KRESGE HEARING RESEARCH INSTITUTE
MICHIGAN MEDICINE

Determining the Structure and Function of Microcircuits in the Auditory Midbrain

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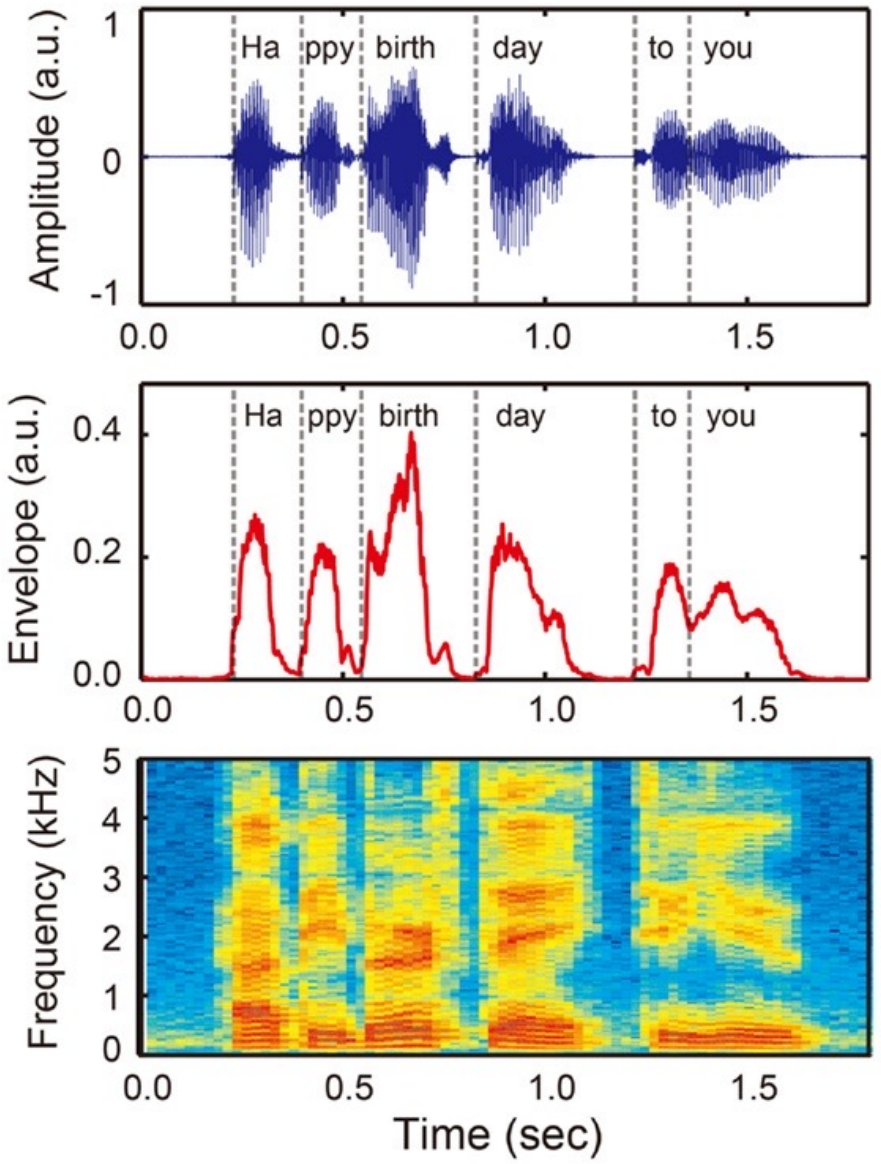
University of Michigan

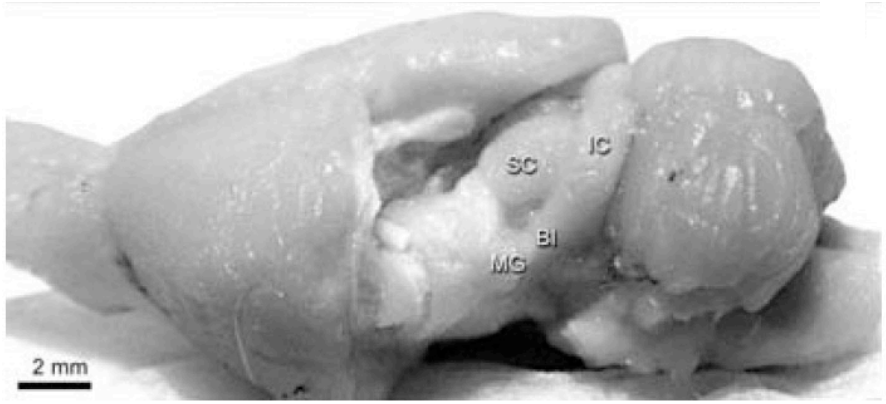
How do neural circuits detect and encode the spectrotemporal features of speech and other vocalizations?

How do auditory circuits change following hearing loss?

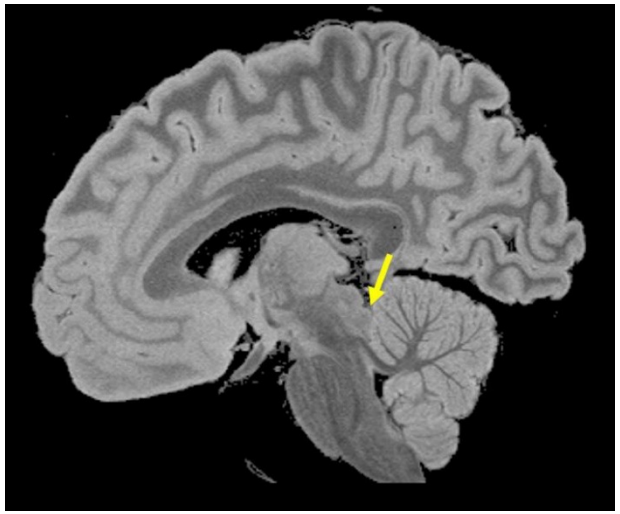


Fujii and Wan, 2014

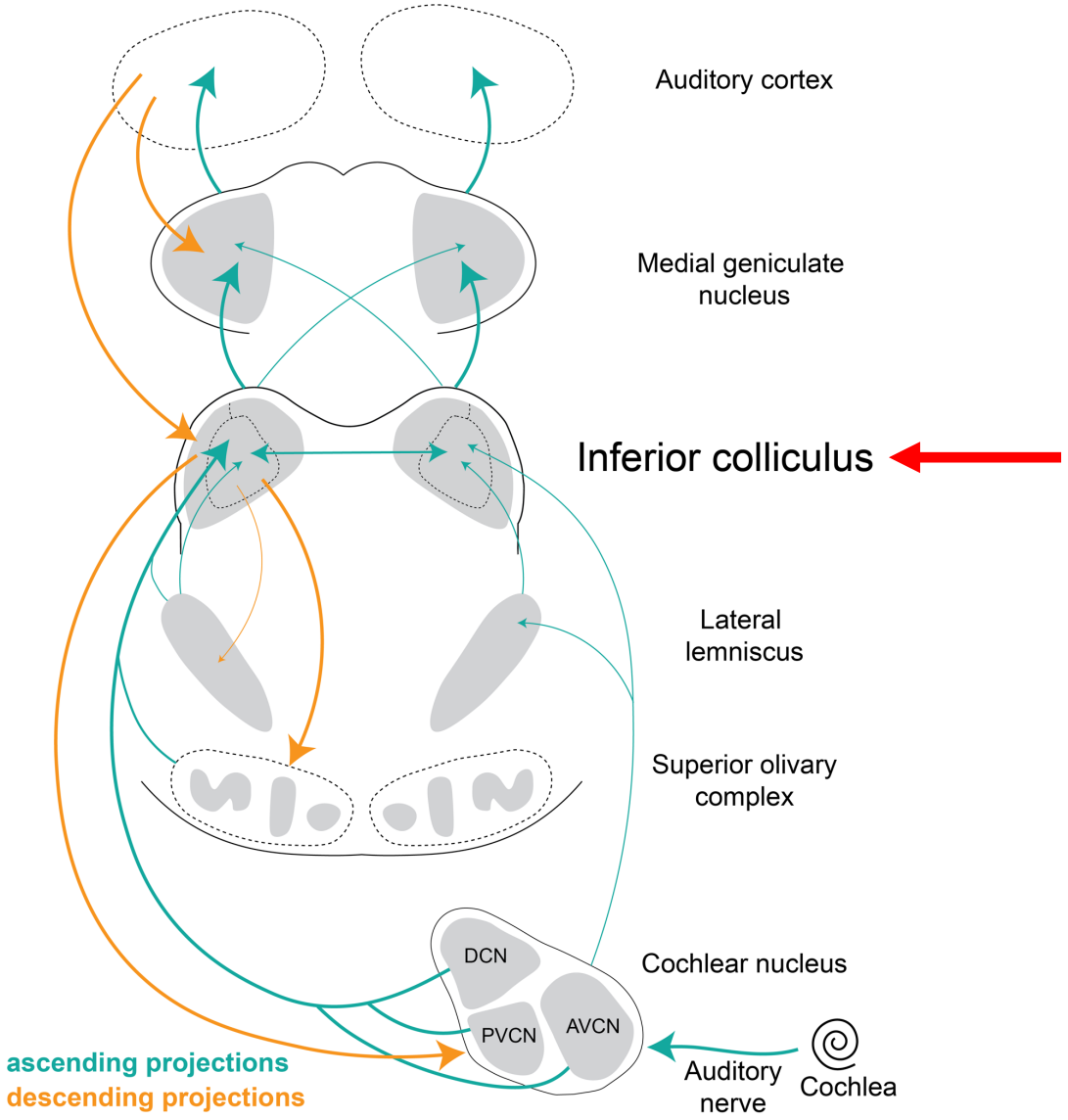
The inferior colliculus (IC) is the hub of the central auditory system



Oliver, 2005



Bordia and Zahr, 2020



Drotos and Roberts, 2024

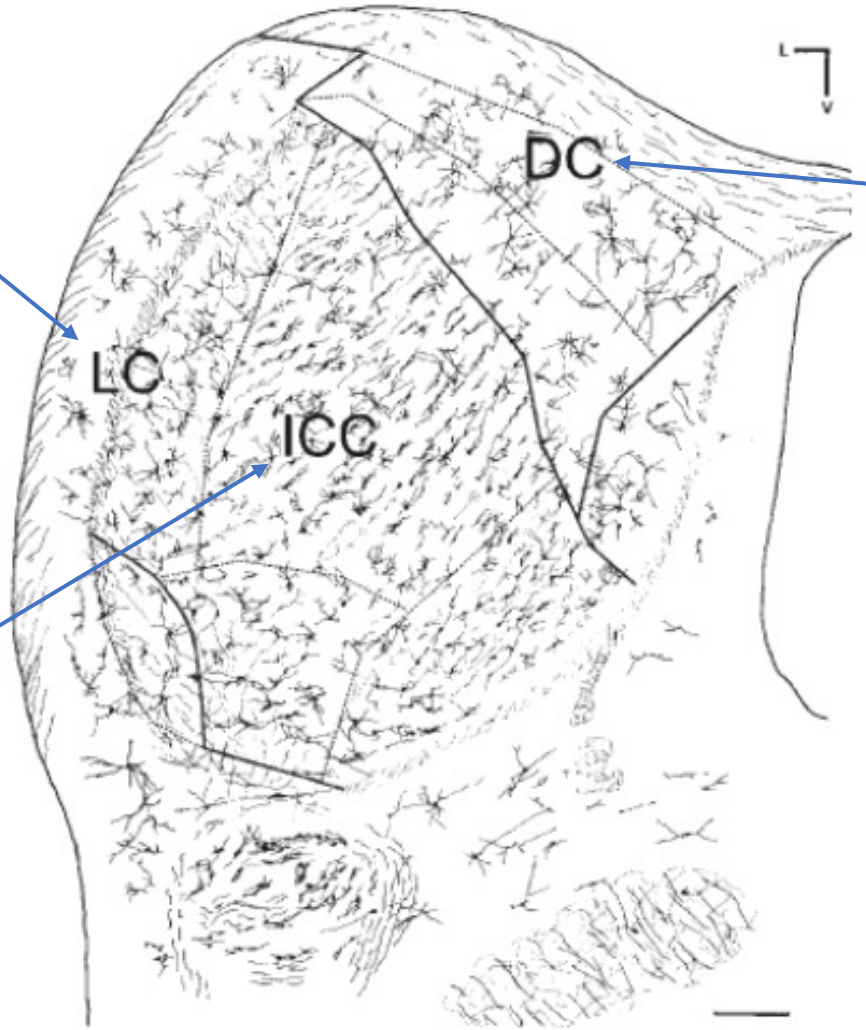
The IC performs computations important for:

- Speech and vocalization processing
- Sound localization
- Echo suppression (and echolocation in bats)
- Signal identification

The IC has 3 main subdivisions

ICIc = lateral cortex

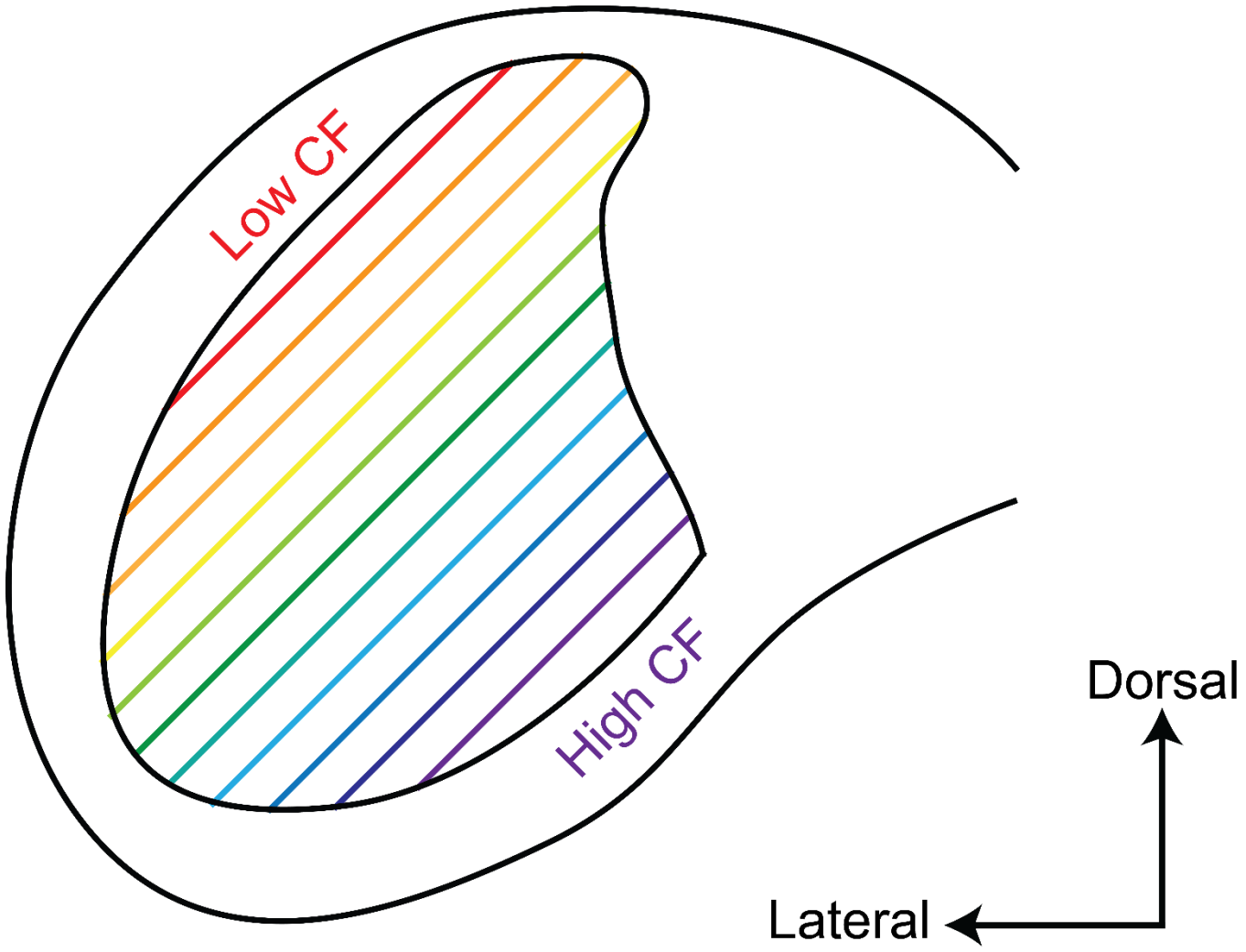
ICc = central nucleus



ICd = dorsal cortex

Oliver, *The Inferior Colliculus*, 2005

The central nucleus of the IC (ICc) is tonotopically organized

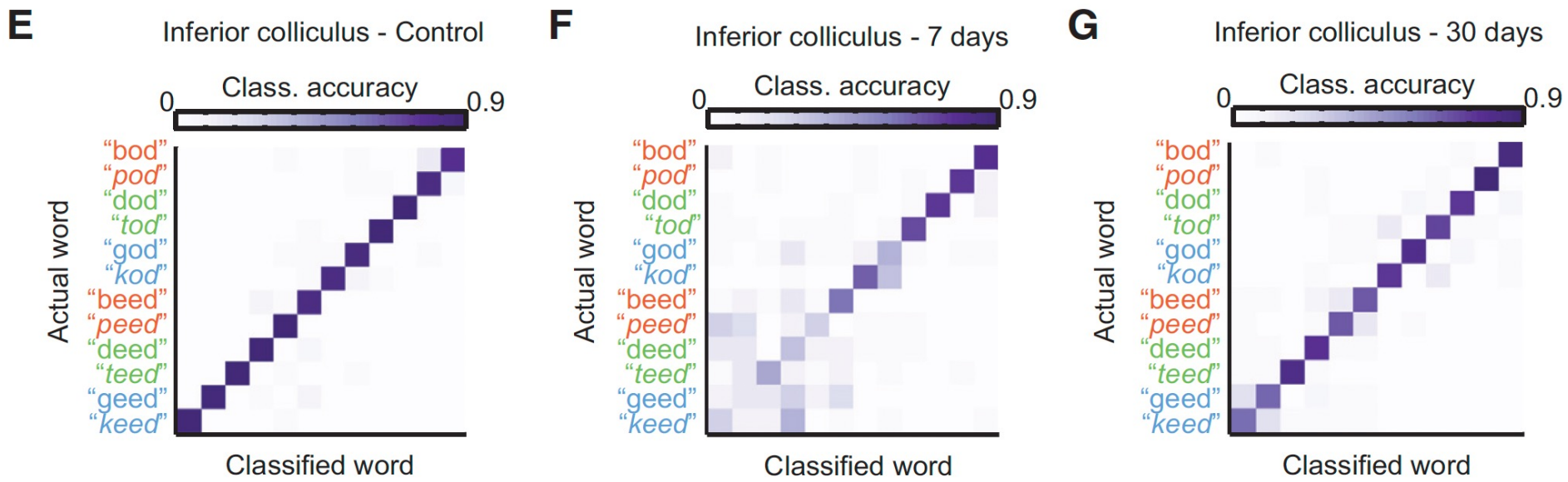


The IC exhibits plasticity following hearing loss

Neuron
Article

Central Gain Restores Auditory Processing following Near-Complete Cochlear Denervation

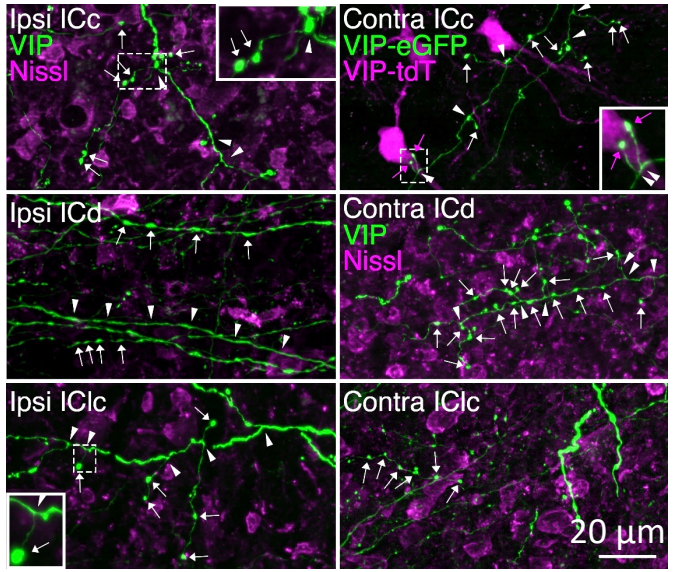
Anna R. Chambers,[1,4] Jennifer Resnik,[1,2] Yasheng Yuan,[3] Jonathon P. Whitton,[1] Albert S. Edge,[1,2] M. Charles Liberman,[1,2] and Daniel B. Polley[1,2,*]



The IC is rich in intrinsic circuitry

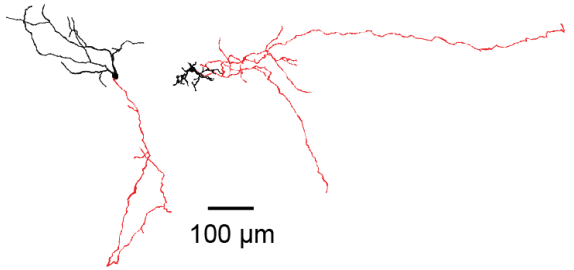
- The rat IC contains ~5x as many neurons as the rest of the auditory brainstem (Kulesza et al., 2002)
- IC neurons can be divided into numerous molecularly defined neuron types, including:
  - VIP (Goyer et al., 2019)
  - NPY (Silveira et al., 2020)
  - CCK_E (Kreeger et al., 2021)
  - SST (Liu et al., 2024)
- Most IC neurons have local axon collaterals (Oliver et al., 1991; Malmierca et al., 1995; Wallace et al., 2012, 2021)

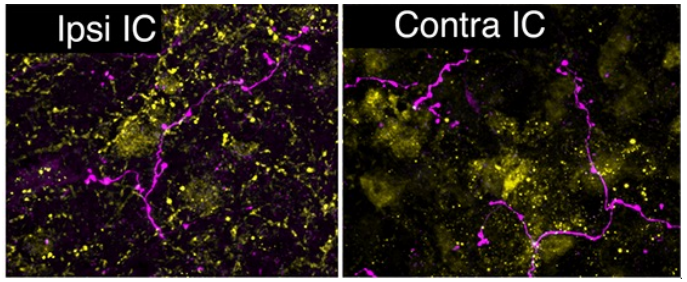
VIP neurons



Beebe et al., 2022

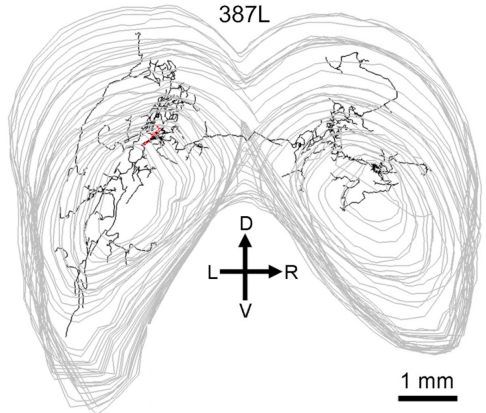
NPY neurons

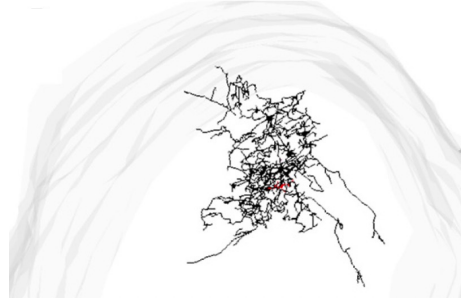




Silveira, Beebe, et al., unpublished

Neurons labeled during in vivo recordings





Wallace et al., 2021

Long-term goals

To determine how neural circuits in the IC detect the spectrotemporal features of speech and other vocalizations.

To determine how IC circuits change in hearing loss and hearing disorders.

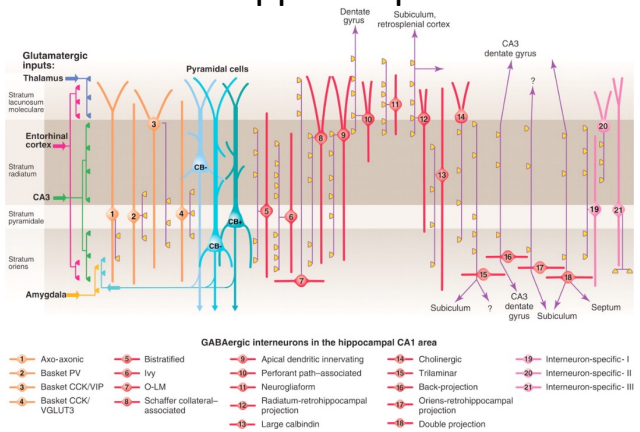
First steps

Probing neural circuits requires the ability to ***identify*** and ***manipulate*** specific classes of neurons.

- What classes of neurons make up the IC?
- How are IC microcircuits organized?

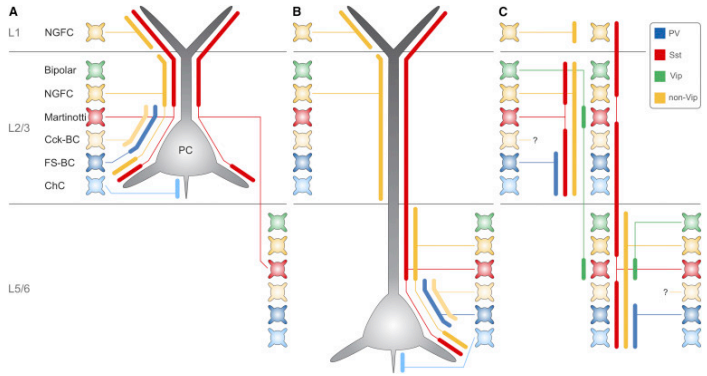
Some examples of well-described neural circuits

Hippocampus



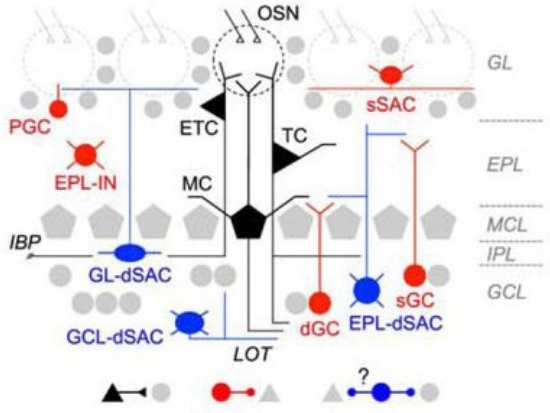
Klausberger and Somogyi, 2008

Cerebral Cortex



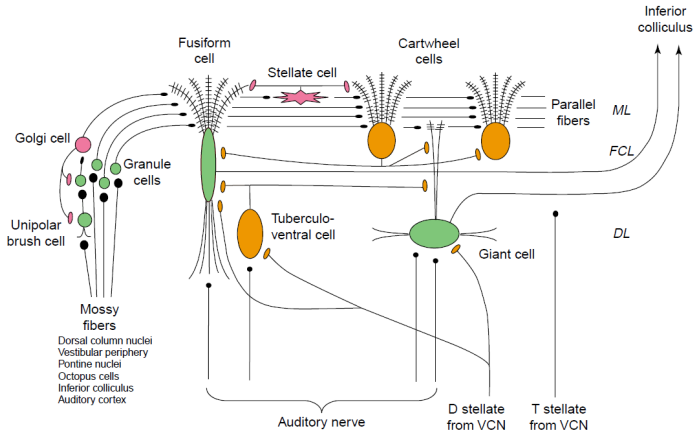
Tremblay et al., 2016

Olfactory bulb



Burton et al., 2016

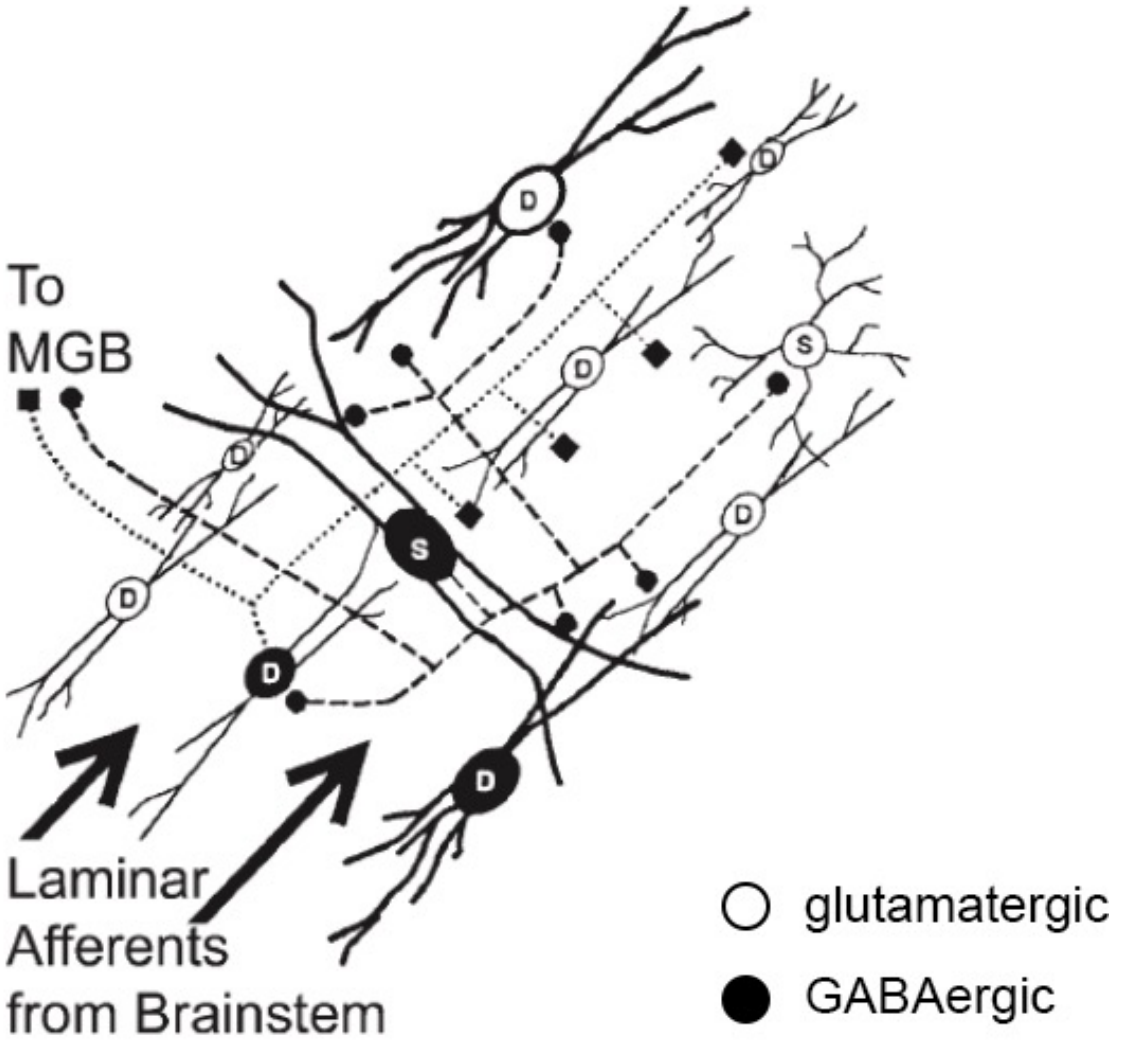
Dorsal cochlear nucleus



Oertel and Young, 2004

The ICc contains disc-shaped and stellate neurons

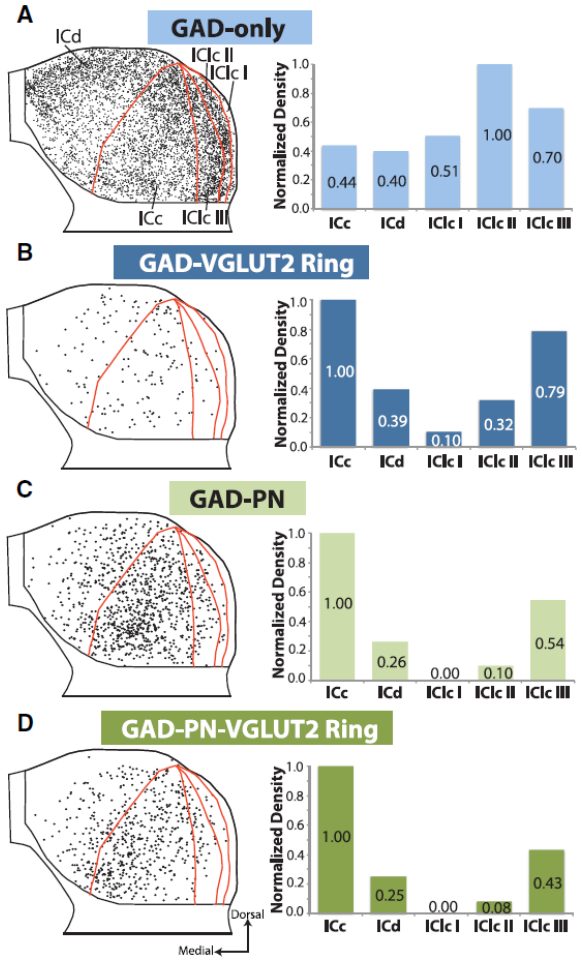
D = disc-shaped neurons
S = stellate neurons



Oliver, *The Inferior Colliculus*, 2005

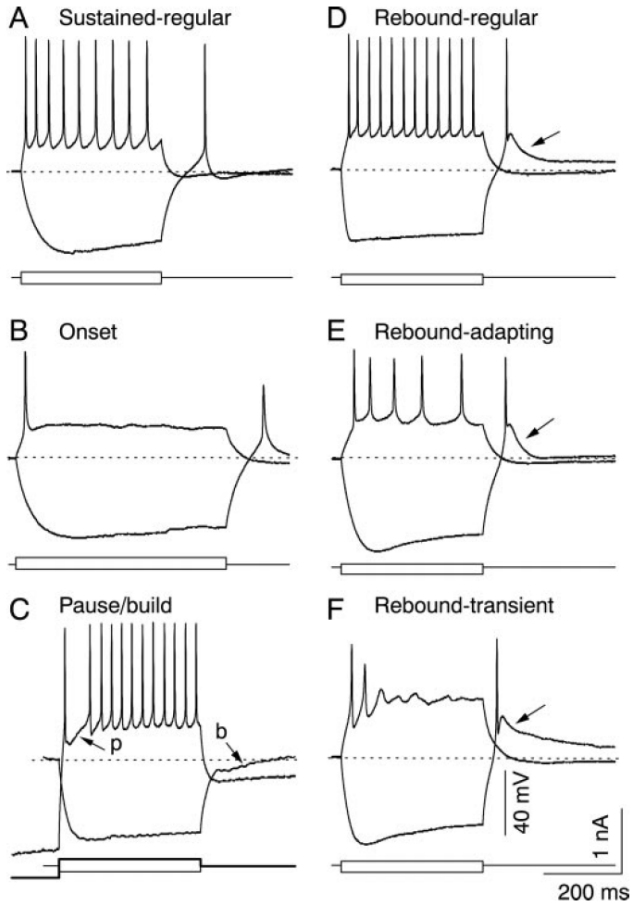
It has proven difficult to identify neuron classes in the IC

Anatomy and neurochemistry



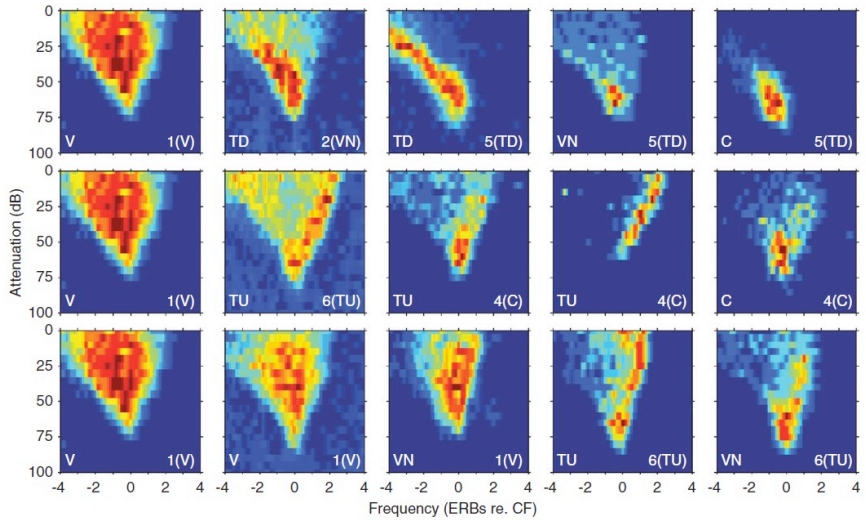
Beebe et al., 2016

In vitro electrophysiology



Sivaramakrishnan and Oliver, 2001

In vivo electrophysiology



Palmer et al., 2013

Outline

- **Identification of VIP and NPY neurons**
- Recurrent and feedforward circuits in the IC
- New approaches to mapping IC local circuits

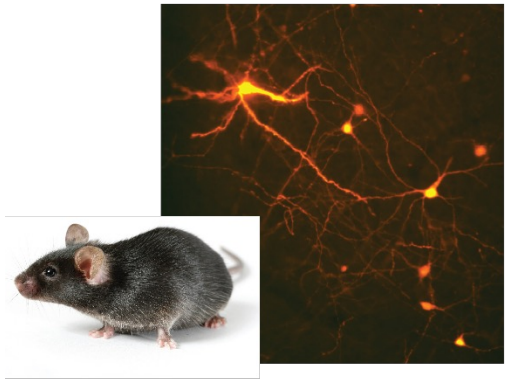

Marina Silveira


David Goyer

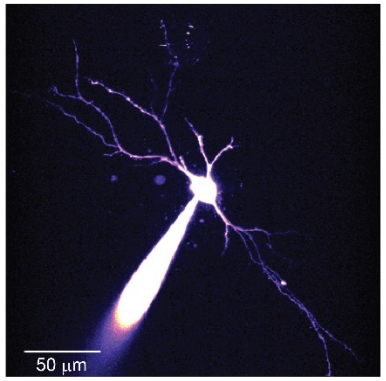

Brett Schofield


Nichole Beebe

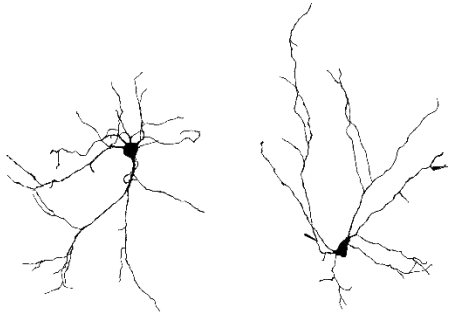
Classification of IC neurons: a multifaceted approach



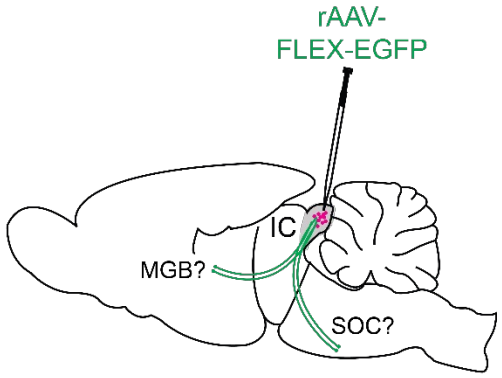
Molecular genetic markers



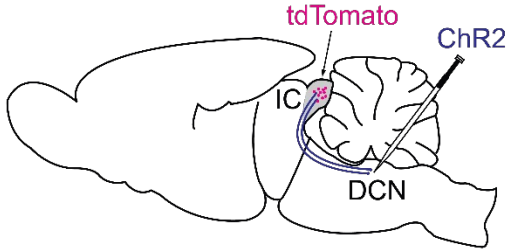
Intrinsic physiology



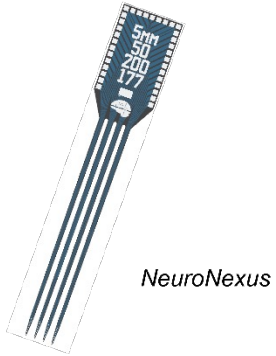
Neuronal morphology



Projections



Inputs

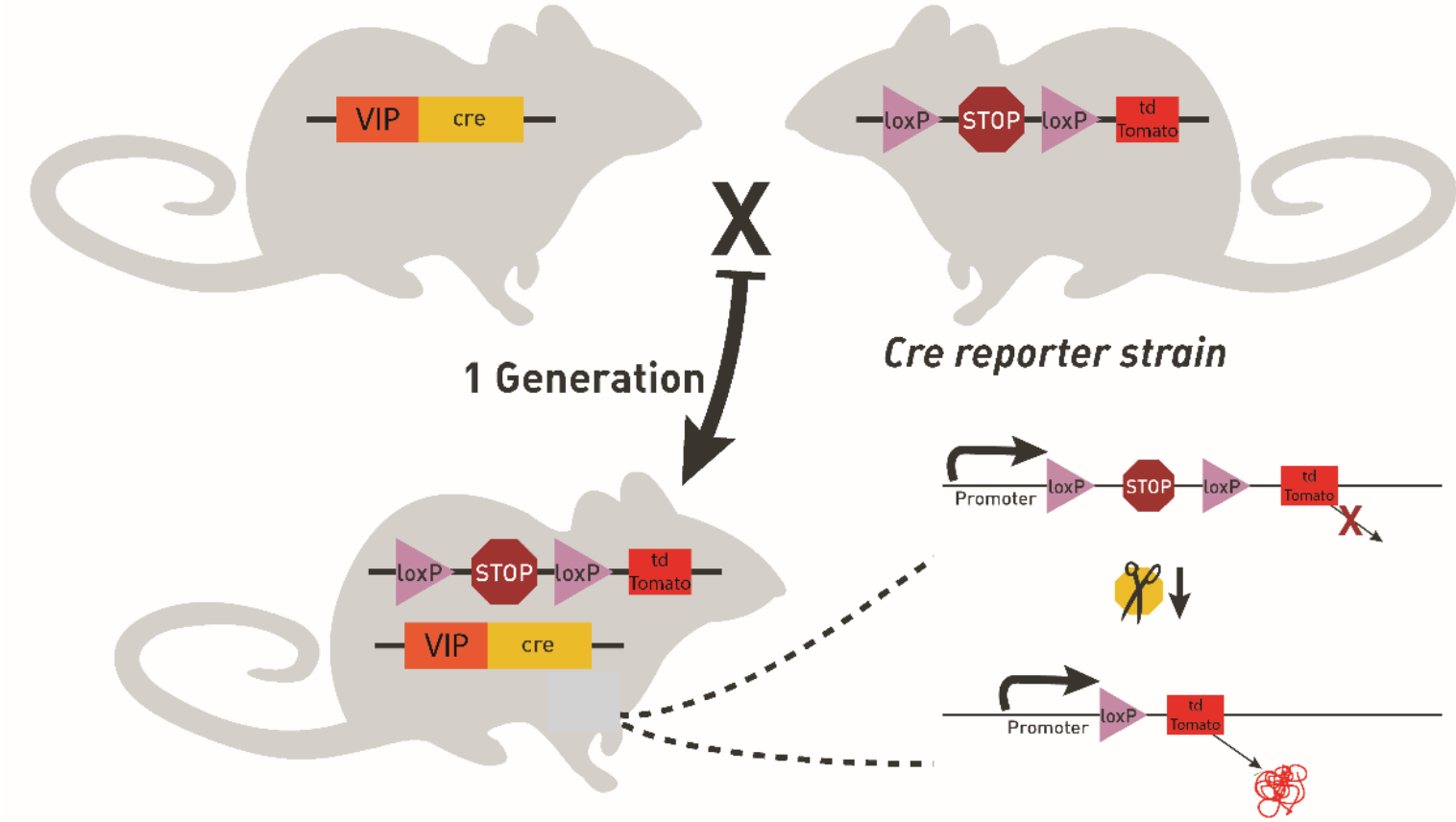


Responses to sound

Neuropeptides

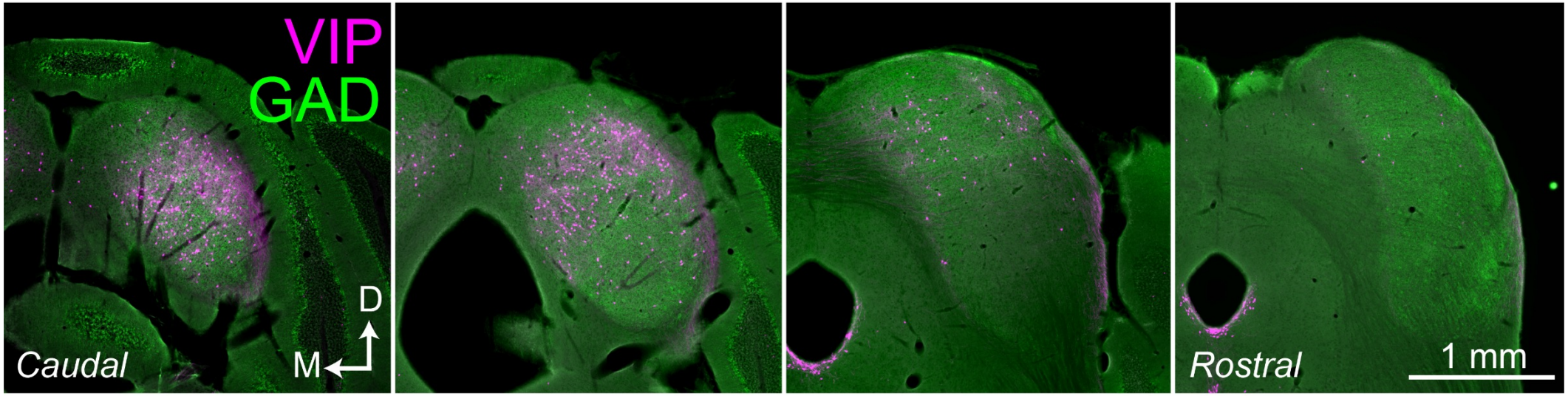
- 3 – 70 amino acid peptides
- Evolutionarily ancient signaling molecules, among the earliest neurotransmitters
- Are important neuromodulators in mammals
- Are markers for neuron types in many brain regions

VIP-IRES-Cre x Ai14 mice



Adapted from Jackson Laboratory

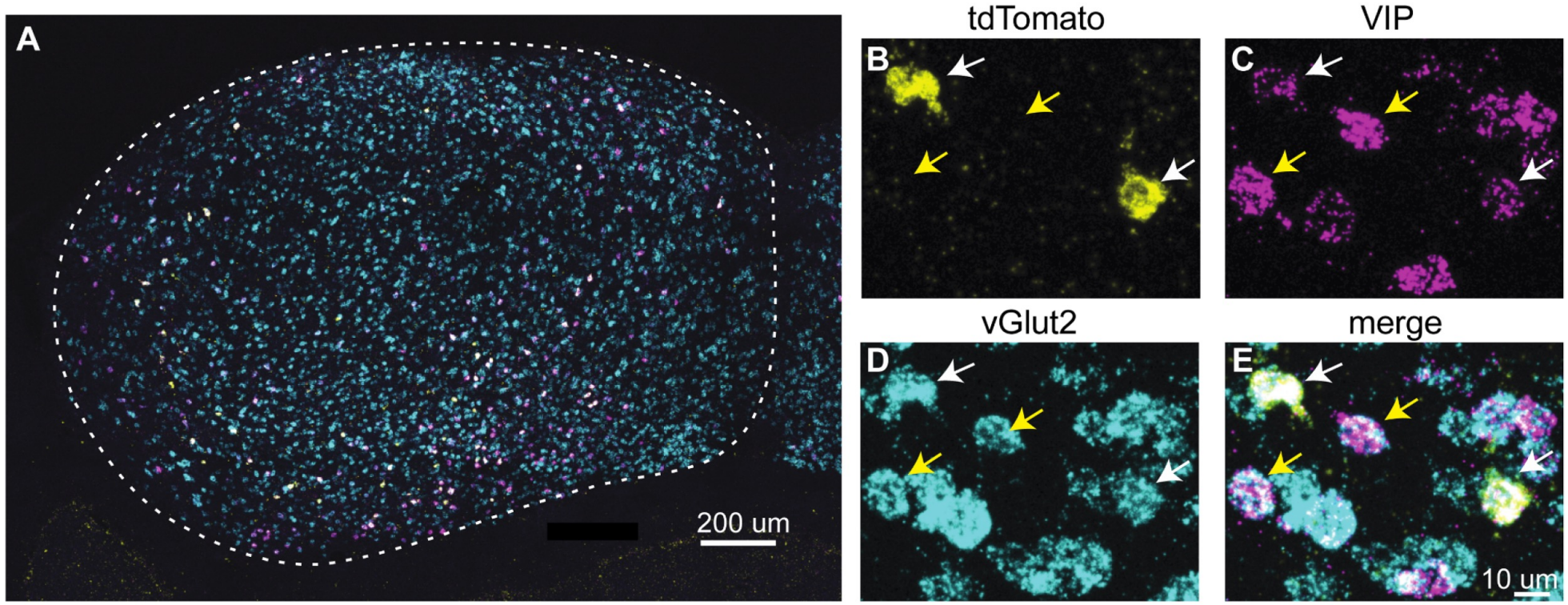
VIP neurons are most common in the caudal ICc and the ICd



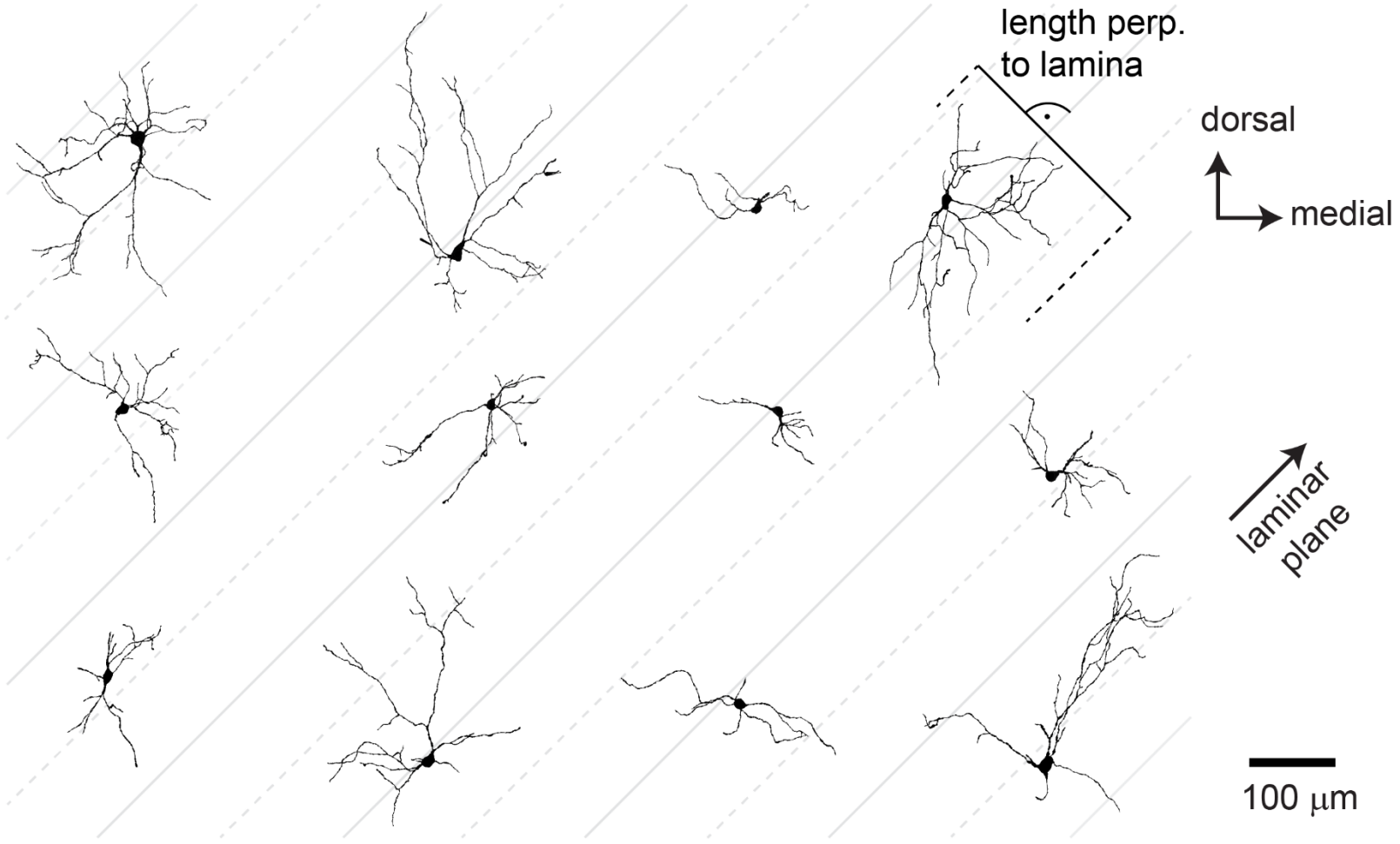
Goyer et al., *eLife*, 2019

VIP neurons are glutamatergic and express *Vip* mRNA

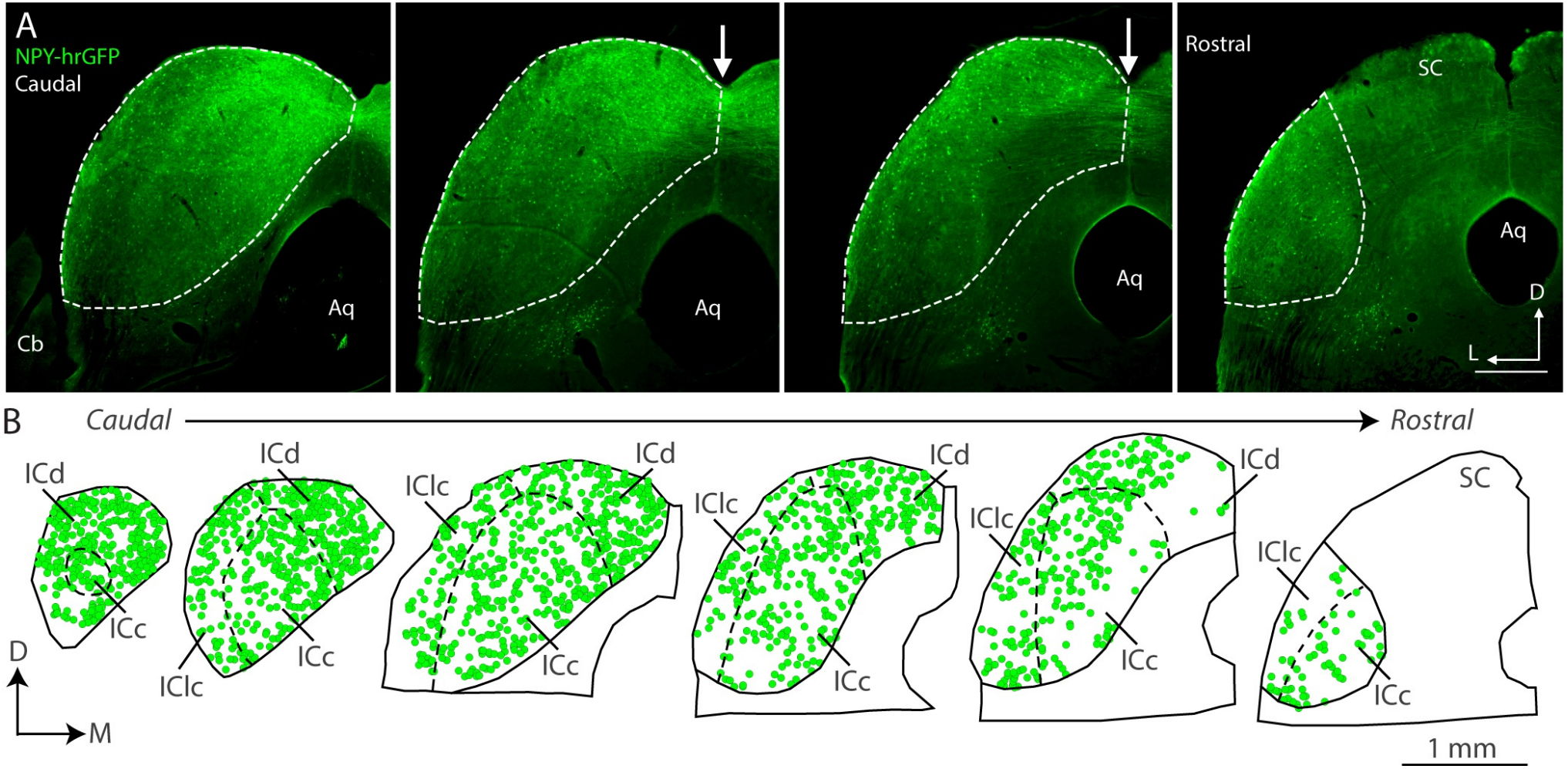
Fluorescent in situ hybridization
(RNAScope)



VIP neurons have a stellate morphology



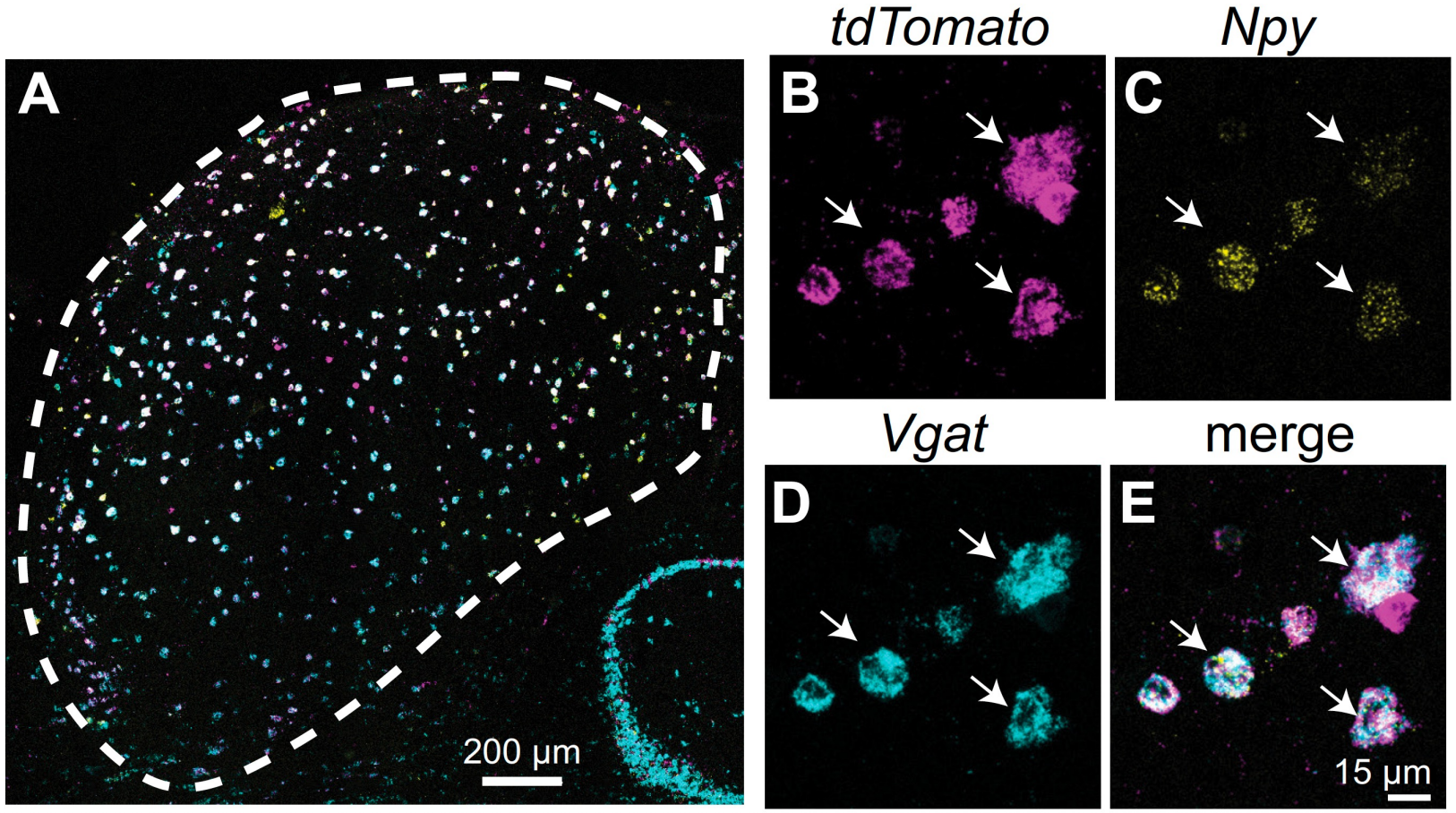
NPY neurons are distributed across the major IC subdivisions



Silveira et al., *J Neurosci*, 2020

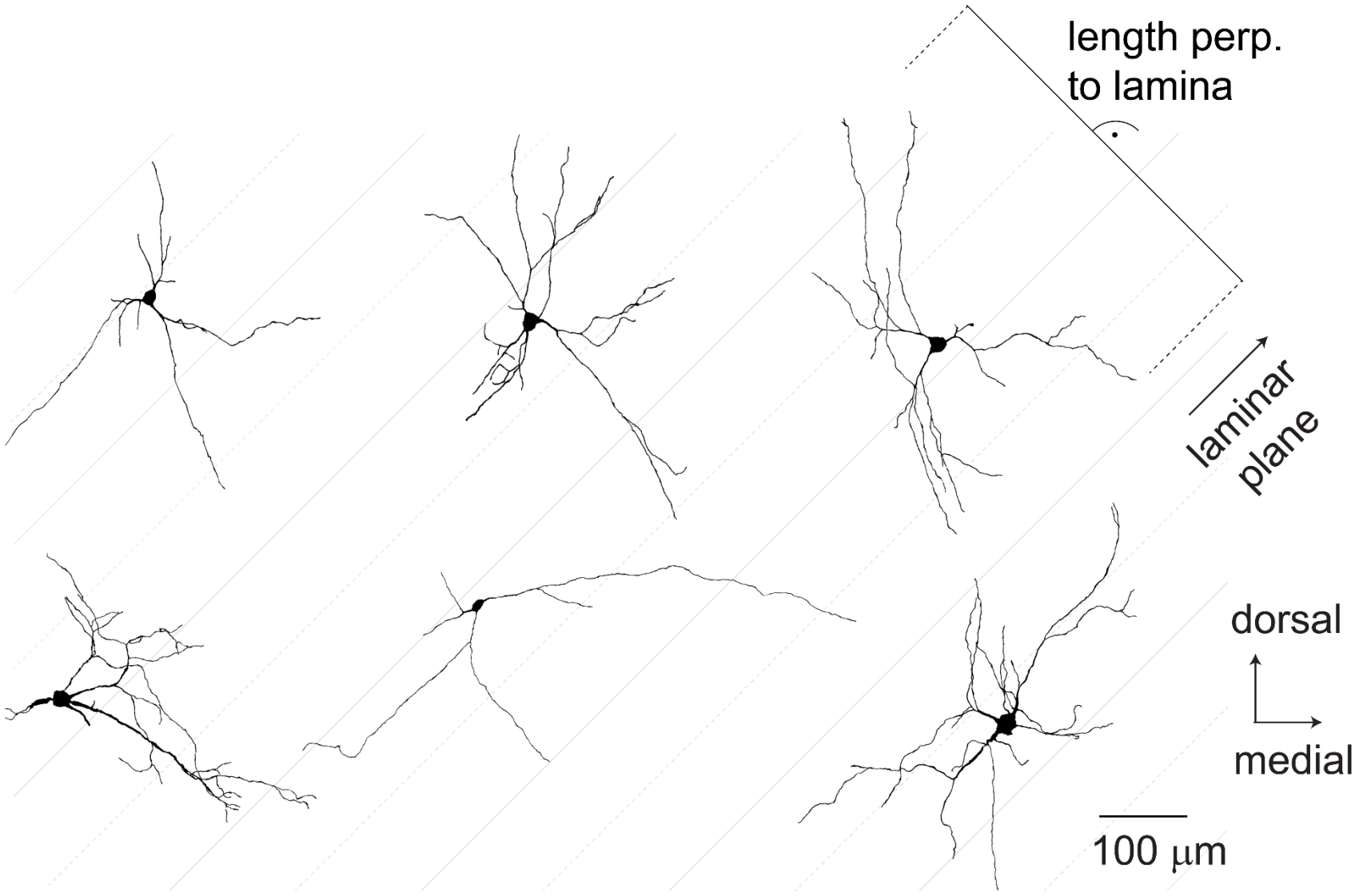
NPY neurons are GABAergic and make NPY

RNAScope in
NPY-FlpO x Ai65F mice

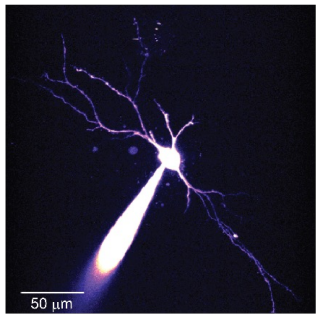


Silveira et al., *J Neurophys*, 2024

NPY neurons are stellate neurons

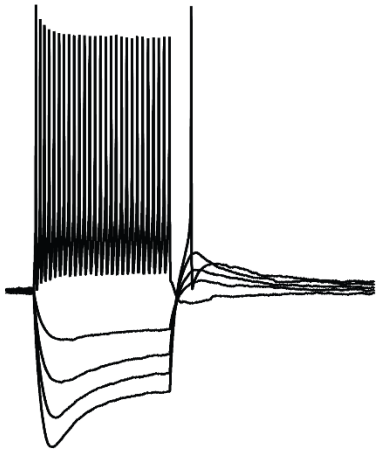


IC neurons exhibit diverse firing patterns

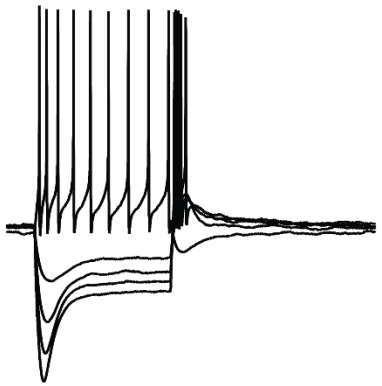


Intrinsic physiology

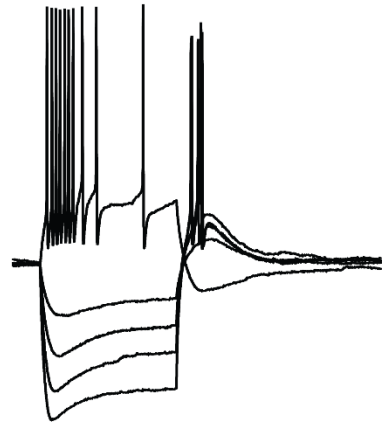
Sustained



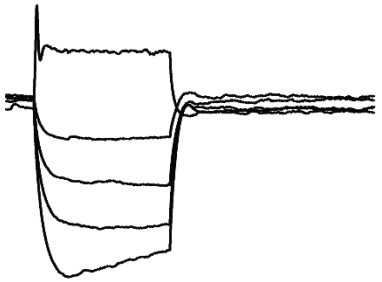
Adapting



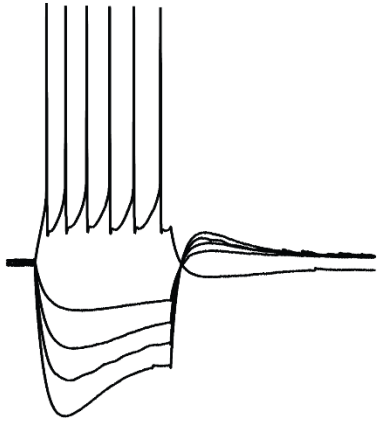
Strongly Adapting



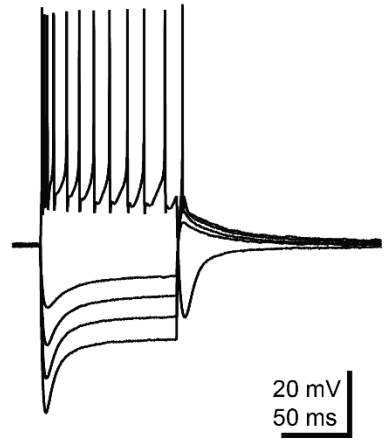
Onset



Double undershoot sustained

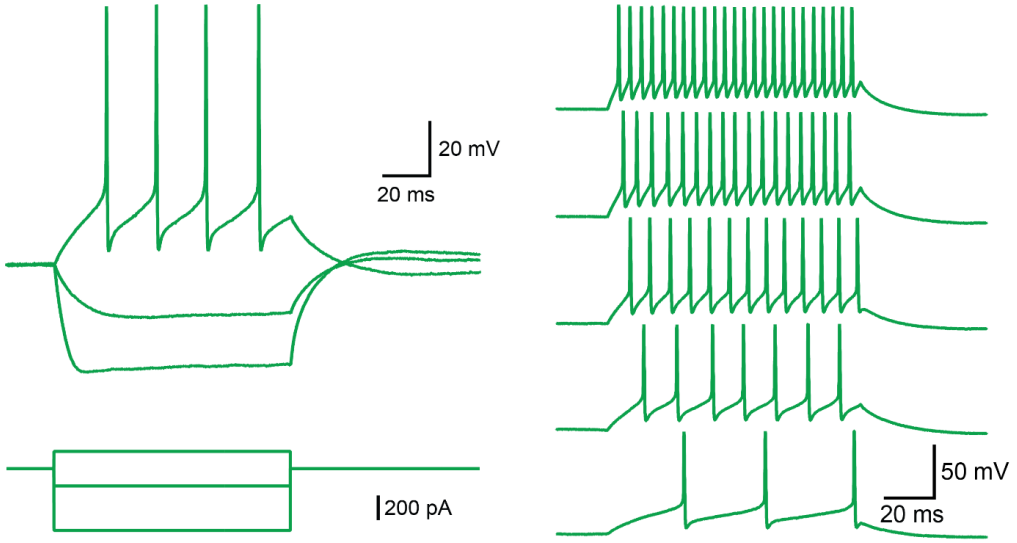


Double undershoot adapting

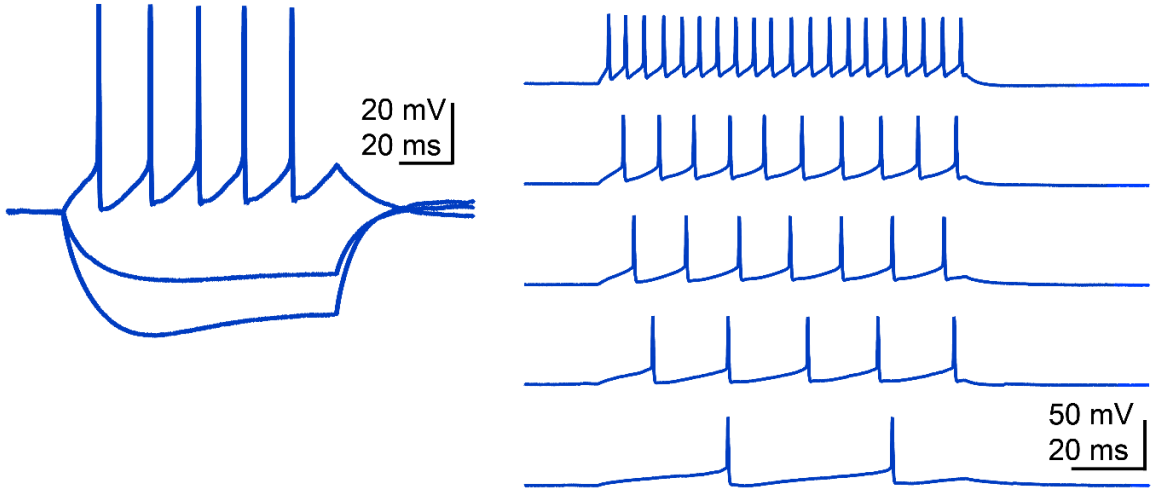


VIP and NPY neurons have sustained firing patterns

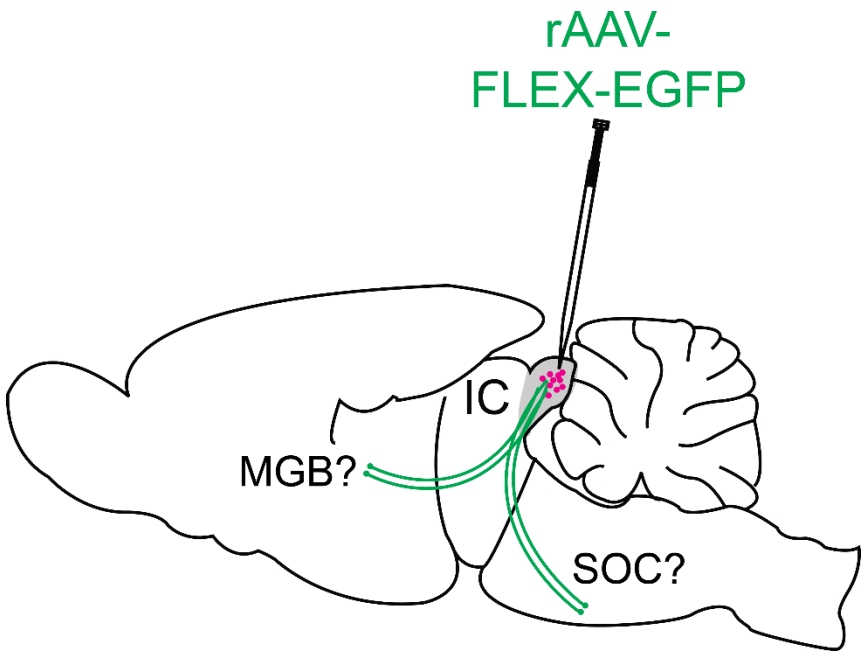
VIP



NPY



Mapping axonal projections with Cre-dependent viral constructs



NEOMED

Brett Schofield

Nichole Beebe

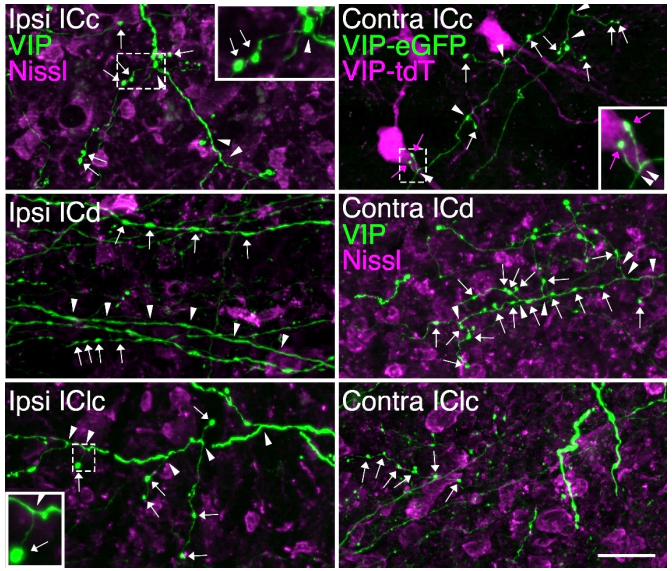
Will Noftz

Pooyan Mirjalili

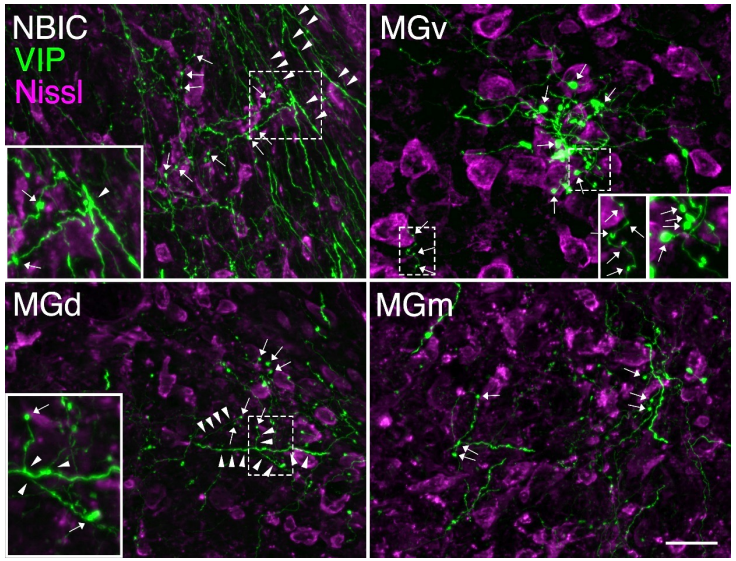
Ryan Edelbrock

VIP neurons project to many targets

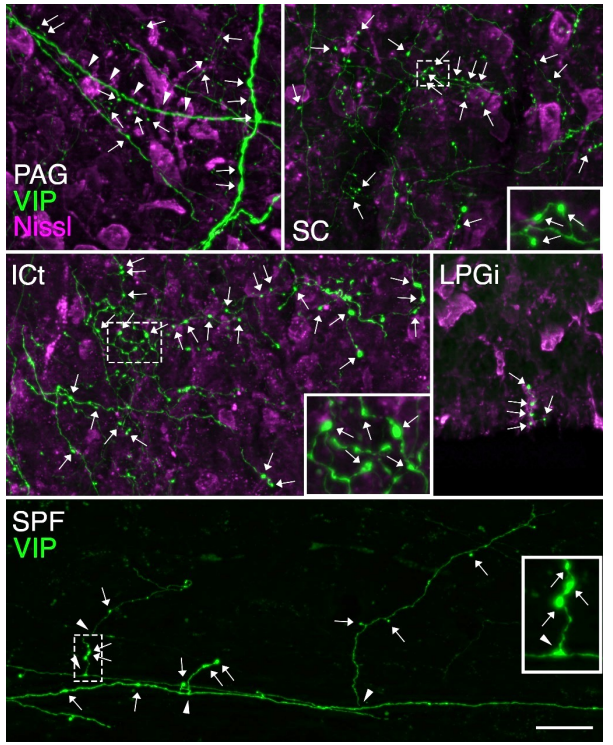
Collicular targets



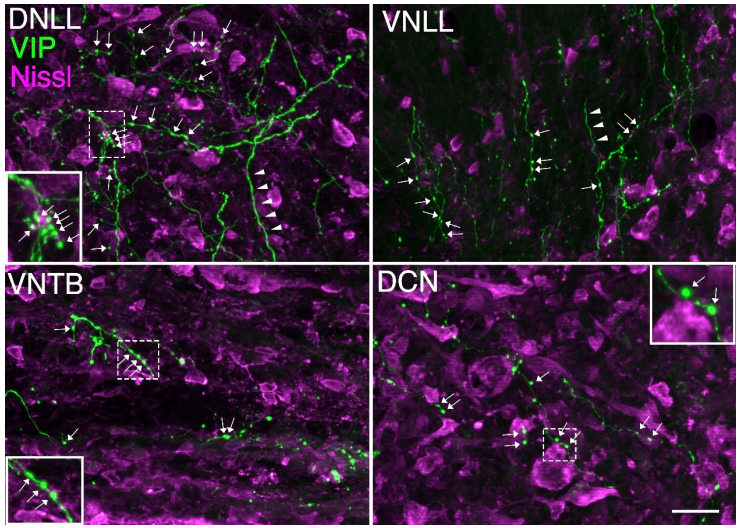
Ascending projections



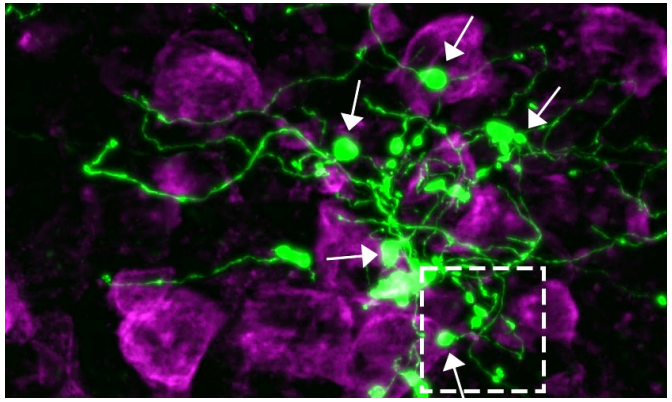
Multisensory and Modulatory targets



Descending projections

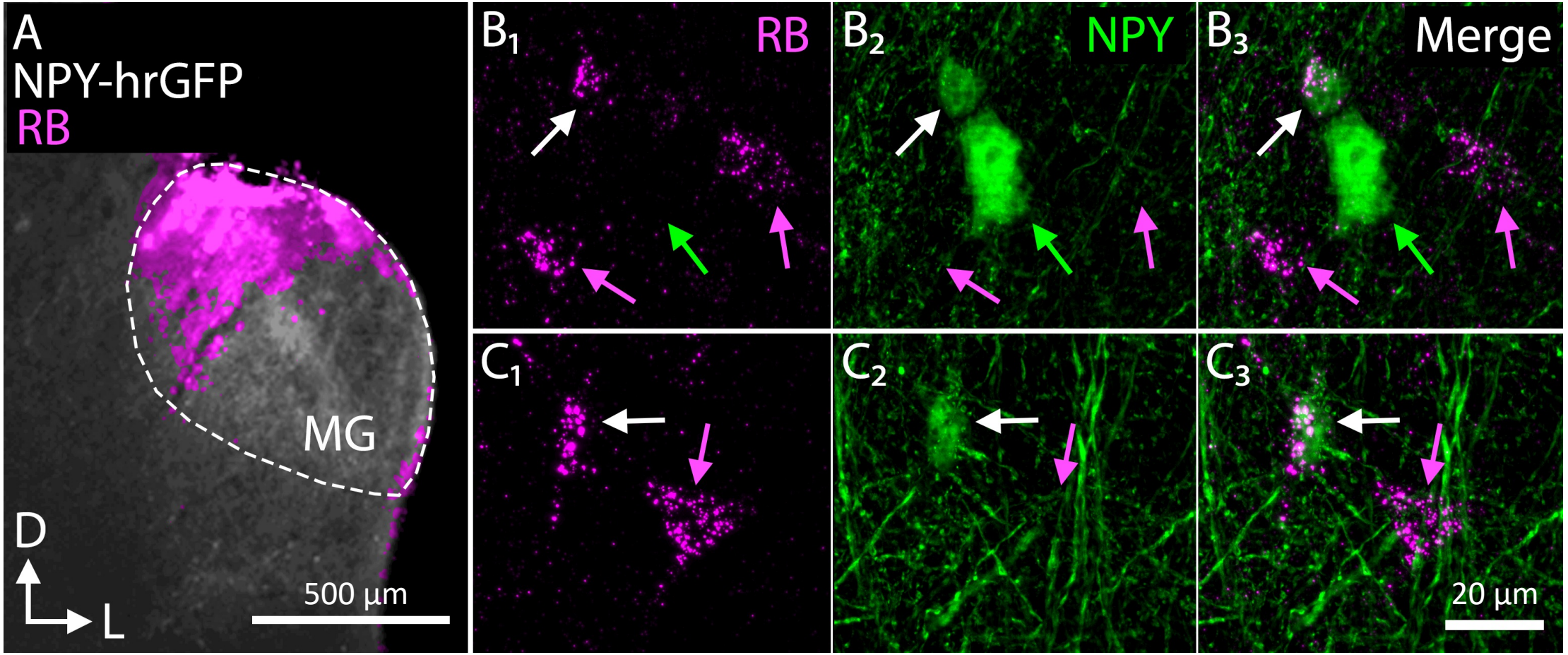


Terminals in MGv

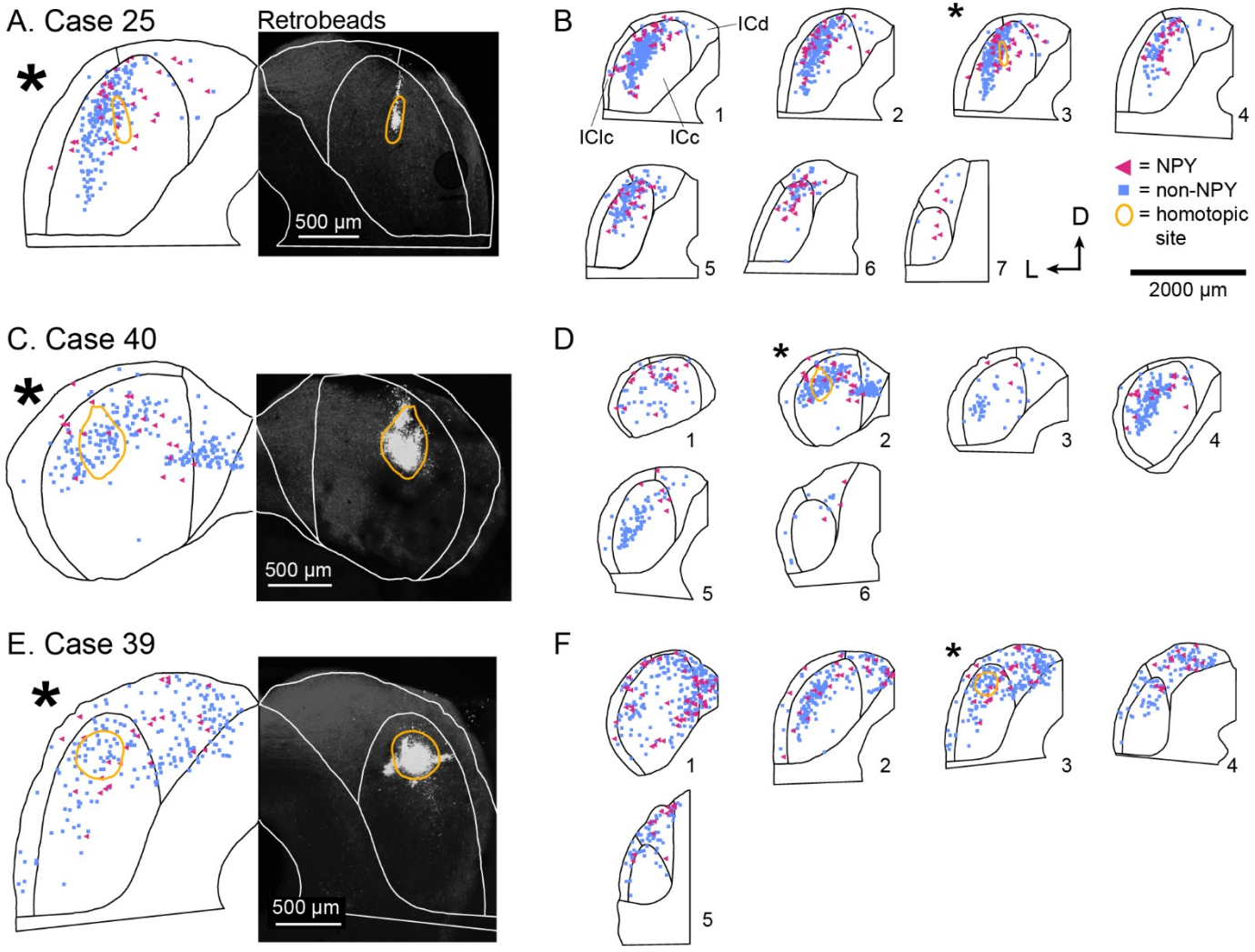


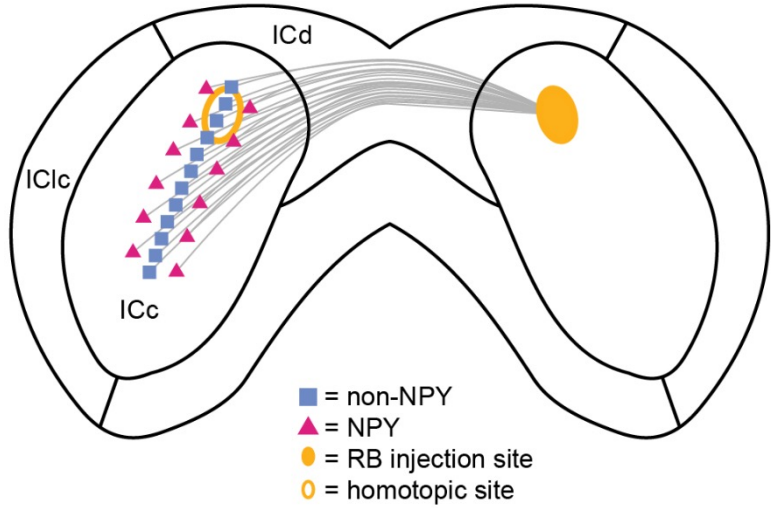
Scale bars = 20 µm

NPY neurons project to the auditory thalamus

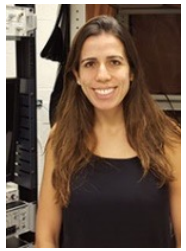


NPY neurons project to the contralateral IC





Justin Anair

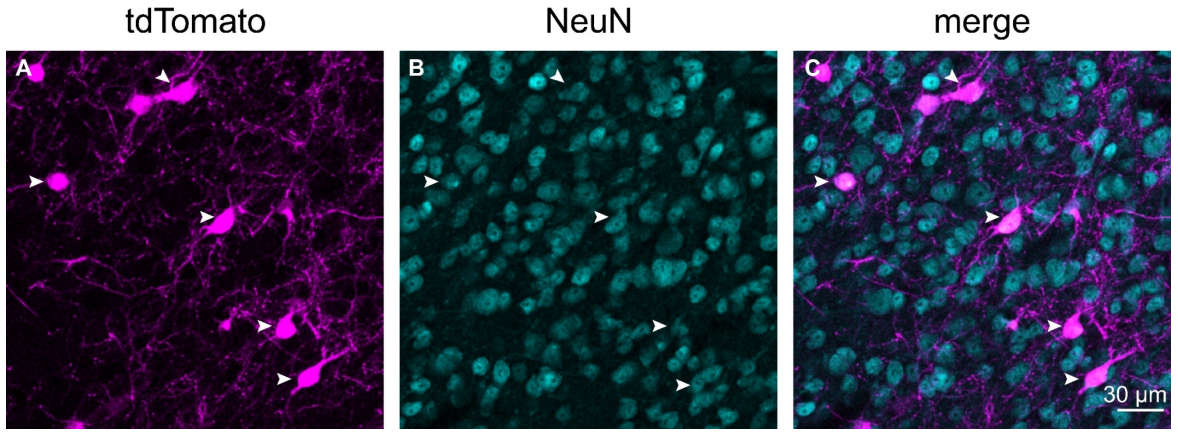


Marina Silveira

VIP and NPY neurons represent ~⅔ of ICc stellate neurons

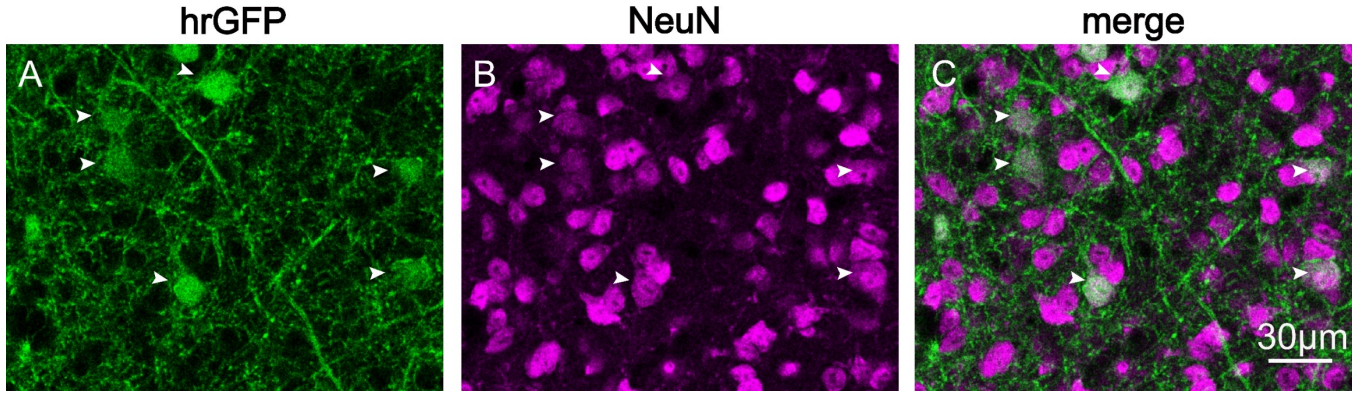
VIP neurons

- ~5% of glutamatergic neurons in the IC
- ~3.5% of ICc neurons
- ~20% of ICc stellate neurons



NPY neurons

- ~33% of GABAergic neurons in the IC
- ~7.5% of ICc neurons
- ~45% of ICc stellate neurons



Outline

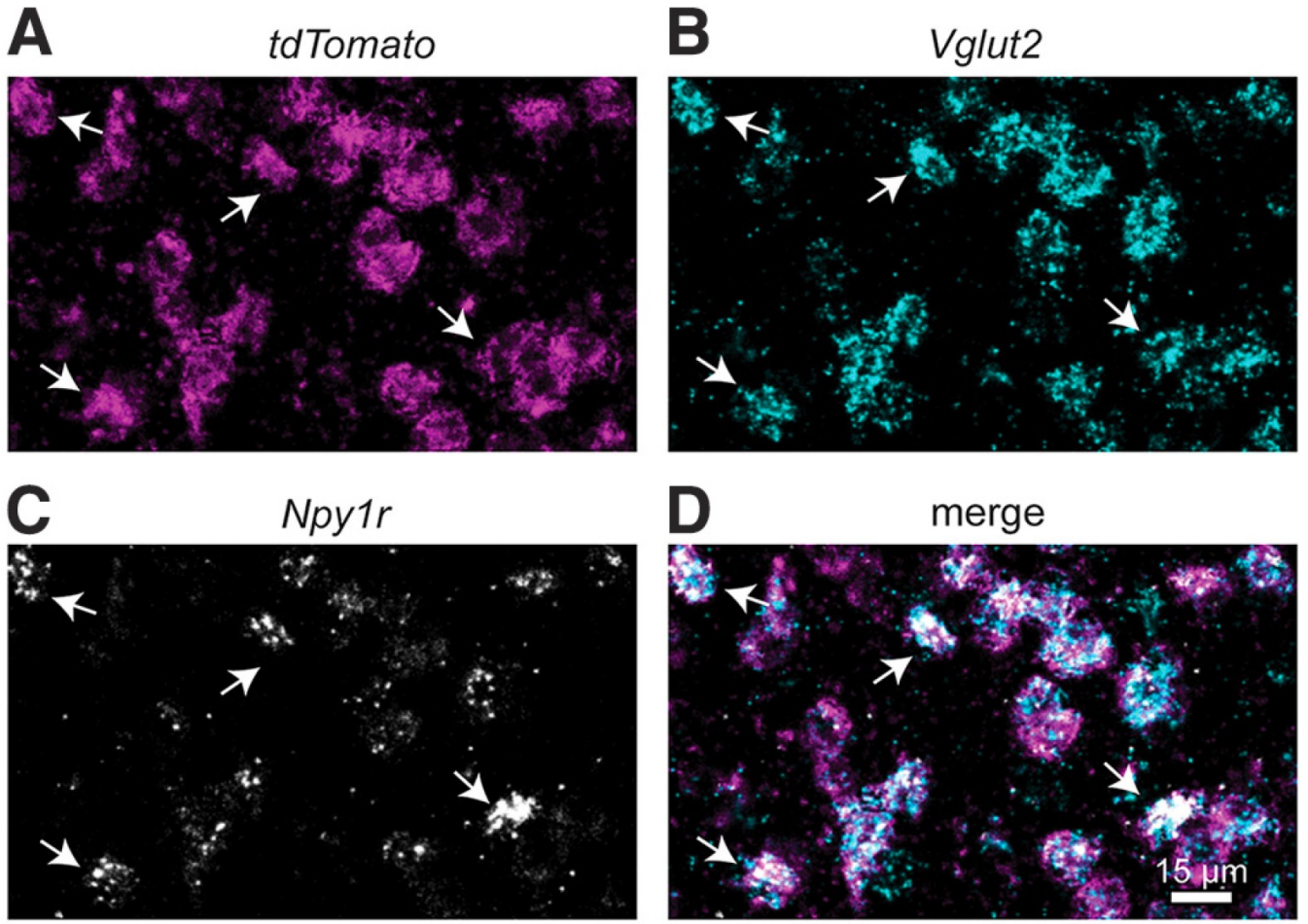
- Identification of VIP and NPY neurons
- **Recurrent and feedforward circuits in the IC**
- New approaches to mapping local IC circuits


Marina Silveira

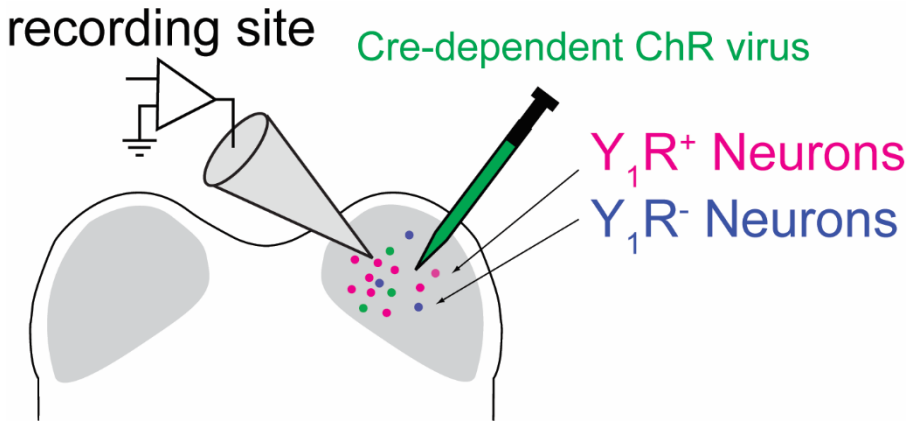

Yoani Herrera

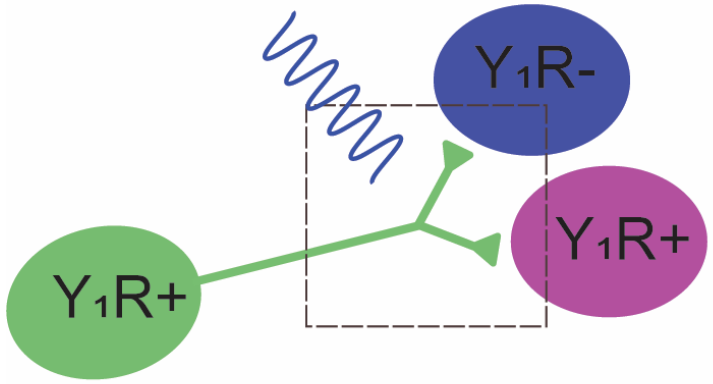

David Goyer

The NPY Y_1 receptor is expressed by ~80% of glutamatergic IC neurons

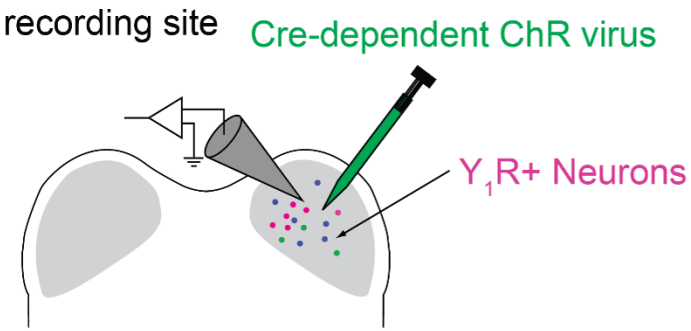


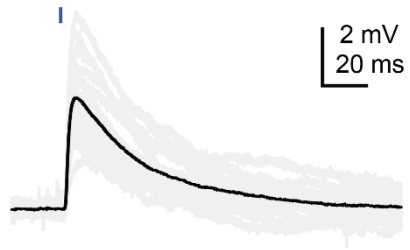
How do glutamatergic IC neurons shape excitation in the IC?

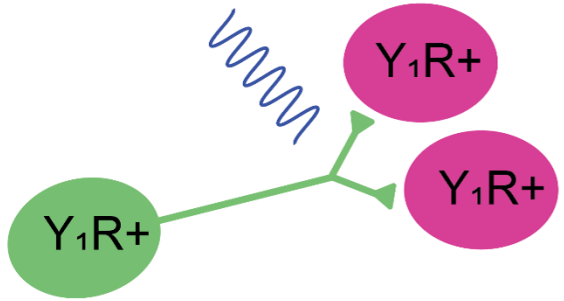


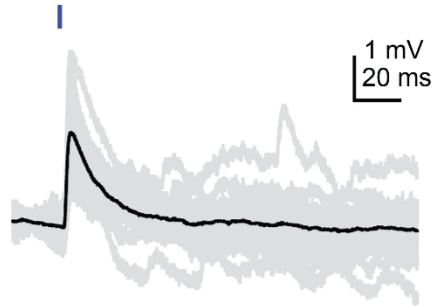


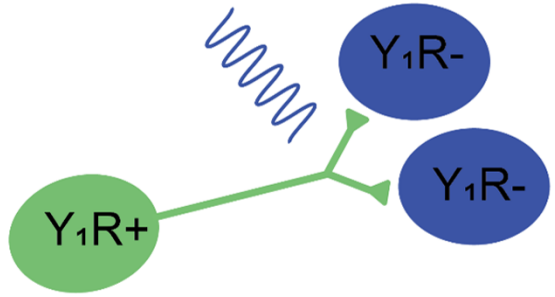
Y_1R^+ neurons synapse onto Y_1R^+ neurons and Y_1R^- neurons



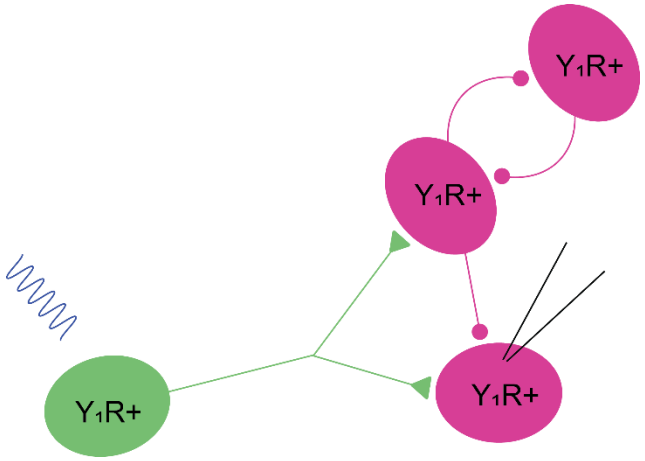




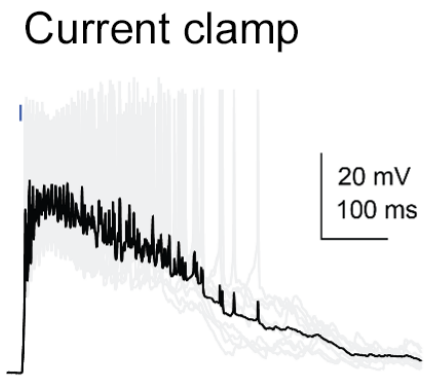


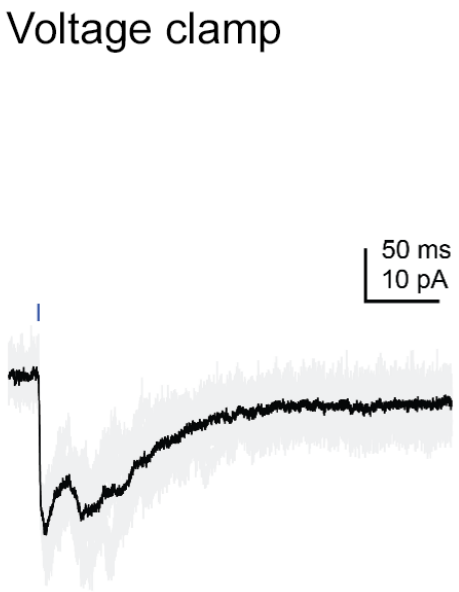


Activating Y_1R^+ neurons can elicit recurrent excitation

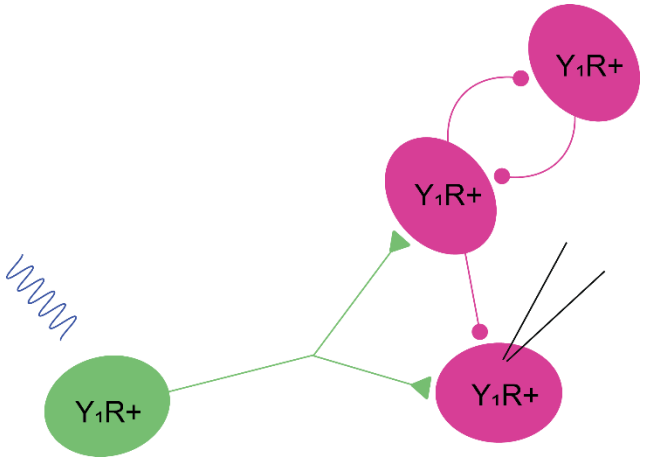


+ 5 µM gabazine and 1 µM strychnine

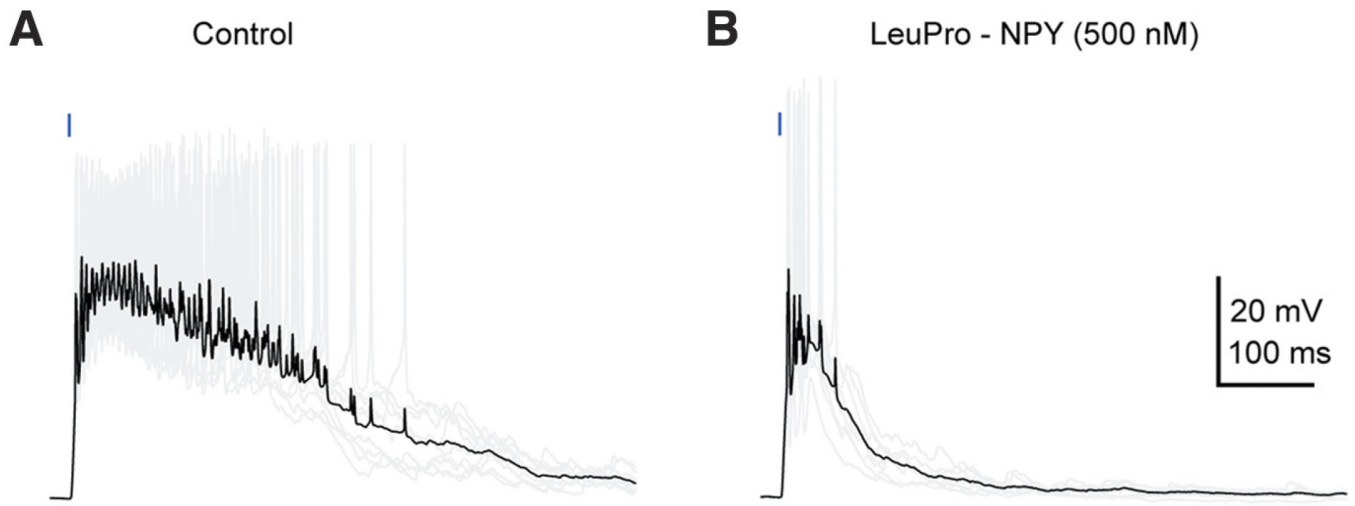




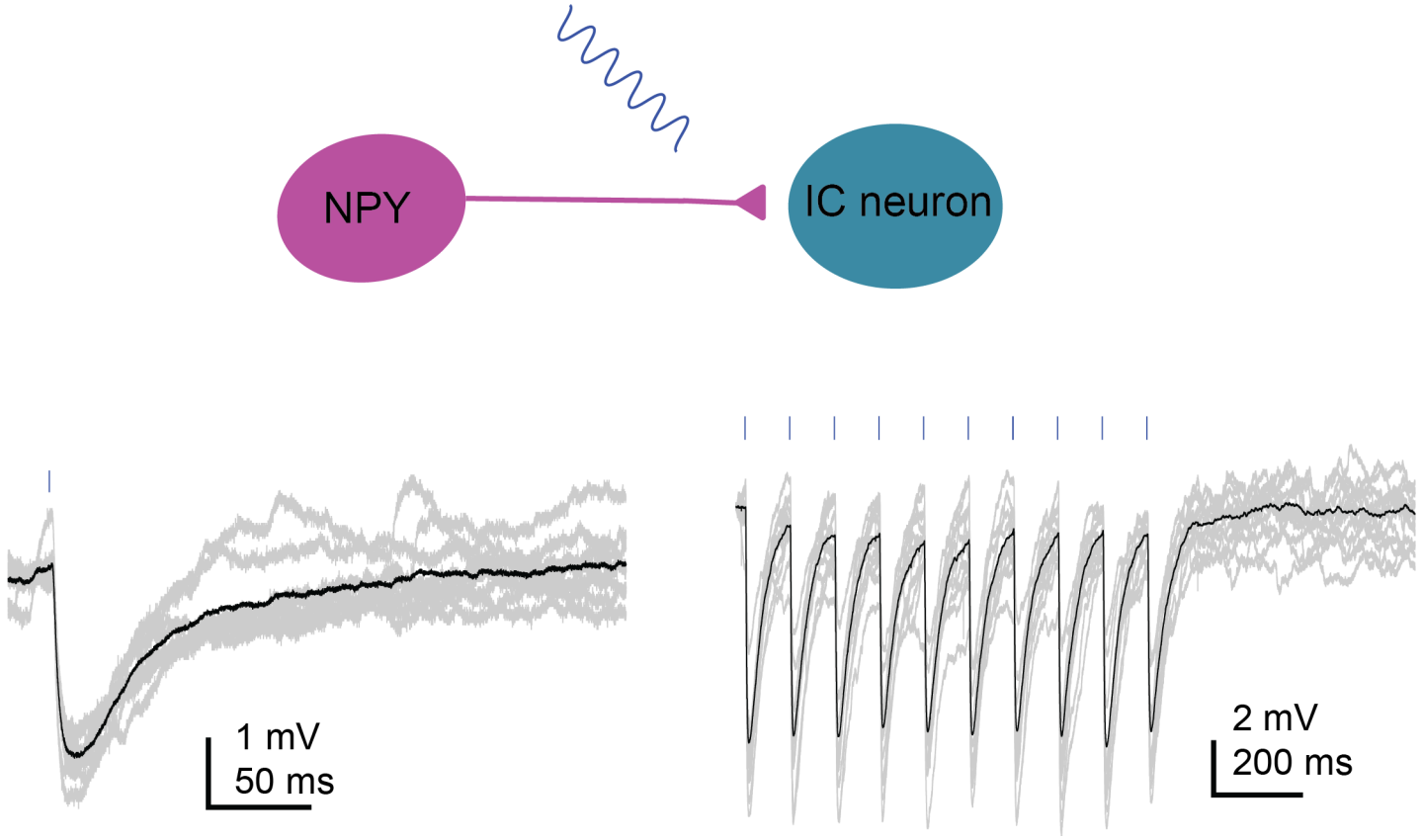
Applying a Y_1R agonist inhibits recurrent excitation



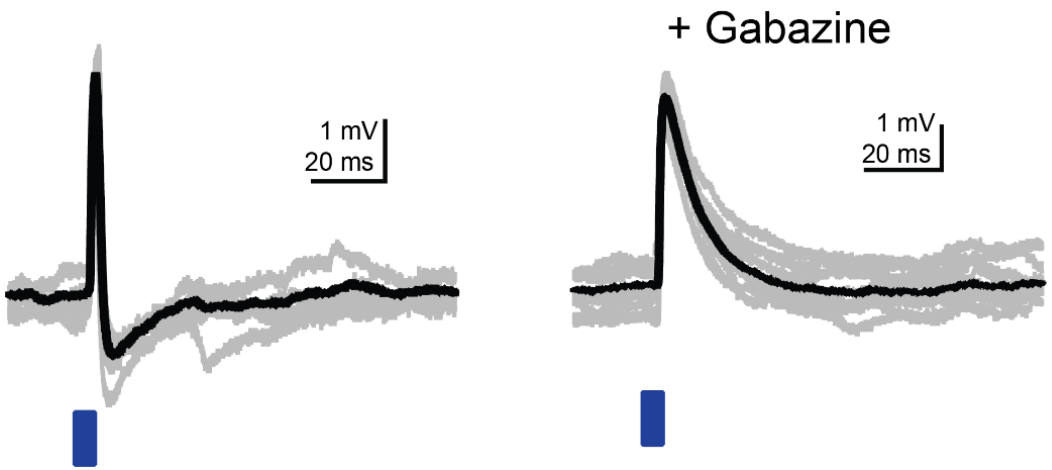
+ 5 µM gabazine and 1 µM strychnine

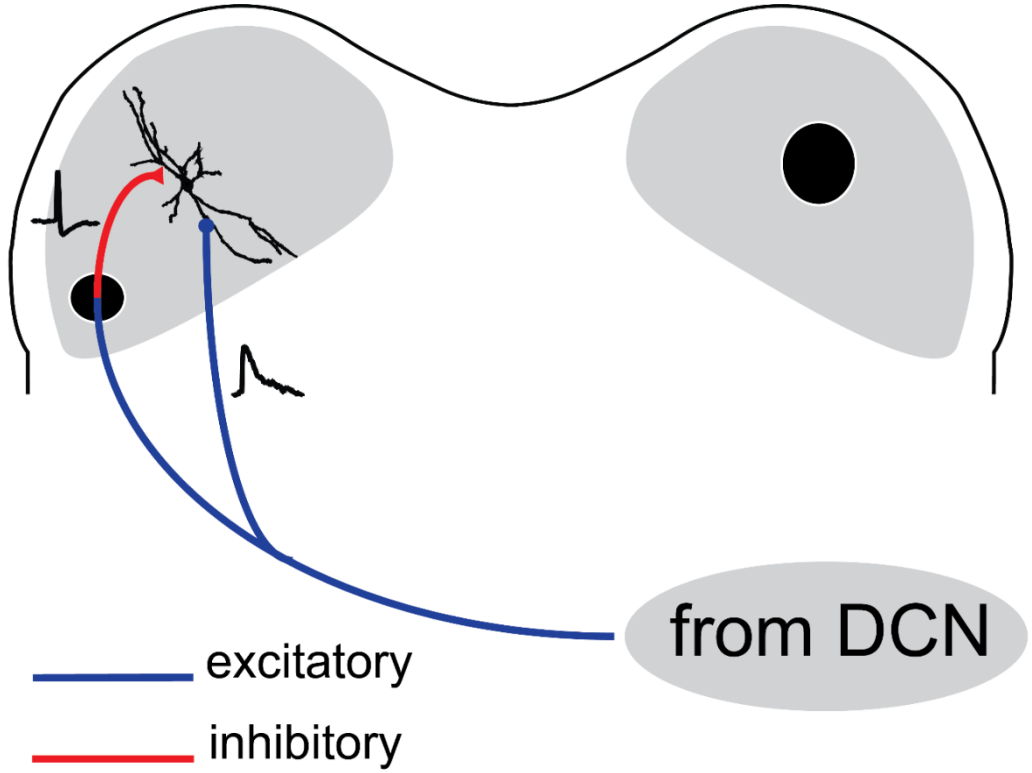


NPY neurons provide inhibitory input to other IC neurons

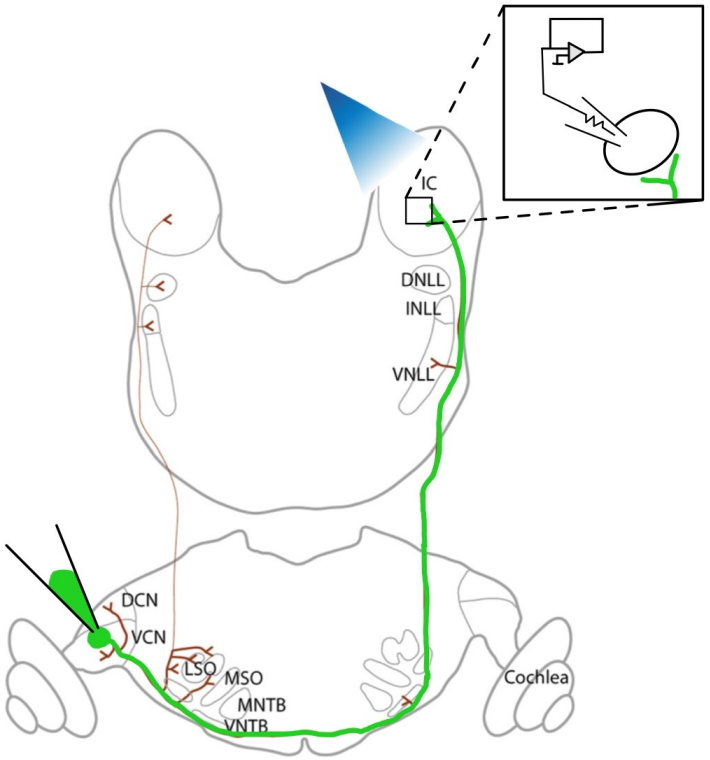


DCN input can elicit feedforward inhibition onto VIP neurons

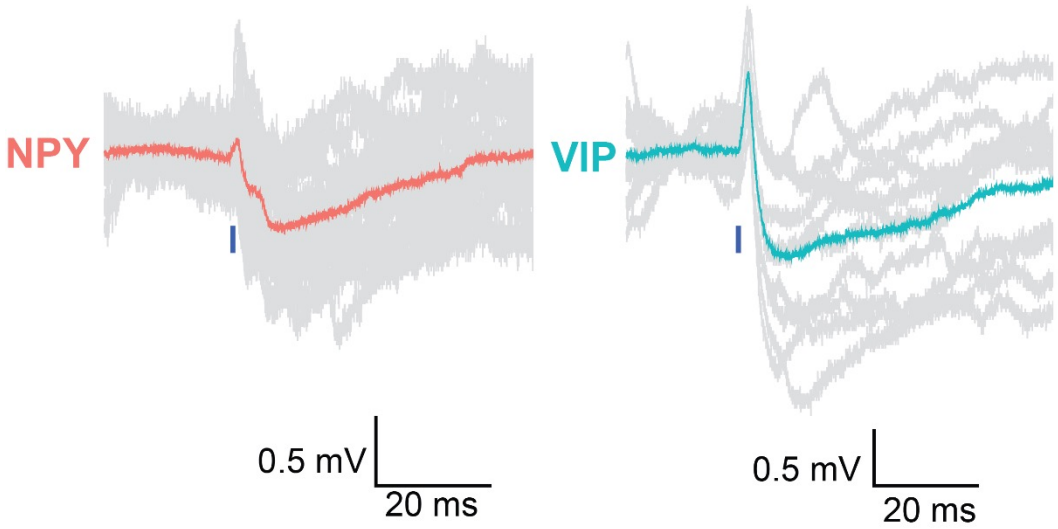


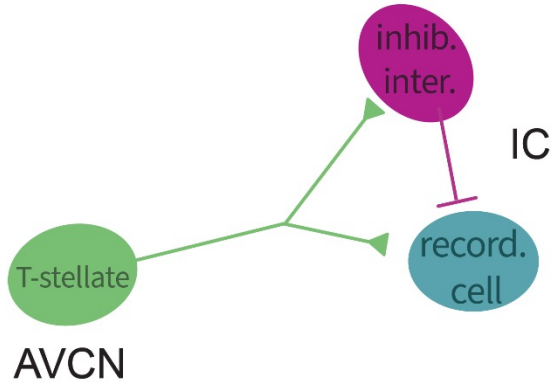


T-stellate input can elicit feedforward inhibition

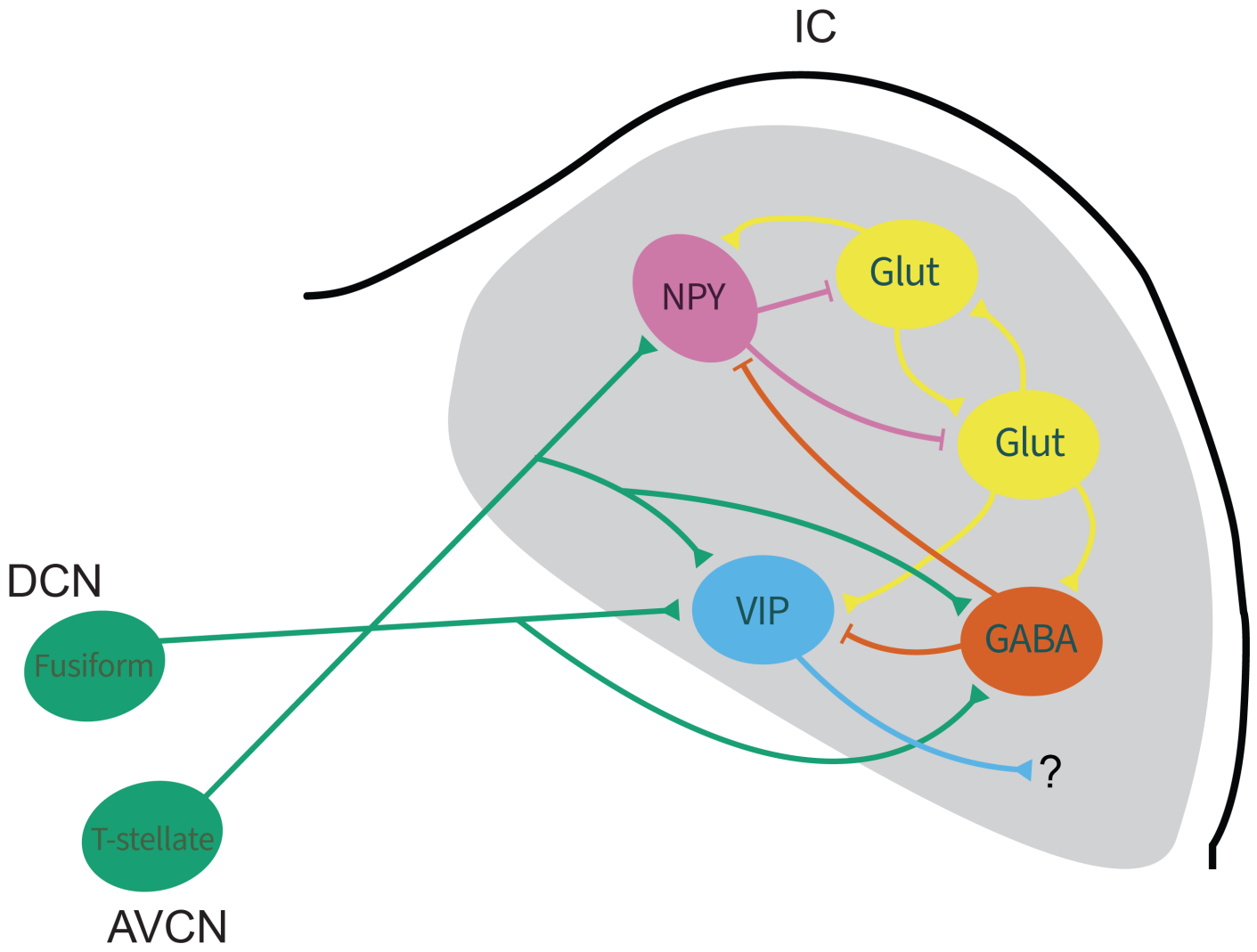


Modified from Oertel et al., 2011

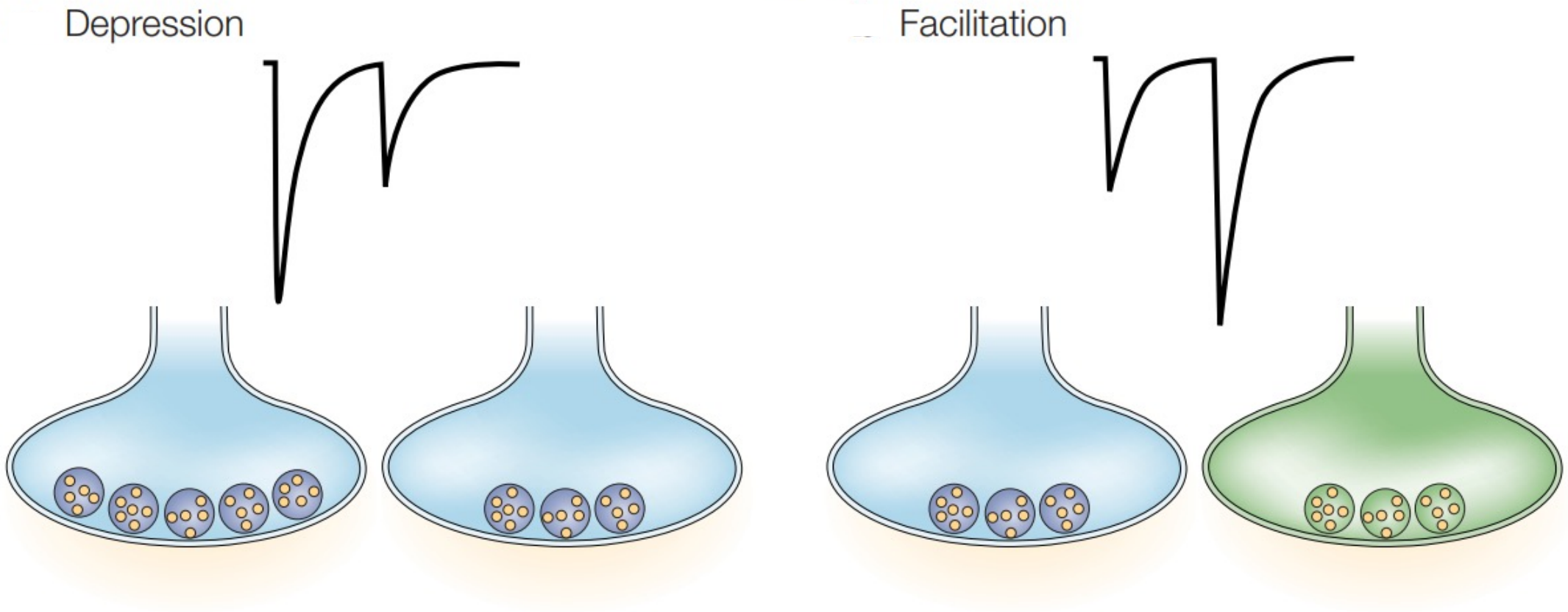




Beginnings of an IC circuit map

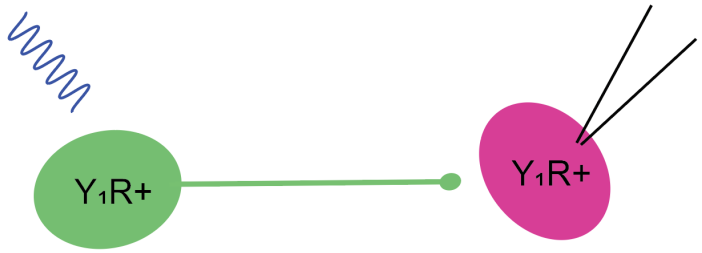


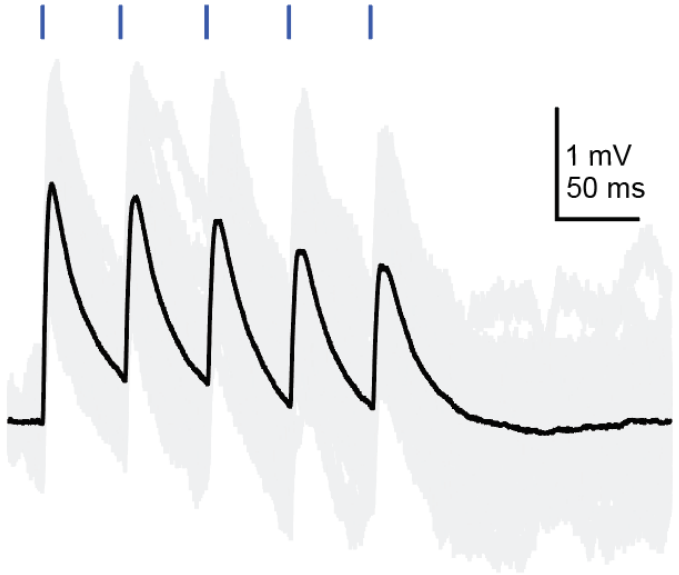
Short-term synaptic plasticity

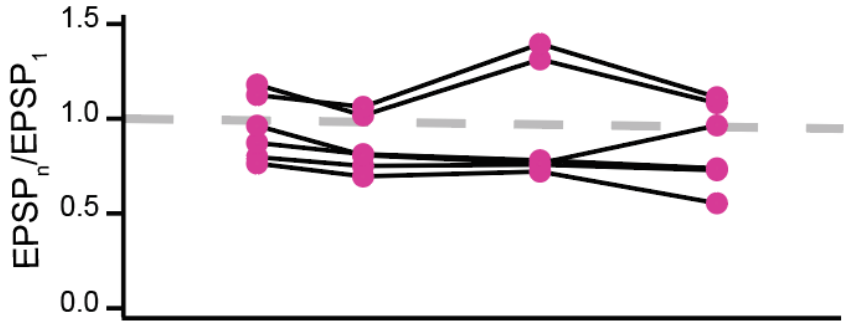


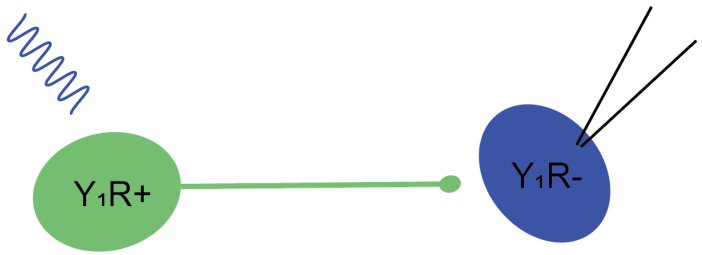
Blitz et al., 2004

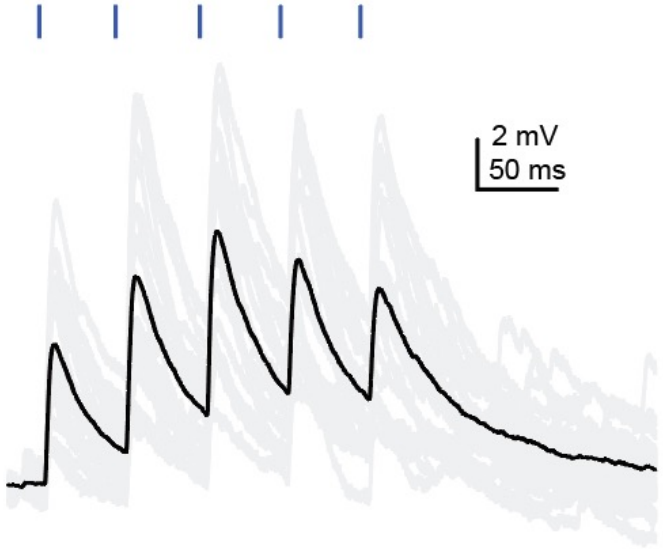
Y_1R synapses have modest short-term plasticity

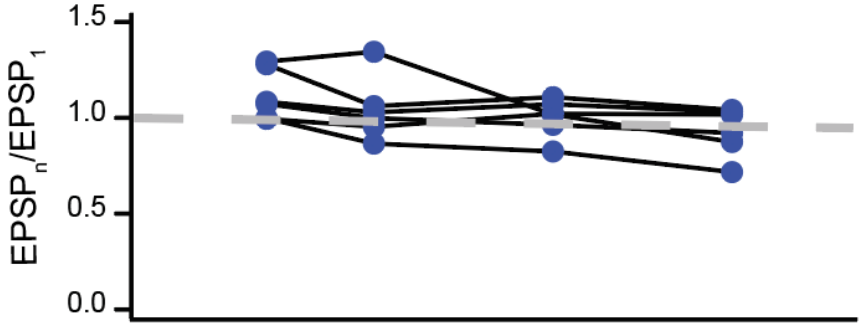




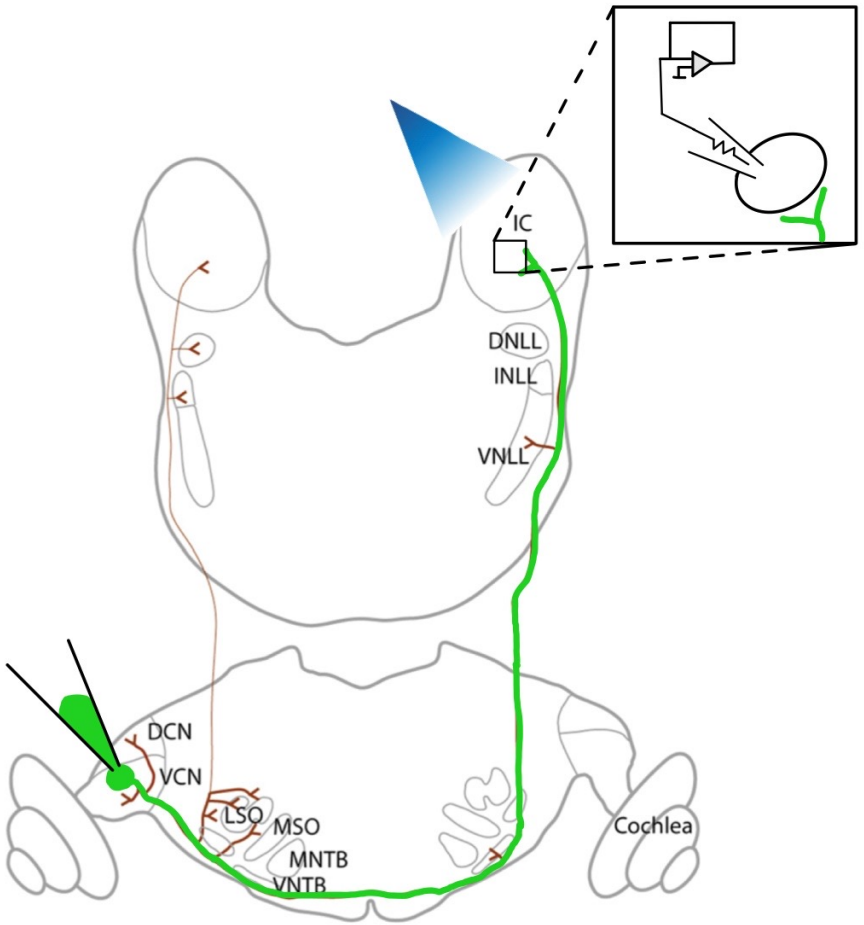






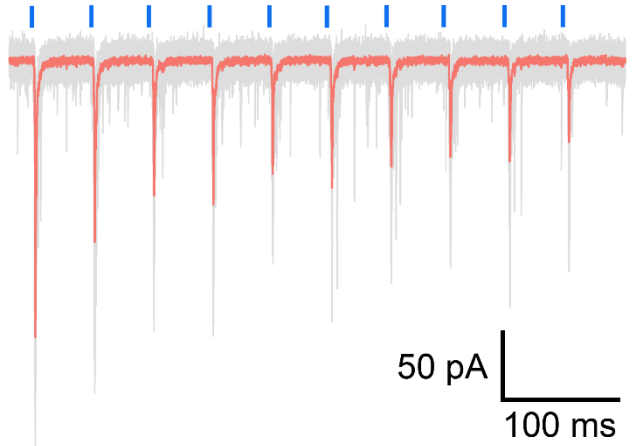


Ascending synapses from the VCN exhibit short-term depression

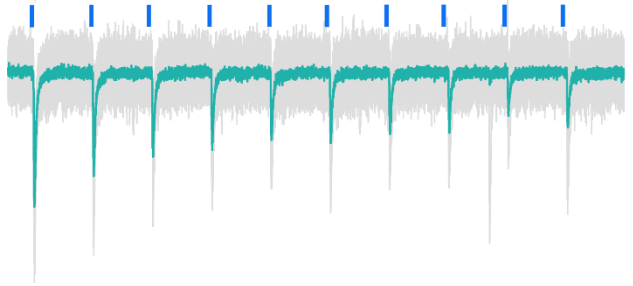


Modified from Oertel et al., 2011

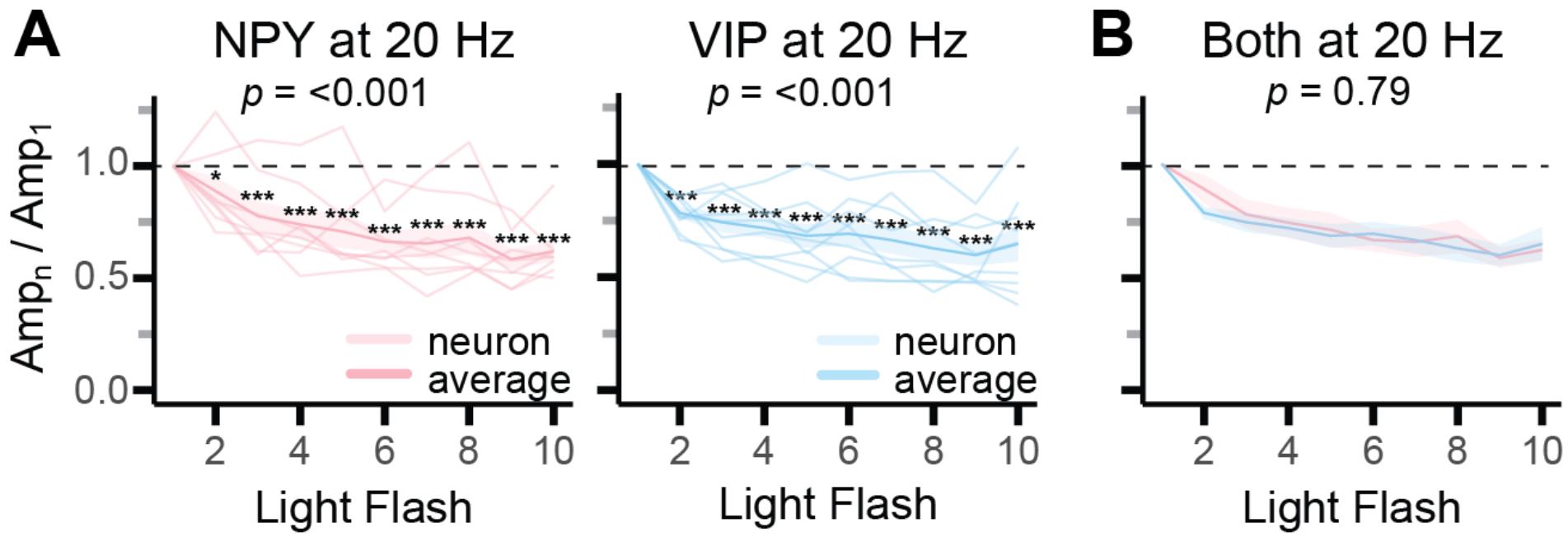
T-stellate to NPY



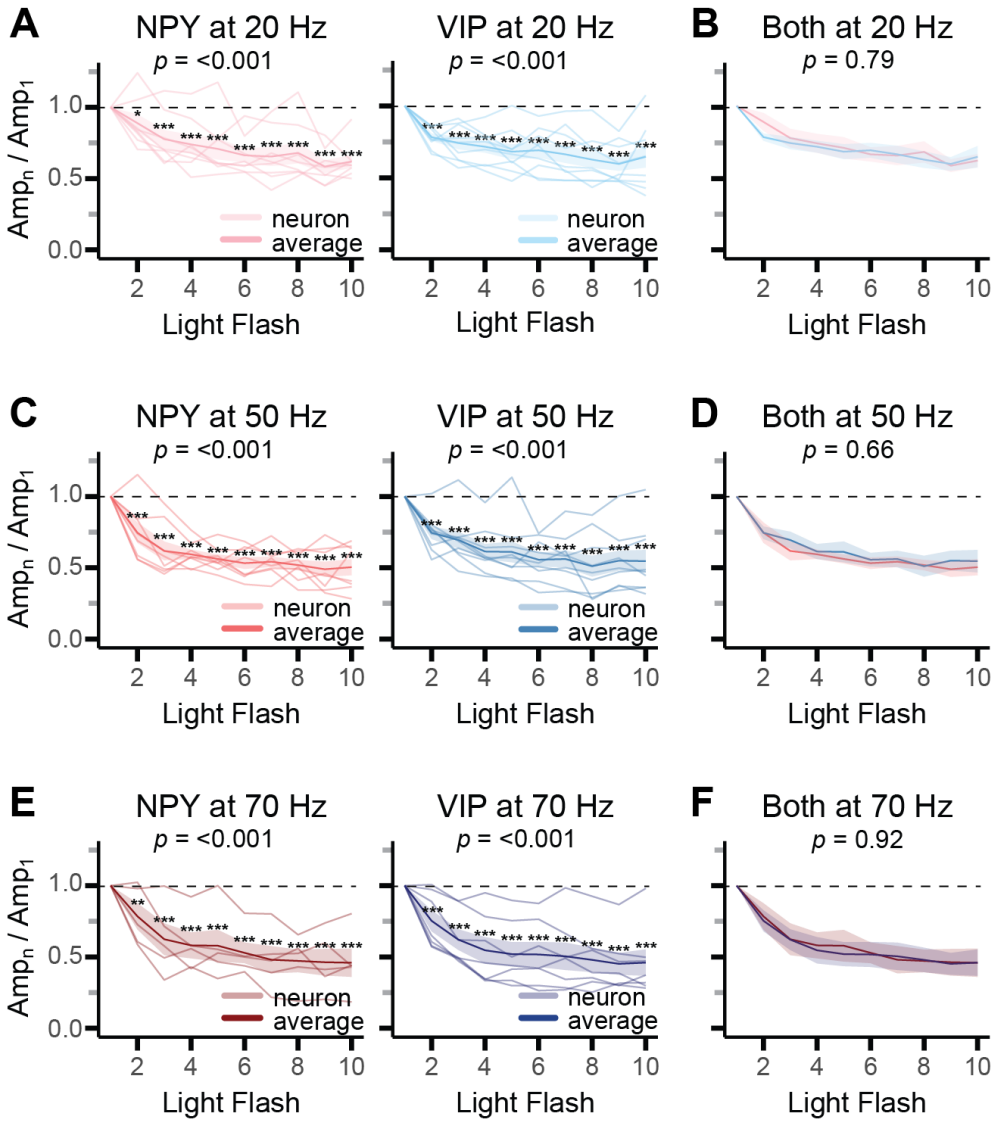
T-stellate to VIP



Ascending synapses from the VCN exhibit short-term depression

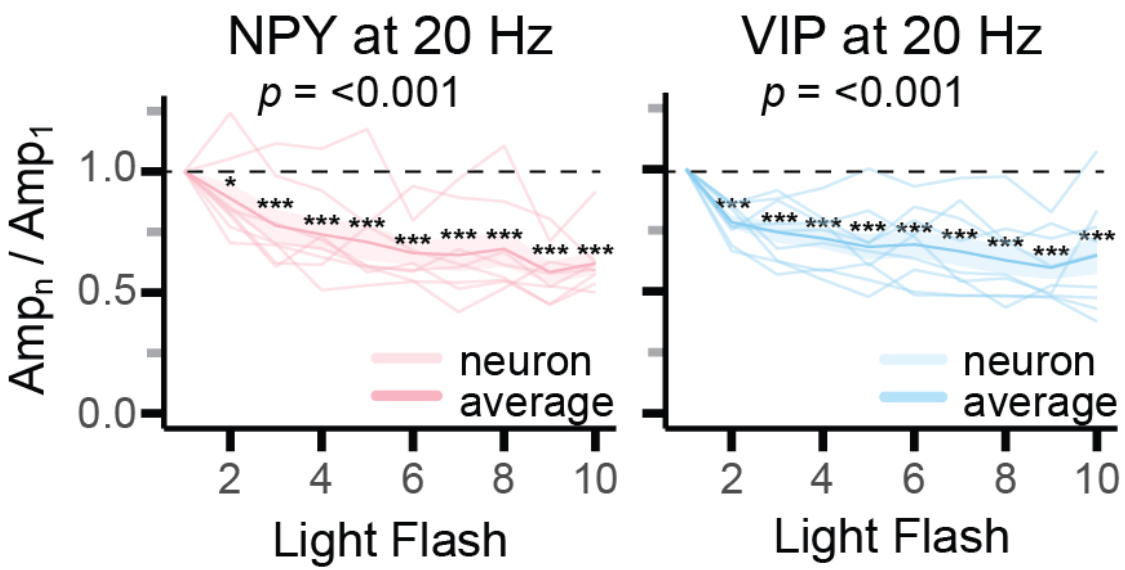


Ascending synapses from the VCN exhibit short-term depression

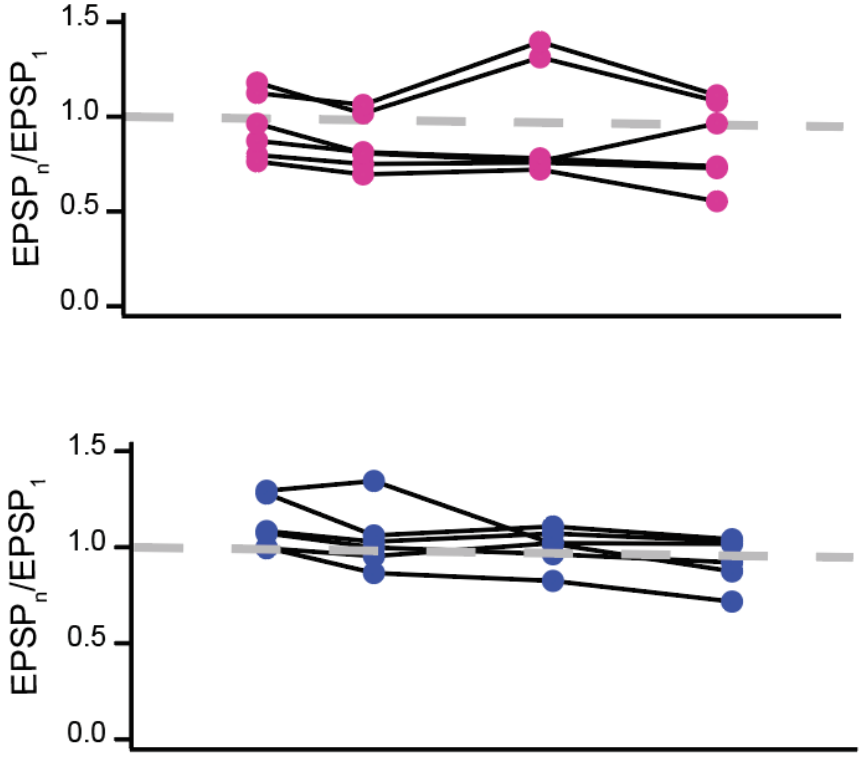


Ascending synapses from the VCN exhibit short-term depression

T-stellate synapses onto NPY and VIP neurons



Y_1R synapses onto local IC neurons



Outline

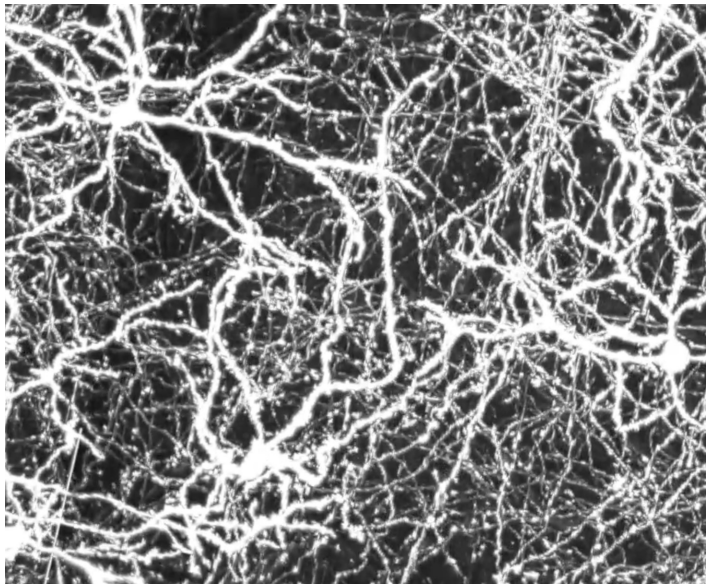
- Identification of VIP and NPY neurons
- Recurrent and feedforward circuits in the IC
- **New approaches to mapping local IC circuits**

Elie Huez

Anatomical mapping of local IC circuits

Problem: IC circuits are incredibly dense.

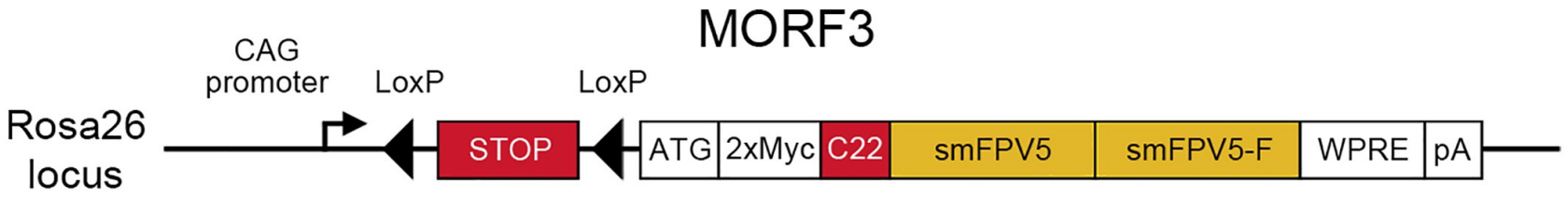
- Local, ascending, and descending axons intermix making it difficult to track the local axonal projections of individual IC neurons.
- Axonal projections have complex, 3-D shapes.



Anatomical mapping of local IC circuits

Solution: Sparse neuron labeling and imaging of axons in the intact IC.

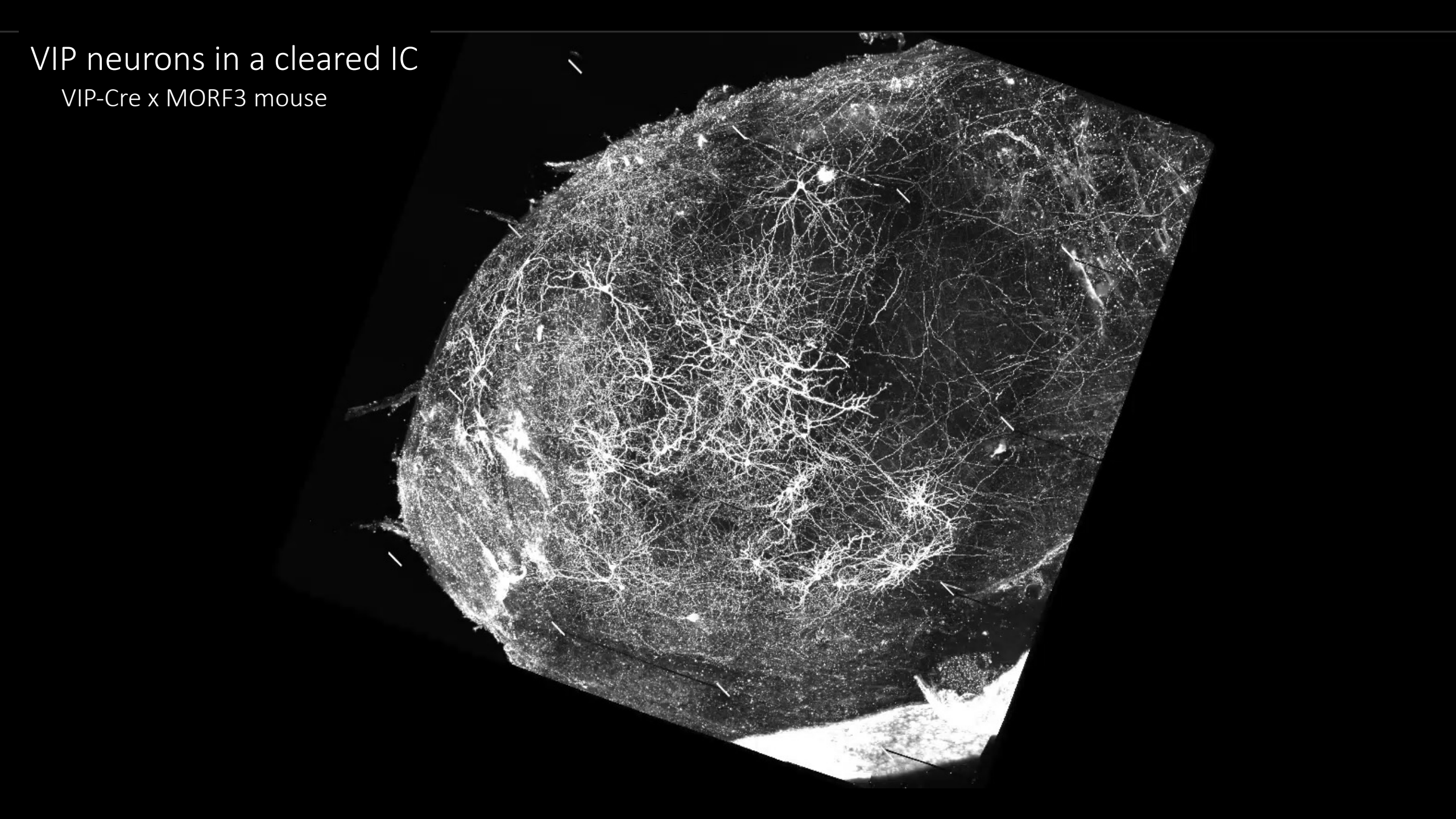
- Sparse labeling using MORF3 mice
- Clearing and immunolabeling of intact brains
- Light-sheet microscopy



Veldman et al., 2020

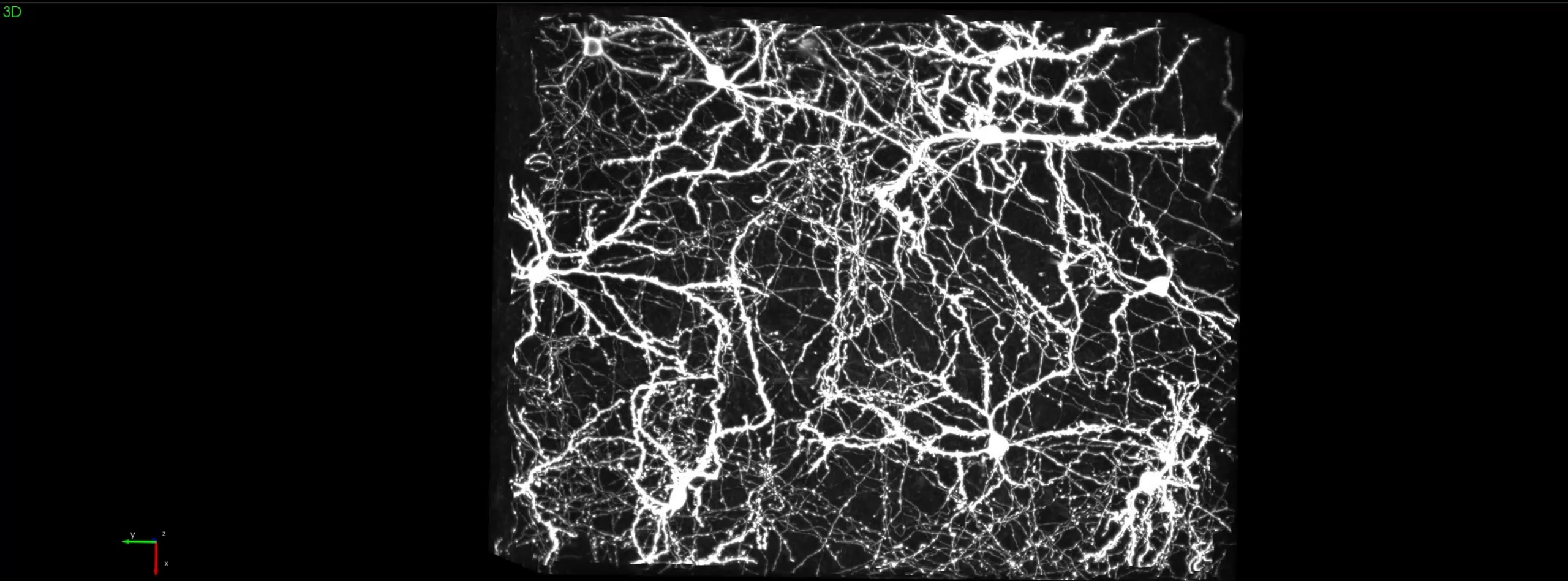
VIP neurons in a cleared IC

VIP-Cre x MORF3 mouse



VIP neurons in a cleared IC

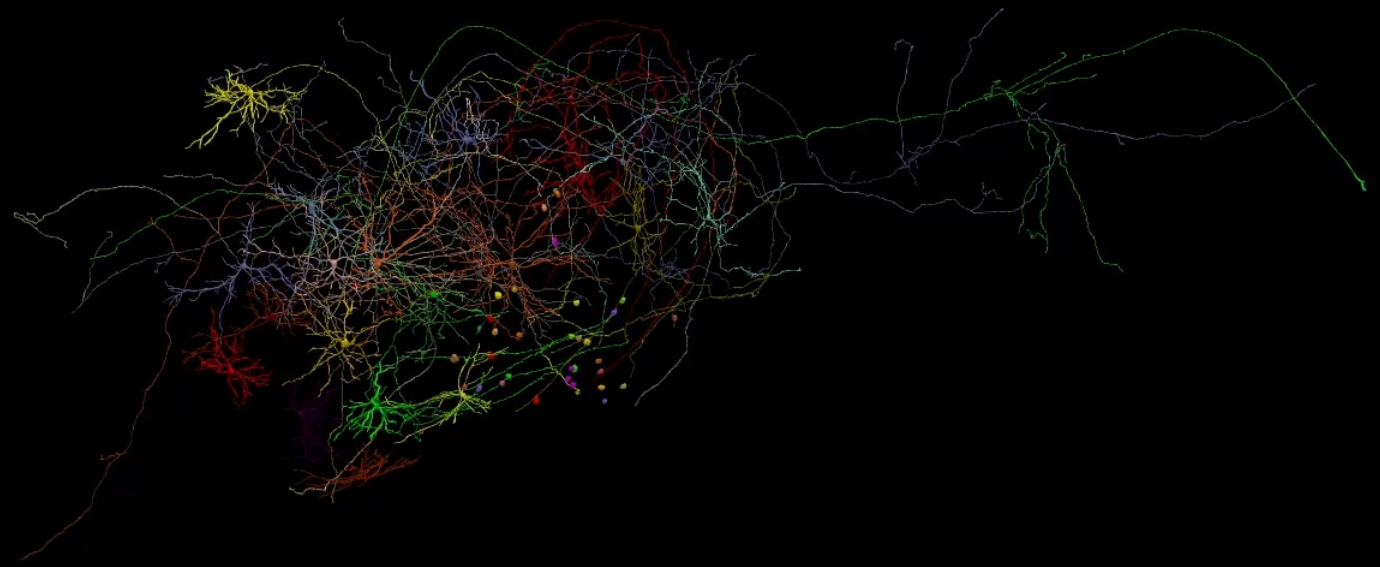
VIP-Cre x MORF3 mouse

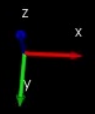


Reconstructions of VIP neurons

VIP-Cre x MORF3 mouse

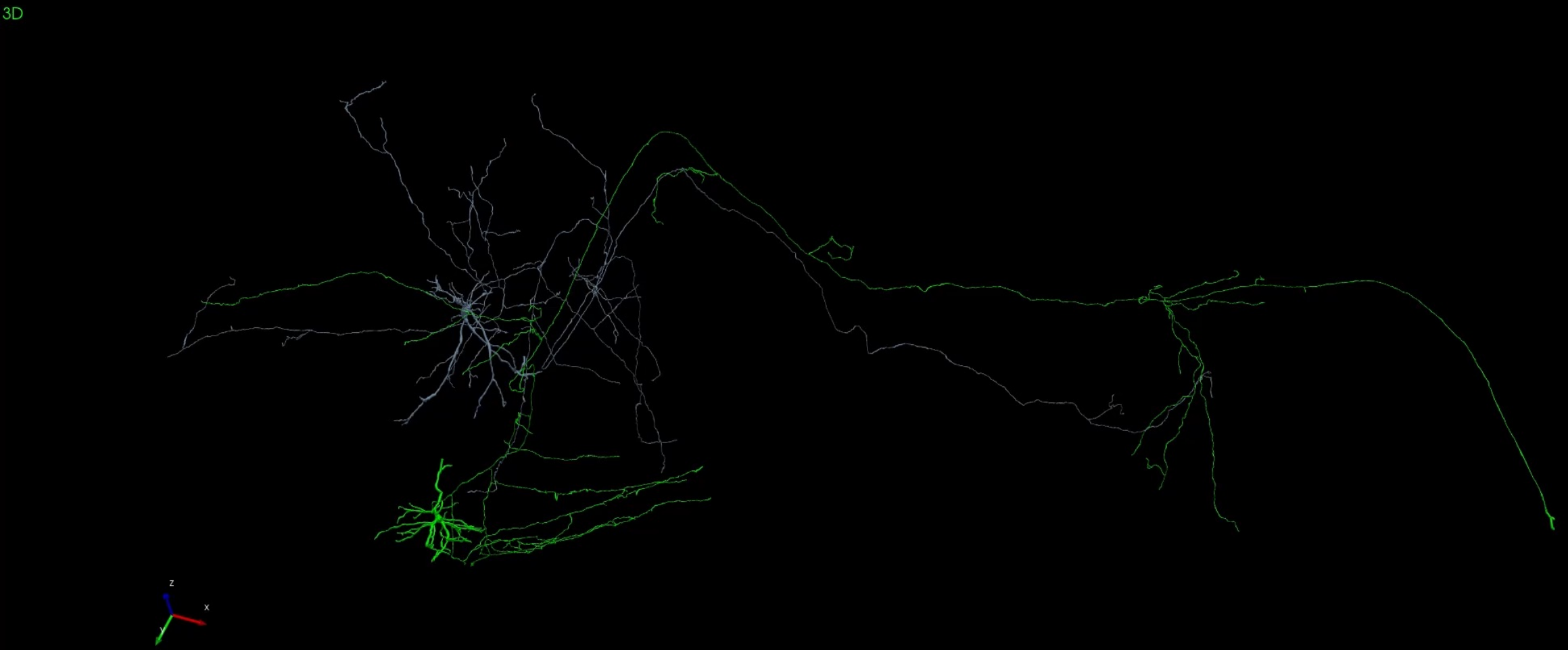
3D





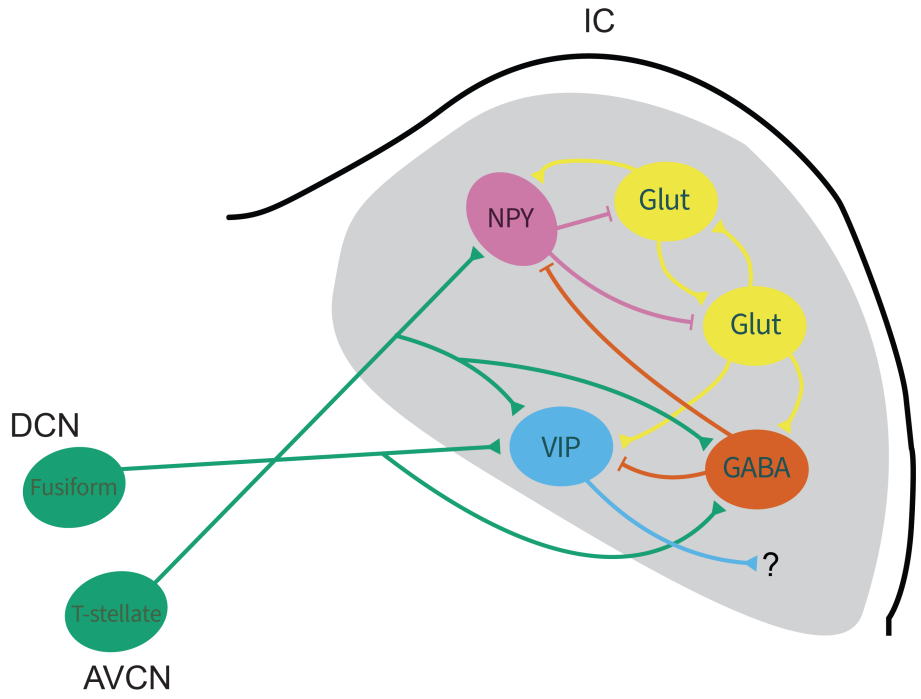
Example VIP neuron with axonal arbor

Example VIP neurons with commissural axons



Conclusions

- VIP and NPY neurons are molecularly identifiable classes of IC stellate cells.
- The IC is rich with recurrent excitatory circuits, and these can be modulated by NPY signaling.
- Ascending input from VCN and DCN elicits feedforward inhibition in the IC.
- Ascending inputs from T-stellate cells undergo short term synaptic depression.
- Sparse labeling and light-sheet microscopy provide a powerful new way to map the local axonal projections of molecularly defined IC neuron types.



Acknowledgements

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- *Audrey Drotos**
- *Justin Anair**
- *Alex George**
- *Julia Kwapiszewski*

Italics = former lab member
* = contributed to work shown today

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Harvard Medical School

- Lisa Goodrich
- Gabriel Romero

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