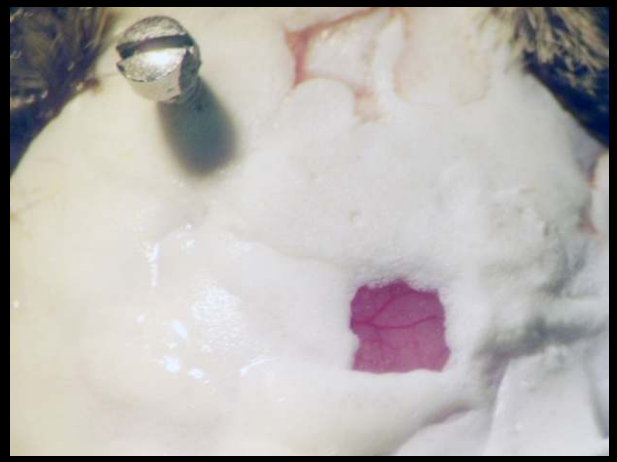


Regeneration Sprouting

Chronic in vivo two photon imaging in mice

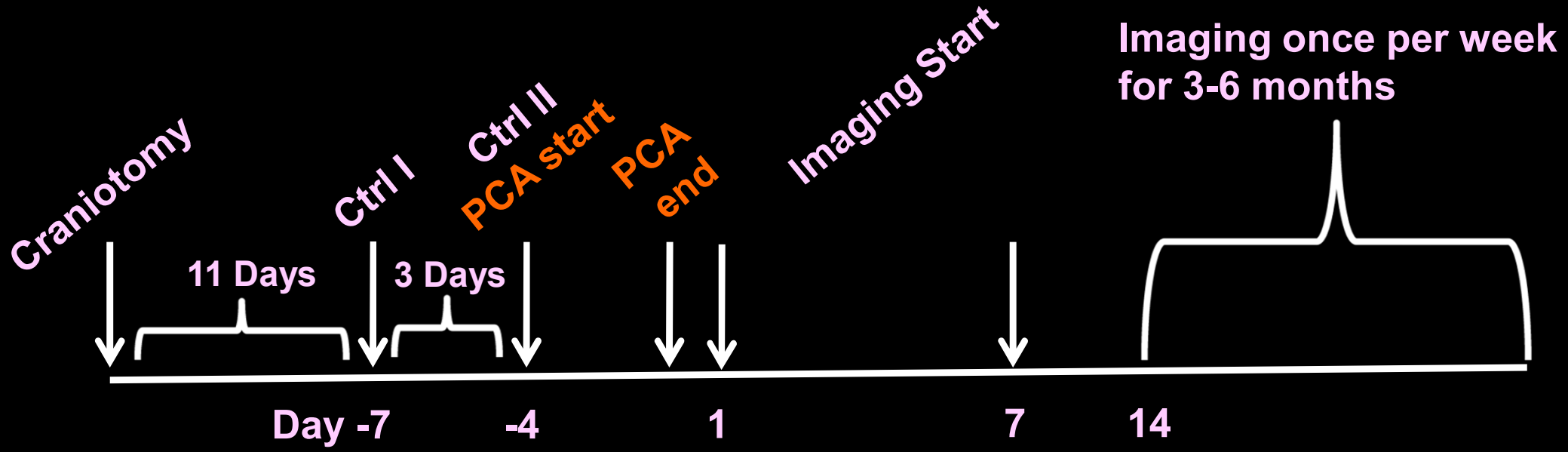


Anterior

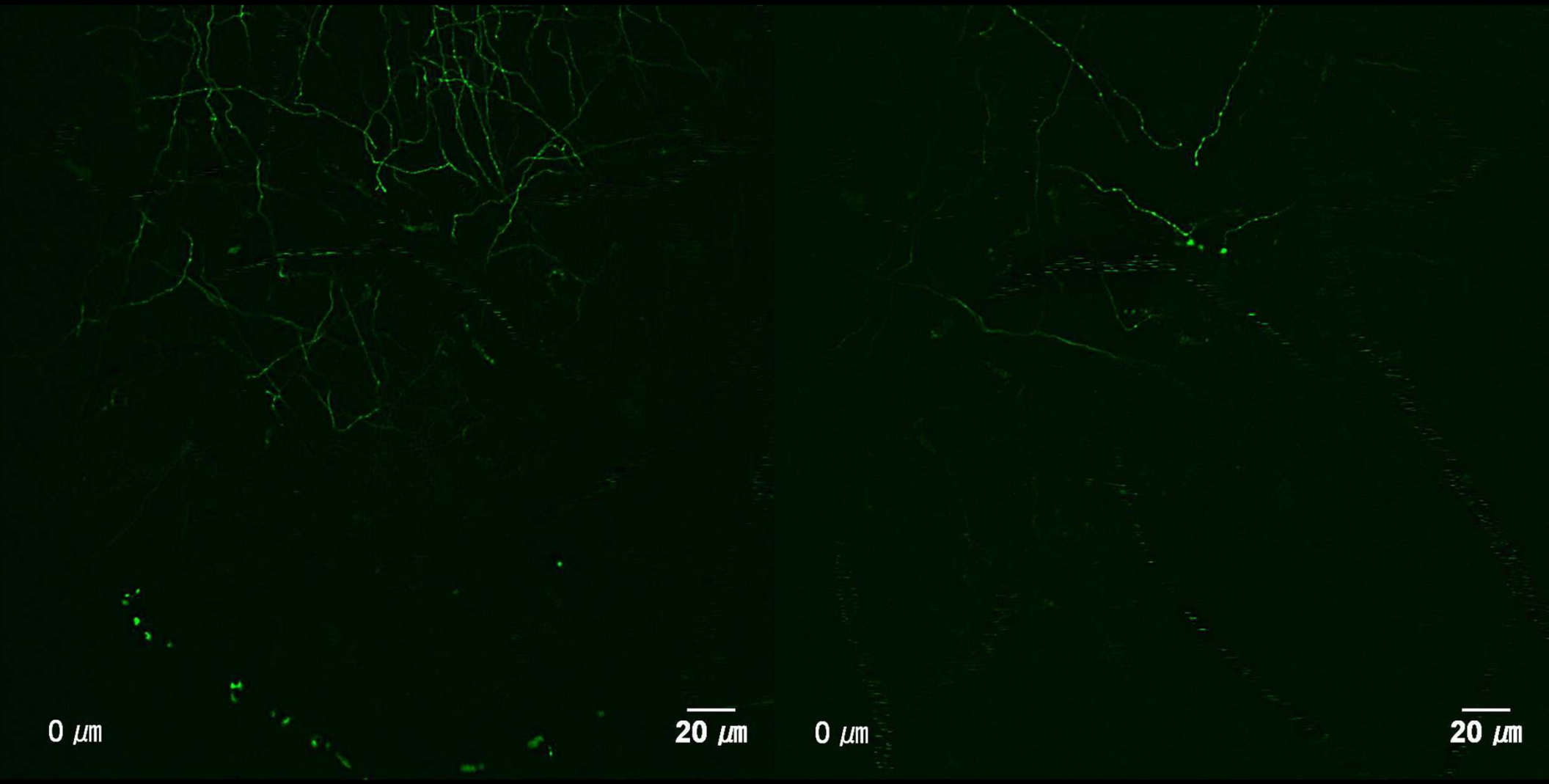


These mice have been genetically engineered
to express Green Fluorescent Protein only in serotonin neurons

Chronic in vivo imaging timeline



Raw exemplar Z-stack images: PCA-treated mouse



2nd Ctrl (Day -4)

1wk After PCA (Day 7)

Some conclusions

1. Do the serotonin axons truly degenerate & regenerate in response to PCA or do they merely transiently stop expressing serotonin? Serotonin axons do degenerate and regenerate (as indicated by slow growth captured in-frame in mice expressing untethered cytoplasmic EGFP.)

2. Do those serotonin axons that survive PCA treatment then undergo compensatory sprouting? No. The spared axons exhibit a small degree of sprouting, but at rates similar to saline controls.

3. Do those serotonin axons that initially survive PCA treatment also survive long-term or do they merely die slowly? Axons that initially survive PCA treatment show ~90% survival 6 months later, a survival rate that is identical to that of saline-treated controls.

4. Do regenerating serotonin axons grow along surviving serotonin axons or blood vessels? The regenerated axons do not grow along spared axons or blood vessels. In fact, they appear to avoid surviving, sprouting and other regenerating axons to recreate the pre-lesion state.

5. What fraction of regenerated serotonin axons survive long-term and do they attain normal morphology and spatial distribution? ~90% of the regenerated axons survive for 6 months after PCA treatment: They survive at the same rate as uninjured serotonin axons. Furthermore, their distribution and shape are indistinguishable from uninjured axons.

Some long-term questions

1. Are the regenerating axons re-growing along trajectories previously laid down by degenerated axons? Does a regenerating axon grow along its very own former trajectory?

2. Are all serotonin neurons equally competent for axonal regeneration or is it just a particular subset?

3. What molecular properties of dorsal raphe serotonin neurons make them unusually successful at axonal regeneration compared to most other neurons in the brain? Could we use such molecular insights to design therapies to promote axon regeneration in other types of neurons in the brain or spinal cord and thereby promote recovery following injury?