Synaptic circuit organization of mouse motor cortex

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How do mammals control their actions?

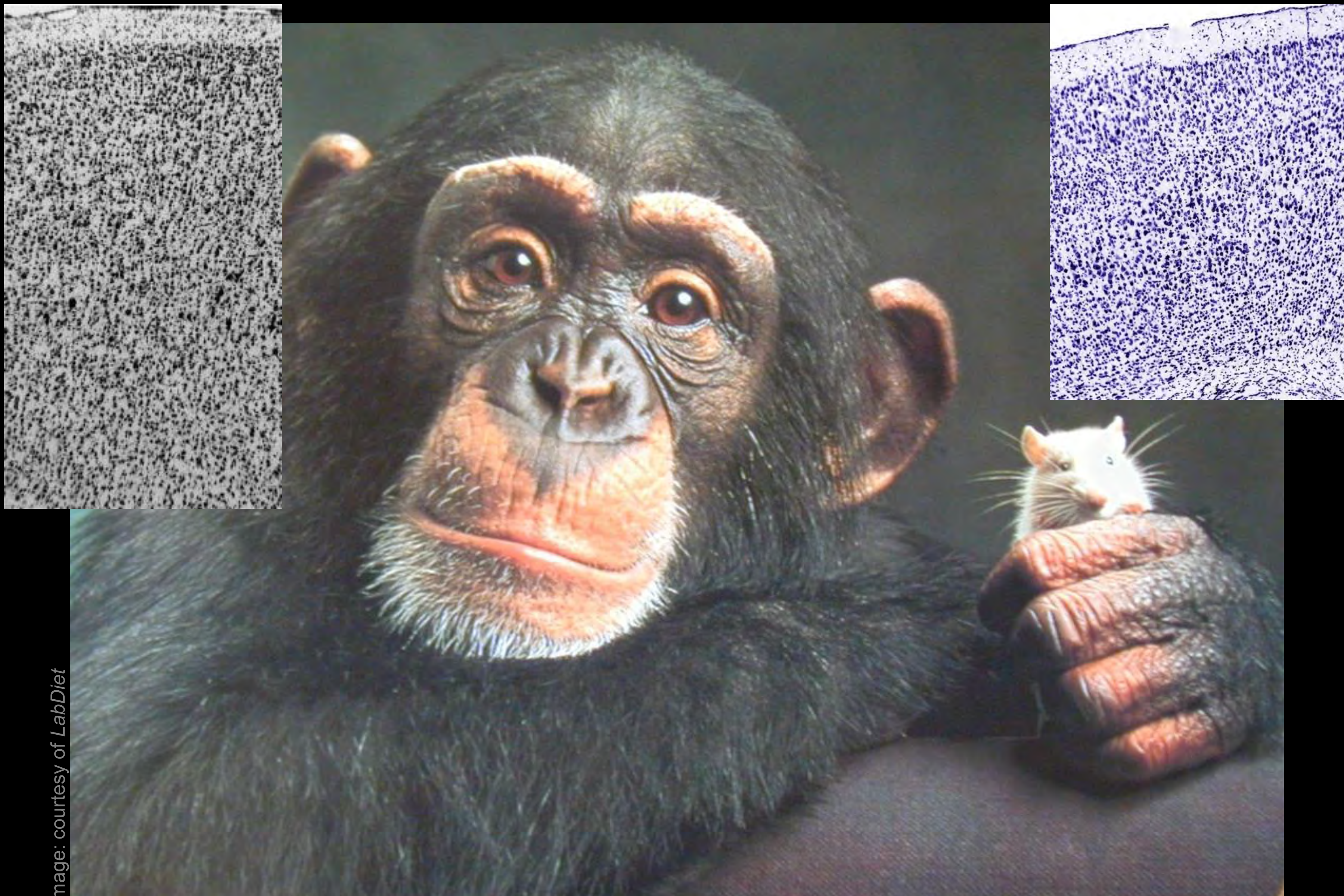
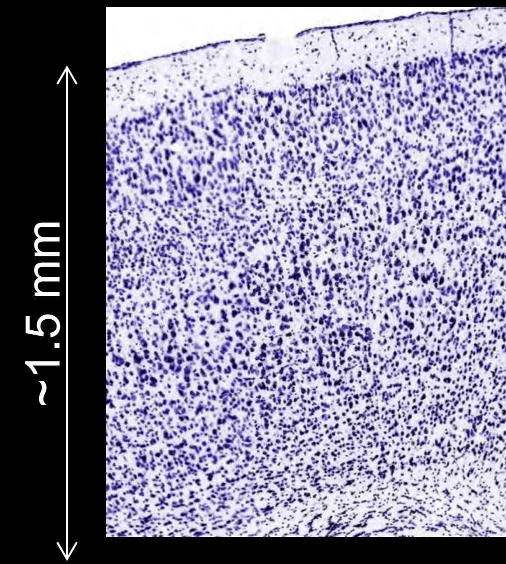


Image: courtesy of *LabDiet*

Some numbers

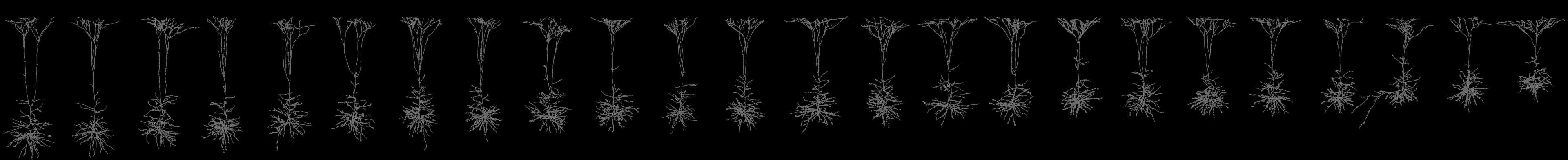


In 1 mm^3 of mouse motor cortex:

- ~10^5 neurons → 10^{10} *potential* connections
- ~4 km of axon, ~0.4 km of dendrites
- But "only" ~10^9 *actual* synapses
- Connections are scarce; presumably also highly selective

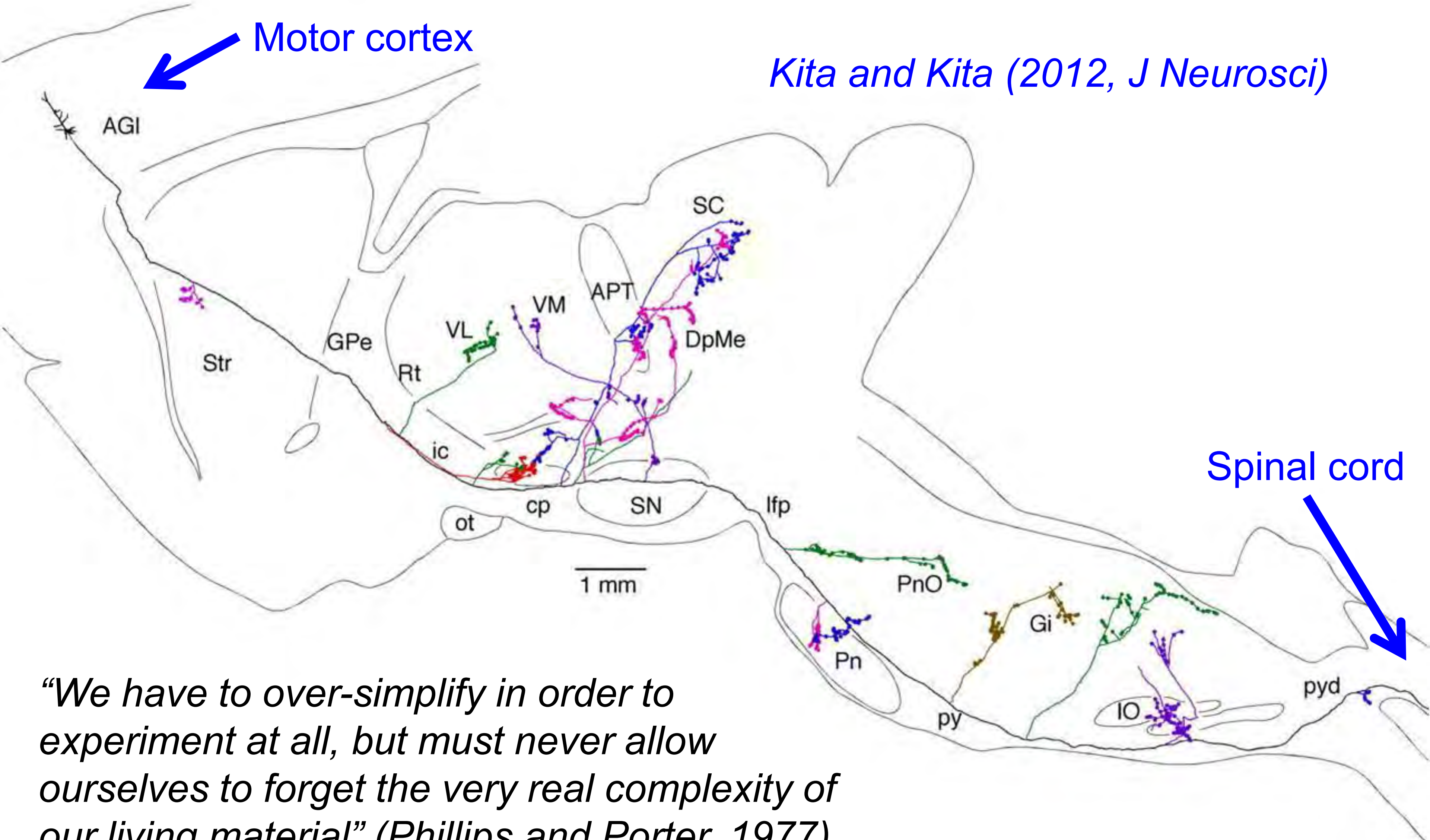
Each corticospinal neuron:

- >1 cm total dendritic length, >10^4 dendritic spines;
- >1 cm intracortical axon, >10^3 boutons



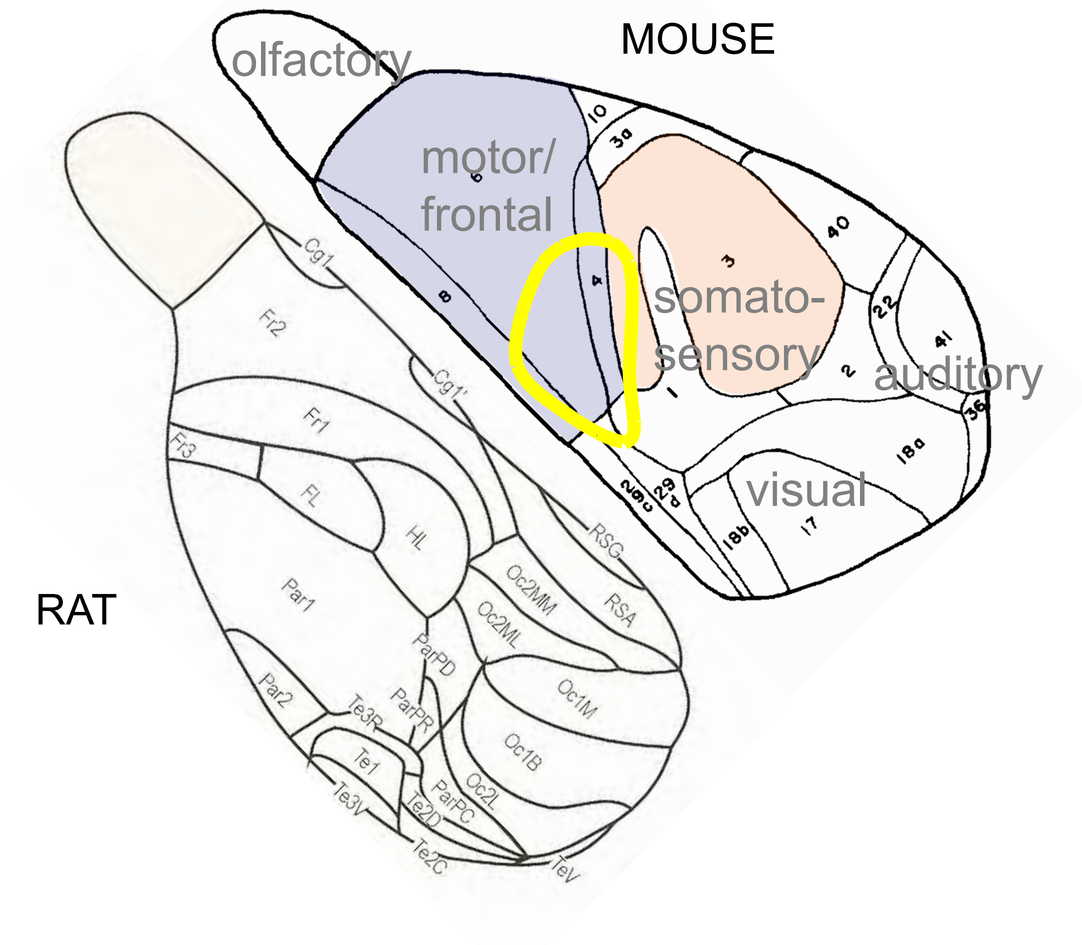
... anatomical complexity!

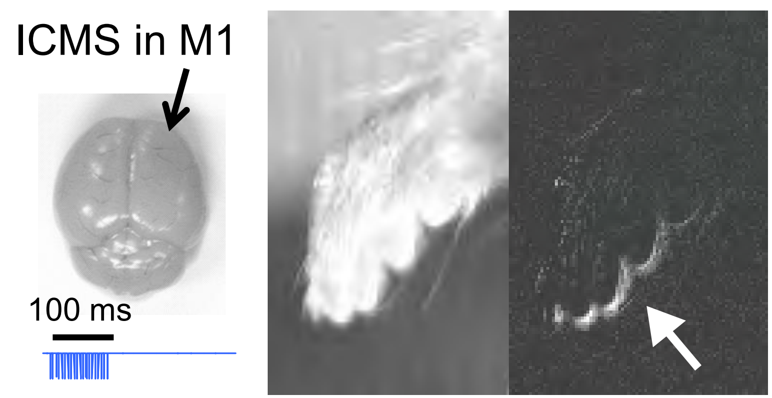
A further note on complexity



"We have to over-simplify in order to experiment at all, but must never allow ourselves to forget the very real complexity of our living material" (Phillips and Porter, 1977)

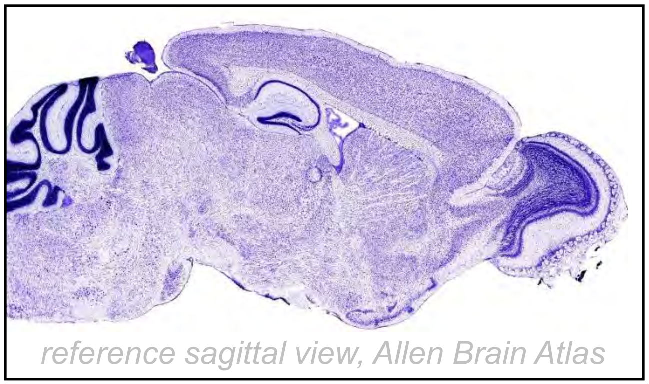
Motor cortex in rodents

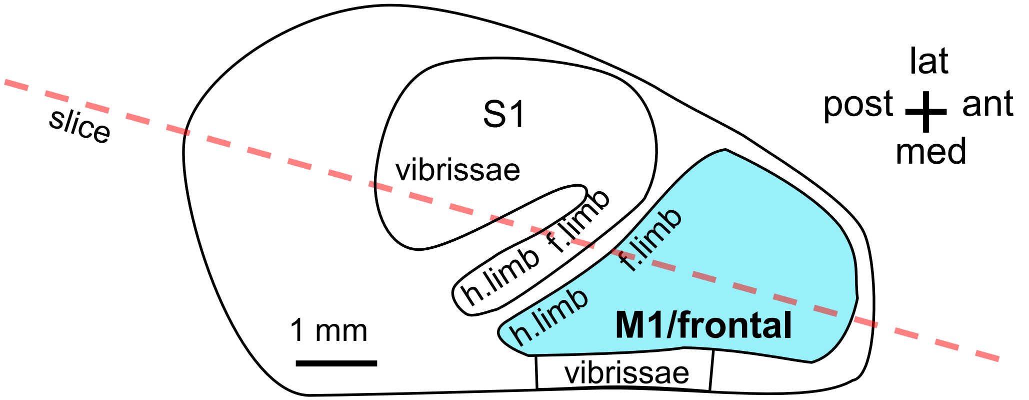


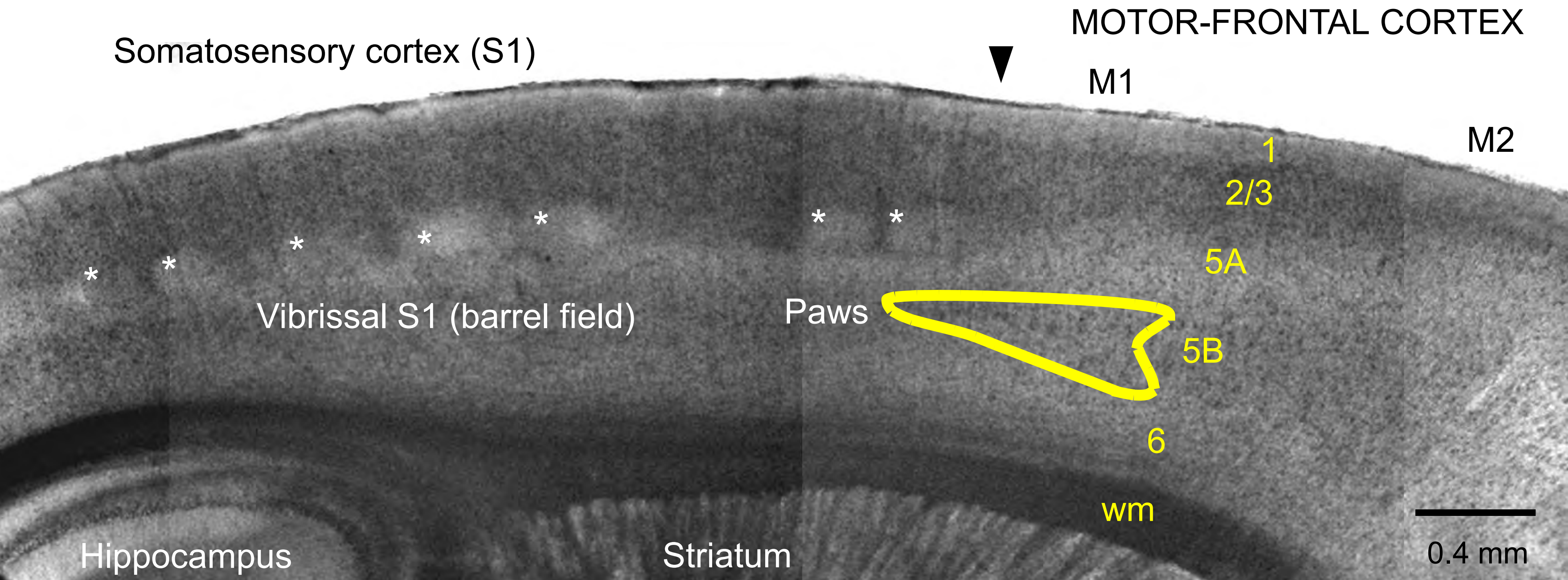


Cytoarchitectonics – mouse: Caviness (1975); rat: Palomero-Gallagher and Zilles (2004)
Microstimulation mapping – mouse: Li and Waters (1991), Ayling et al. (2009)

Motor cortex brain slice





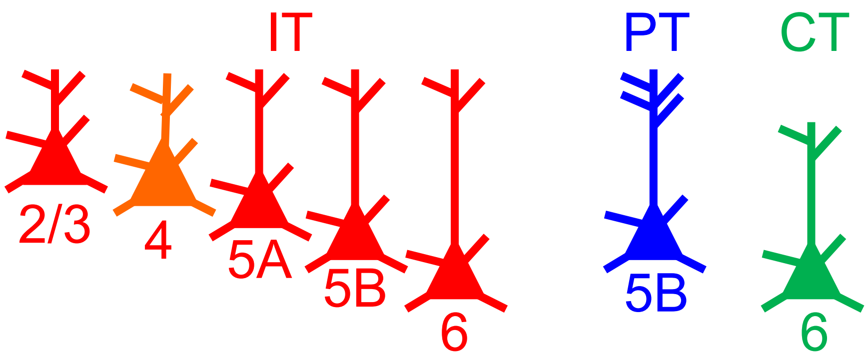


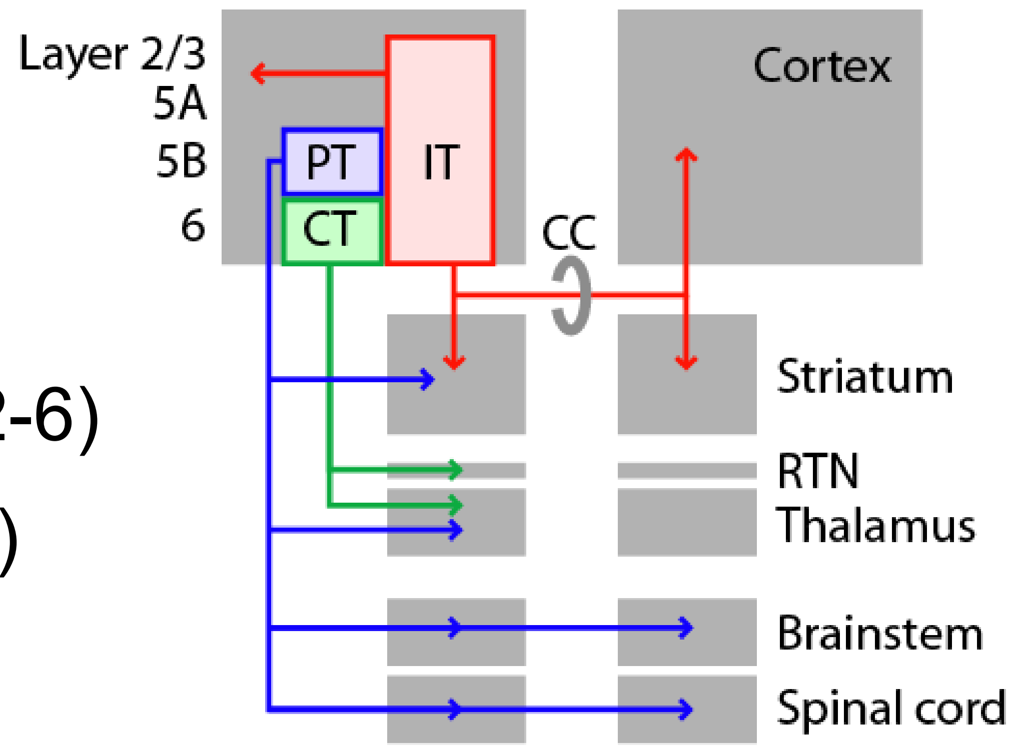
Questions about M1 circuits

Who talks to whom to form the basic local and long-range input-output circuits?

- **How are excitatory neurons interconnected?**
- Interneurons and disynaptic inhibition?
- Motor cortex ↔ thalamus?
- Is there a layer 4 in “agranular” M1?

Excitatory = pyramidal = projection neurons





IT: intratelencephalic (L2-6)

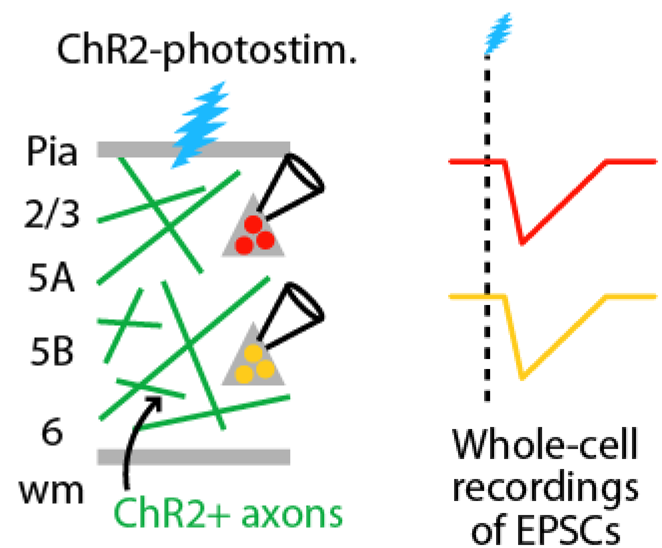
PT: pyramidal tract (L5B)

CT: corticothalamic (L6)

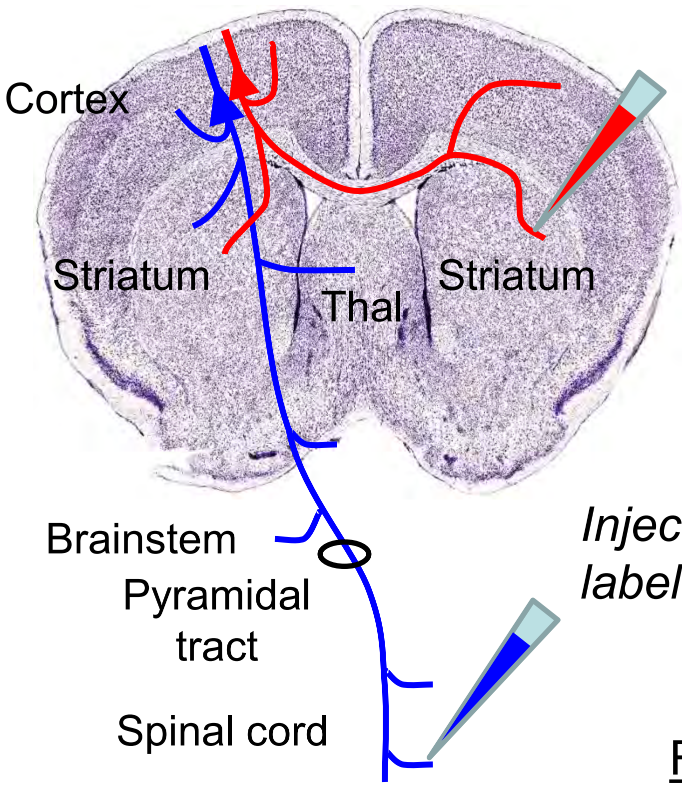
By targeting identified projection neurons, can assess input-output organization of M1 at the cellular level

General strategy for optogenetic circuit mapping in M1

- <u>Cell-type specific photostimulation</u>:
  - Express ChR2 in presynaptic cells/axons of interest
  - LED/wide-field stimulation
- <u>Targeted postsynaptic recordings</u>:
  - Label projection neurons in vivo with retrograde tracers
  - Record in brain slices from multiple identified projection neurons
  - To quantify, compare (normalize) responses



Labeling IT and PT neurons



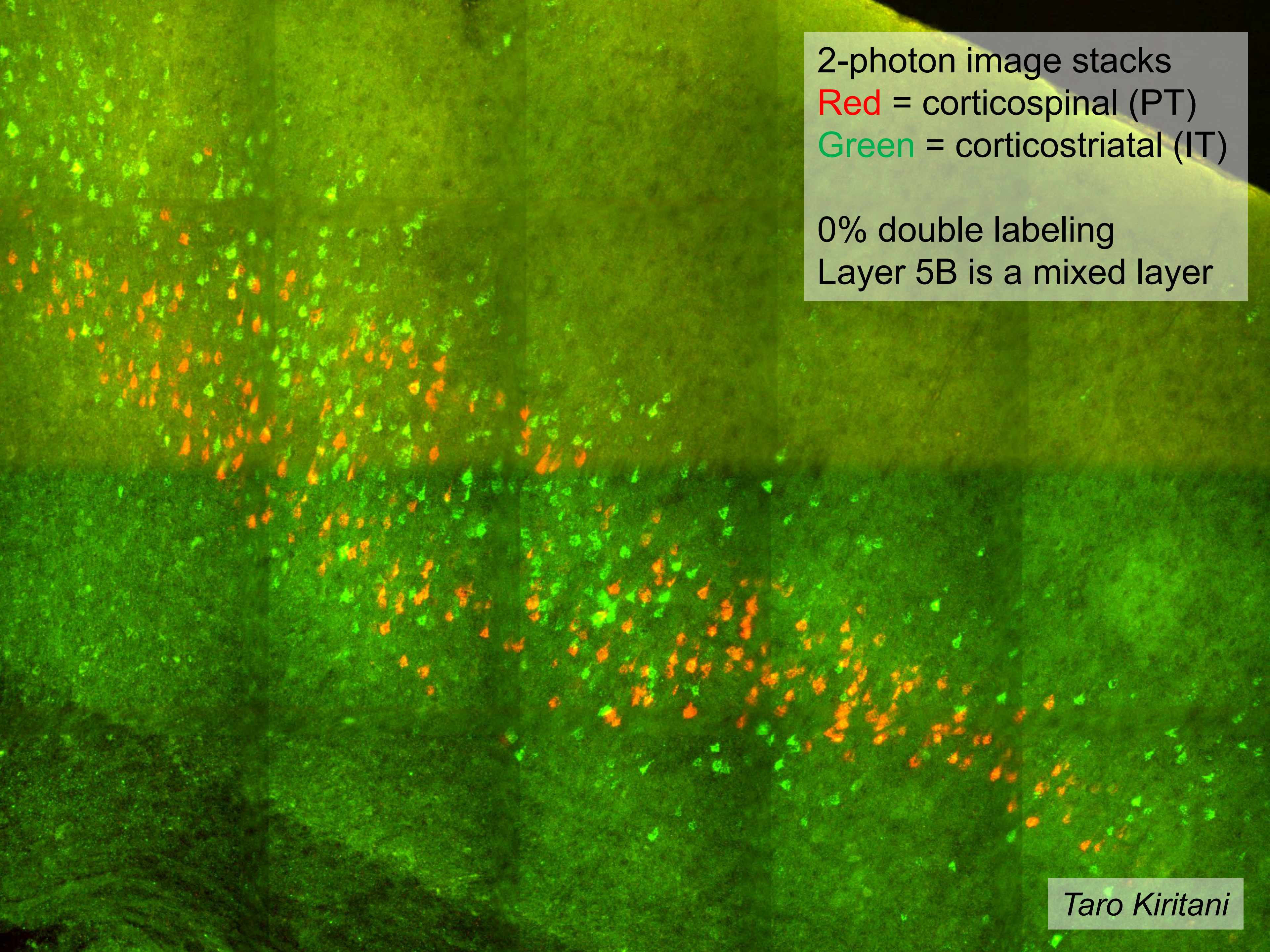
Inject contralateral striatum → labels callosally projecting corticostriatal-type IT neurons

Inject contralateral cervical spinal cord → labels corticospinal-type PT neurons

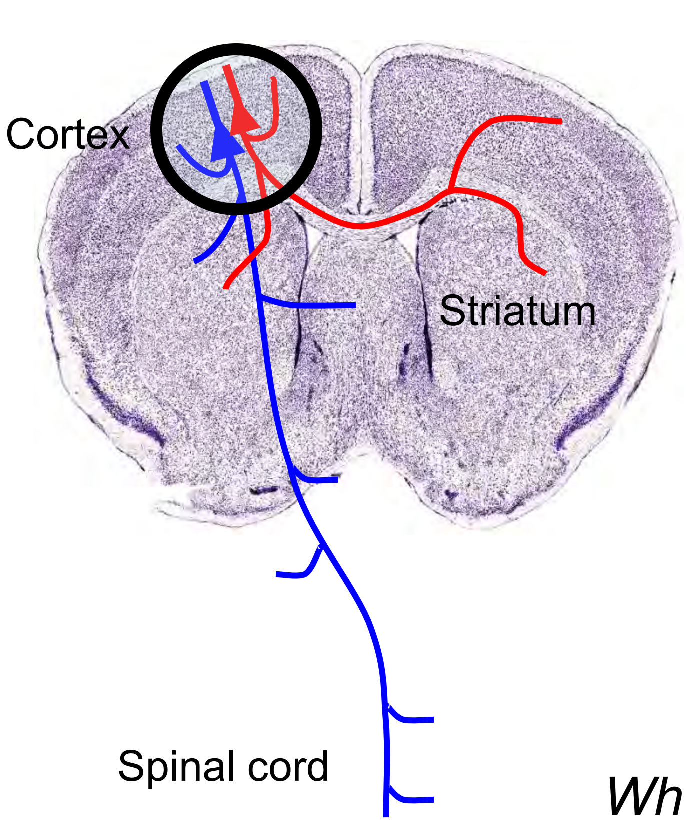
Retrograde labeling reagents:

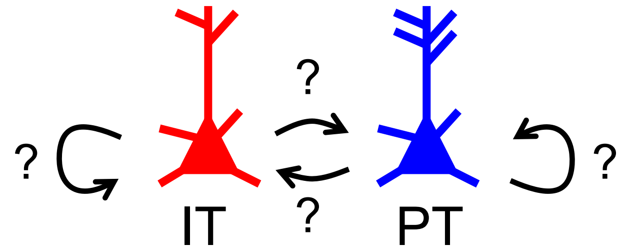
- Inert retrograde tracers (for targeted recordings)
- Rabies virus (for optogenetic photostimulation)

(Wilson, Jones, Parent, Deschenes, Reiner, etc.)


2-photon image stacks
Red = corticospinal (PT)
Green = corticostriatal (IT)
0% double labeling
Layer 5B is a mixed layer
Taro Kiritani

Intracortical IT-PT connectivity



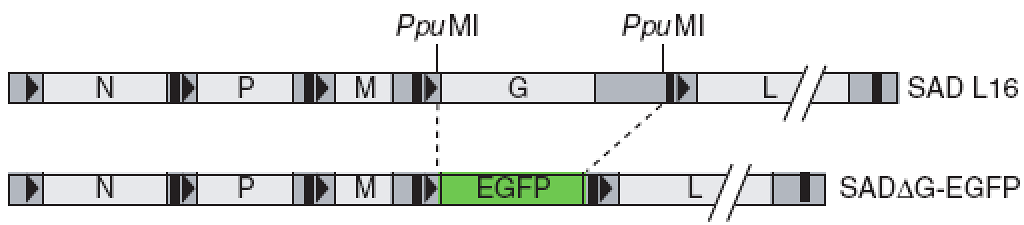


		Presynaptic	
		IT	PT
Postsynaptic	IT	?	?
	PT	?	?

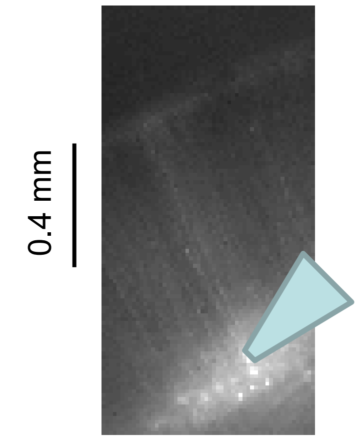
Who talks to whom?

- *Recurrent excitation within each class?*
- *Cross-talk across classes?*

Retrograde labeling with rabies



Wickersham et al. (2007)



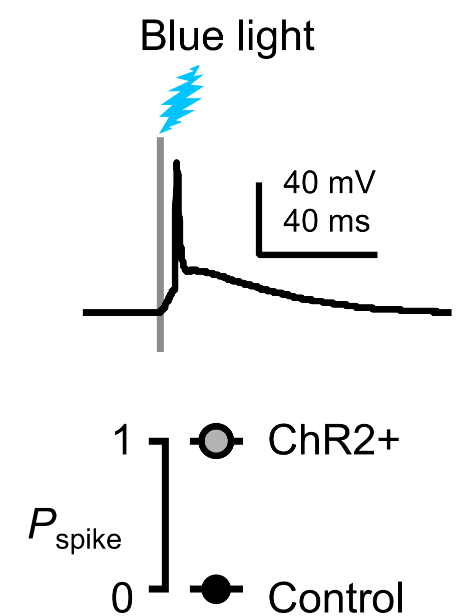
<u>Deletion-mutant rabies virus (RV):</u>

•Glycoprotein gene deleted; no trans-synaptic spread

•Ideal for retrograde labeling

•Refs: Wickersham, Callaway, Seung

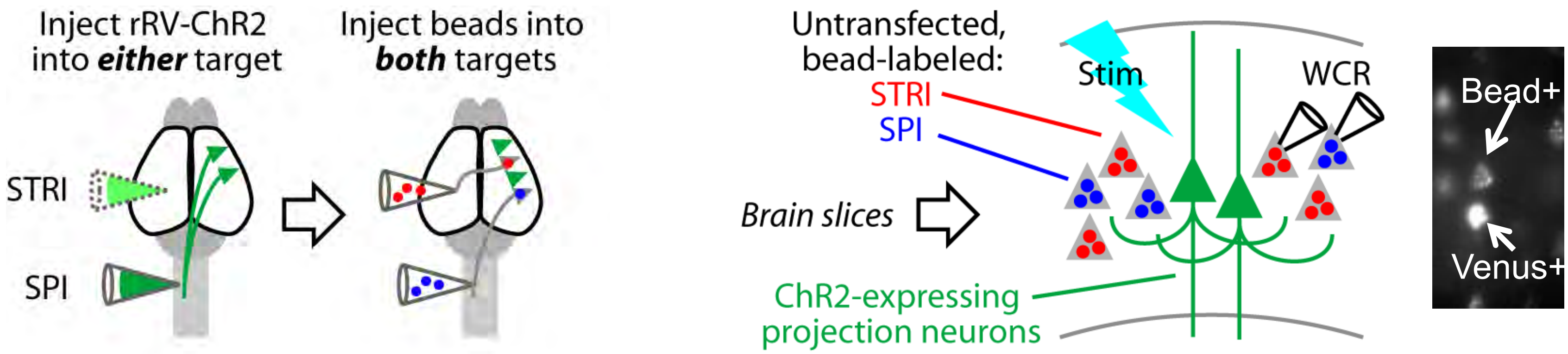
•cf. Rathelot, Strick (transsynaptic)

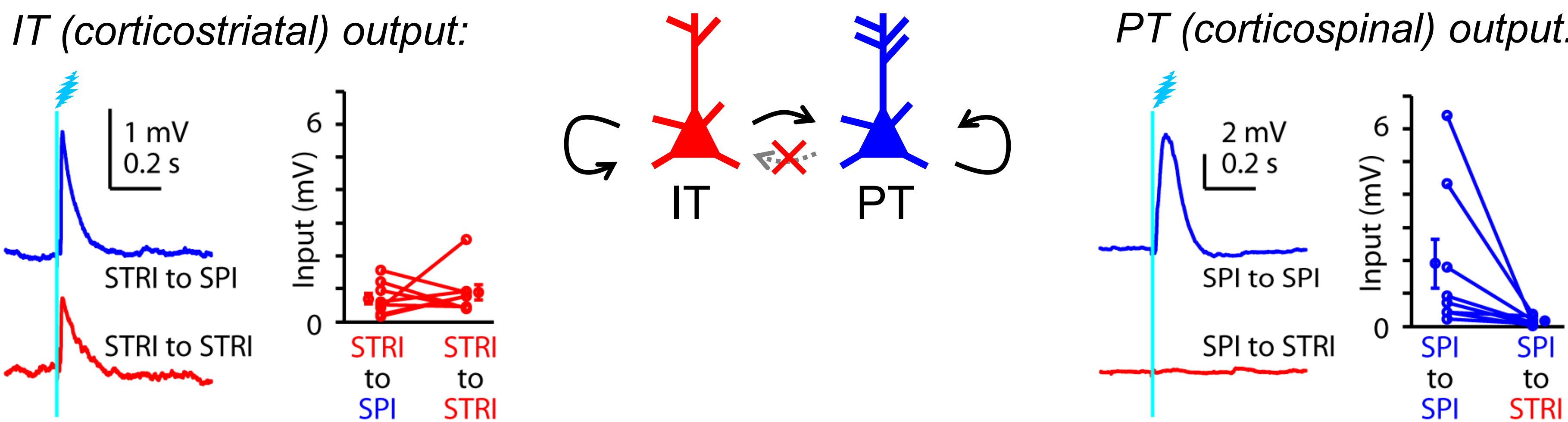


<u>RV-ChR2-Venus</u> for retrograde transfection of projection neurons with channelrhodopsin-2 (ChR2)

Taro Kiritani, collaboration with
Ian Wickersham & Sebastian Seung

RV-ChR2 connectivity analysis

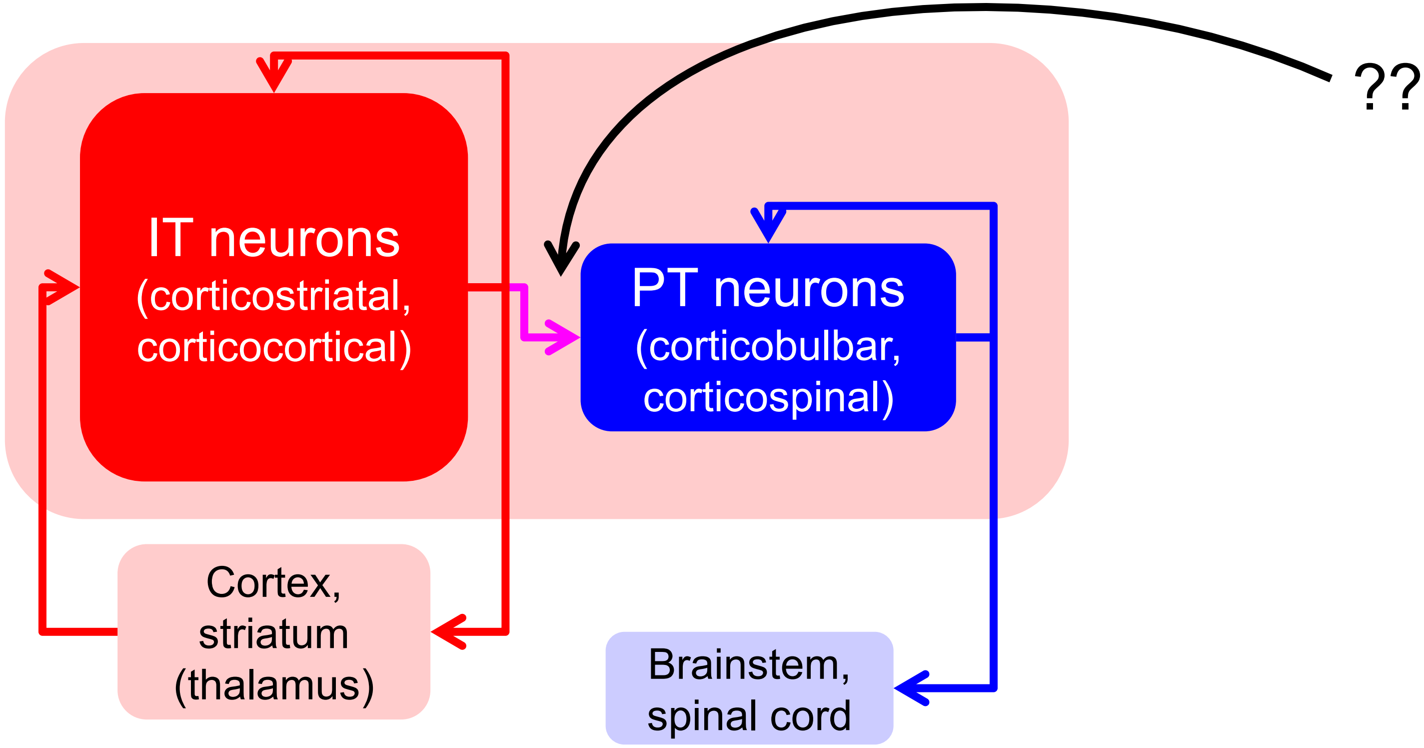




- *IT neurons talk to PT neurons, but not vice versa*
- *Confirmed with quadruple recordings*
- *Similar findings in rat PFC (Morishima Kawaguchi 2006)*

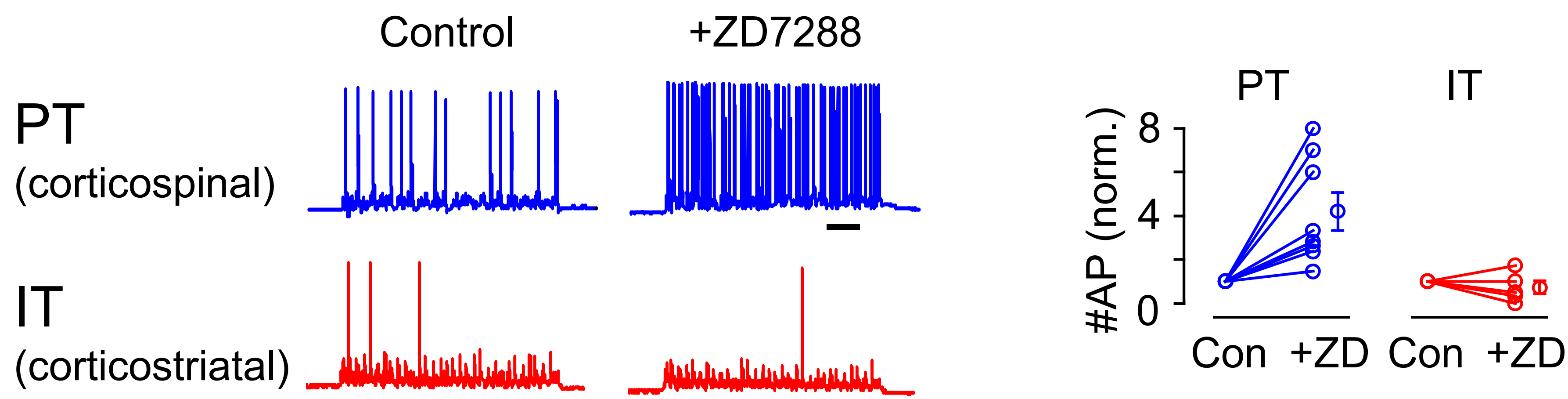
Taro Kiritani

Hierarchical circuit organization

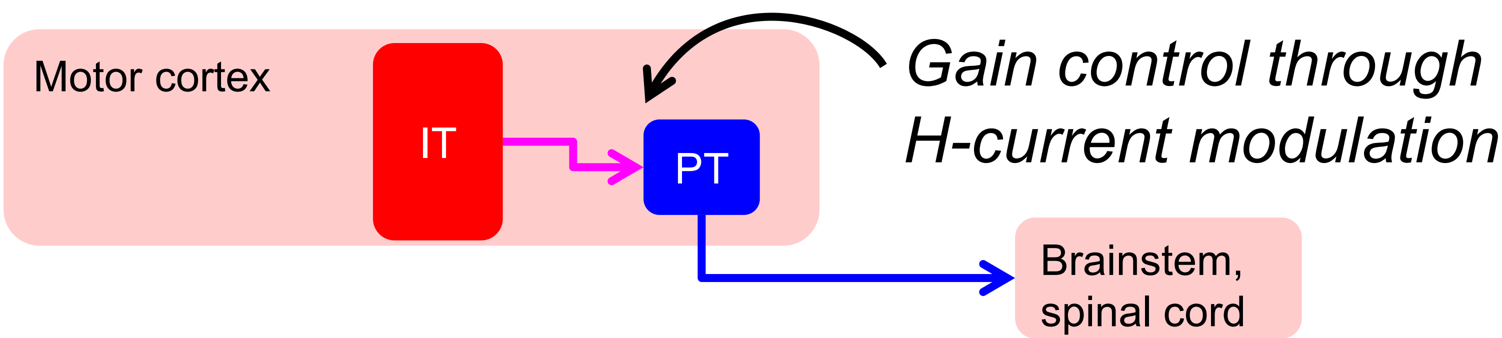


- *Both IT and PT make intra-class (recurrent) connections*
- *Interclass connectivity: unidirectional IT → PT*
- *PT neurons are downstream*
- *Are they simply slaved to IT activity?*

H-current (I_h) controls IT→PT



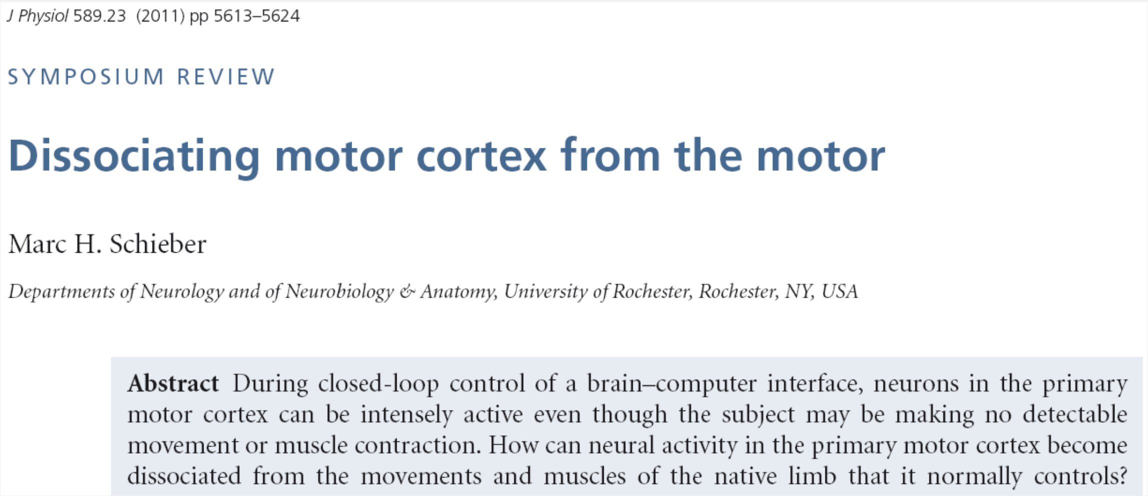
- *L2/3-driven PT (but not IT) firing increases when I_h is blocked*
- *Noradrenergic neuromodulation also closes HCN channels*



Patrick Sheets

Potential relevance to motor behavior

M1 activity is flexibly related to muscle activity (e.g. BMI)



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SYMPOSIUM REVIEW

Dissociating motor cortex from the motor

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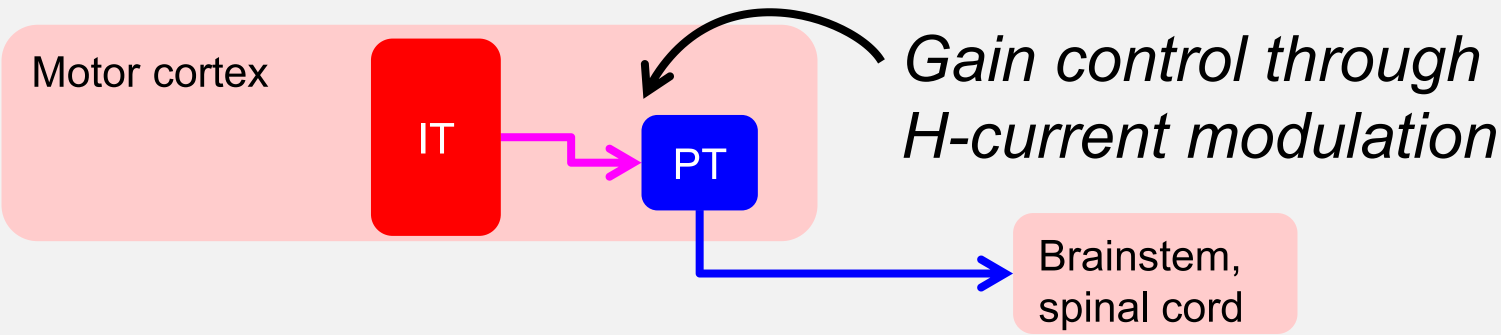
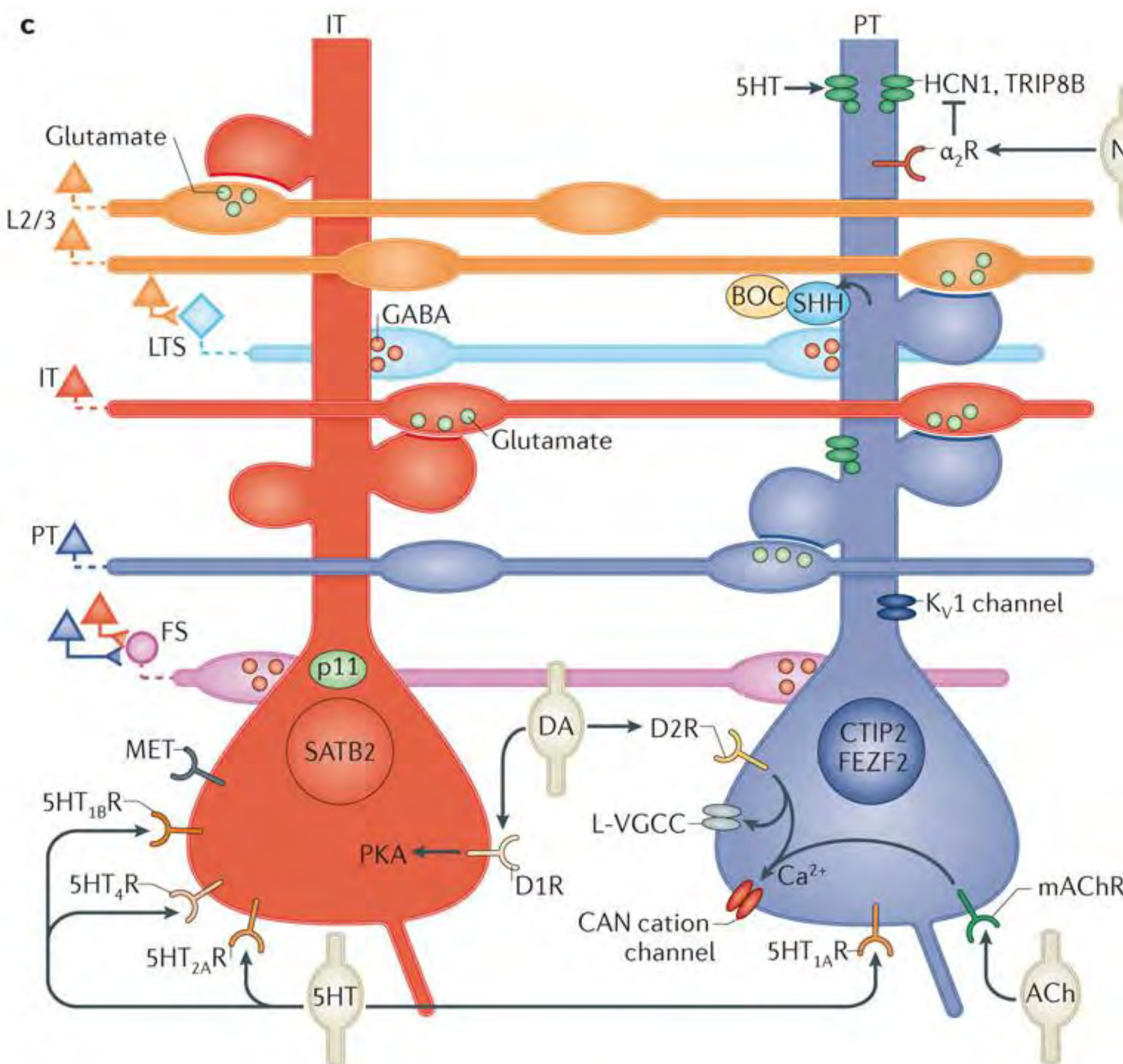
Abstract During closed-loop control of a brain–computer interface, neurons in the primary motor cortex can be intensely active even though the subject may be making no detectable movement or muscle contraction. How can neural activity in the primary motor cortex become dissociated from the movements and muscles of the native limb that it normally controls?



IT/PT molecular differentiation



<u>Neuromodulation</u>
Norepinephrine, dopamine, serotonin, acetylcholine

<u>Ion channels</u>
HCN, others

<u>Cell fate specification</u>
Satb2, Ctip2, others

IT/PT-related studies and data sets

Weiler et al. (2008, Nat Neurosci) – laminar organization of excitatory connections
Anderson et al. (2010, Nat Neurosci) – layer 2/3 inputs to IT/PT
Sheets et al. (2011, J Neurophysiol) – H-current in PT neurons
Kiritani et al. (2012, J Neurosci) – IT → PT connectivity
Apicella et al. (2012, J Neurosci) – inhibitory circuits of IT/PT
Suter et al. (2013, Cerebral Cortex) – spiking properties of IT/PT
Kress et al. (2013, Nature Neurosci) – IT/PT inputs to striatal neurons
Yamawaki et al. (2014, eLife) – layer 4 in M1
Joshi et al. (2015, J Neurosci) – IT/PT in auditory cortex
Suter et al. (2015, J Neurosci) – Inter-areal connectivity of M1/S2 incl IT/PT
Yamawaki et al. (2015, J Neurosci) – CT connectivity with IT/PT/TC in M1

Reviews:
Shepherd (2013, Nature Reviews Neurosci) – broad review of IT/PT
Harris and Shepherd (2015 Nature Neurosci) – broad review of cortical circuits