Basic Mouse Neuroanatomy and the Allen Connectivity Atlas

August 25th, 2014

Julie Harris

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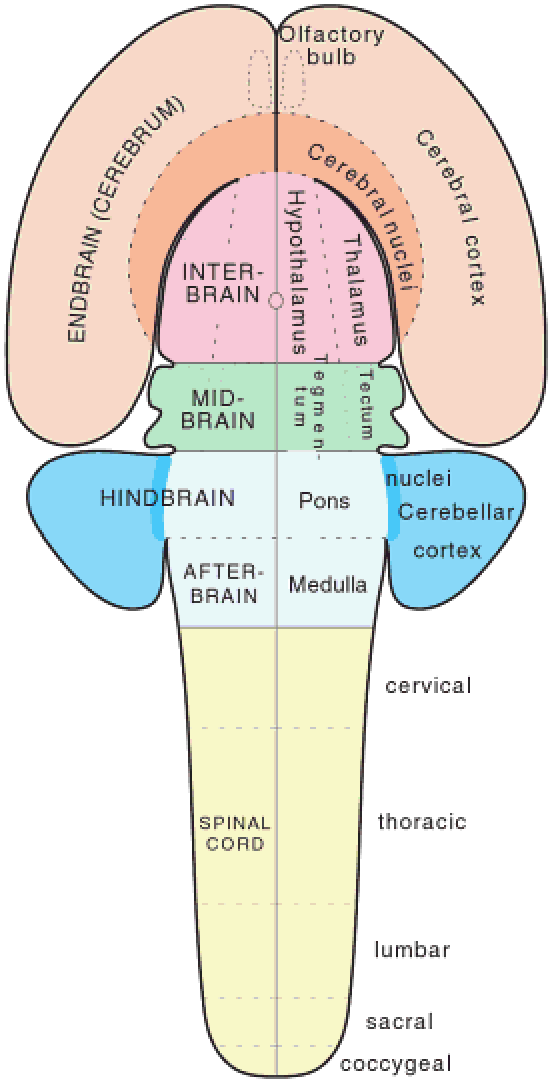
Three Major Divisions of the Mammalian Brain

- Forebrain:
  - Isocortex
  - Cerebral nuclei
  - Olfactory bulb
  - Hippocampus
  - Interbrain (hypothalamus and thalamus)

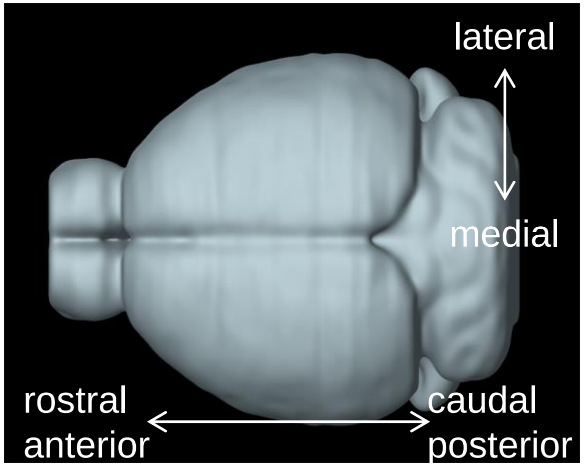
- Midbrain:
  - Dorsal tectum (sensory functions)
  - Ventral tegmentum (motor functions)

- Hindbrain:
  - Pons
  - Medulla (sensory and motor nuclei)
  - Cerebellum

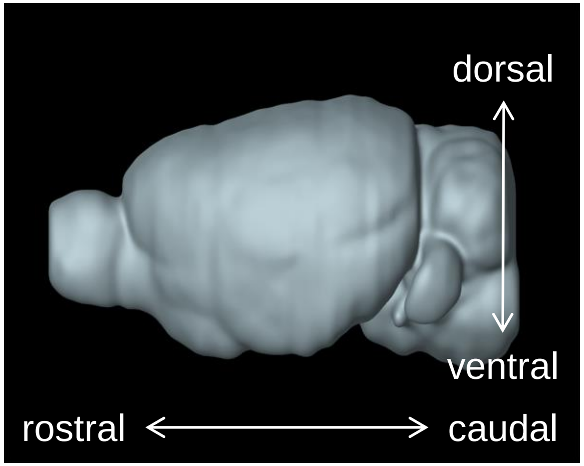


Swanson, 2008, 2012

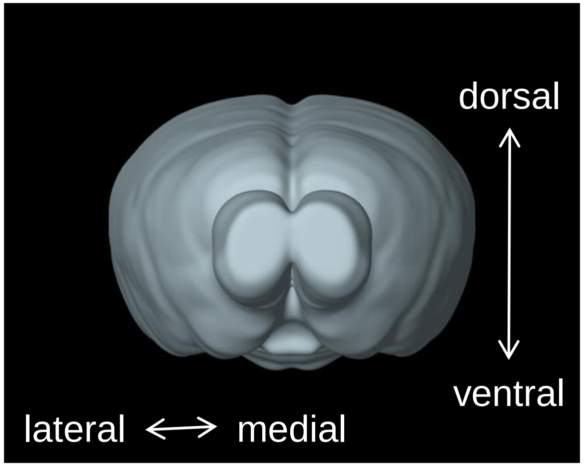
Nomenclature – Axes of Reference



Horizontal



Sagittal



Coronal

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Mouse Anatomical Atlases

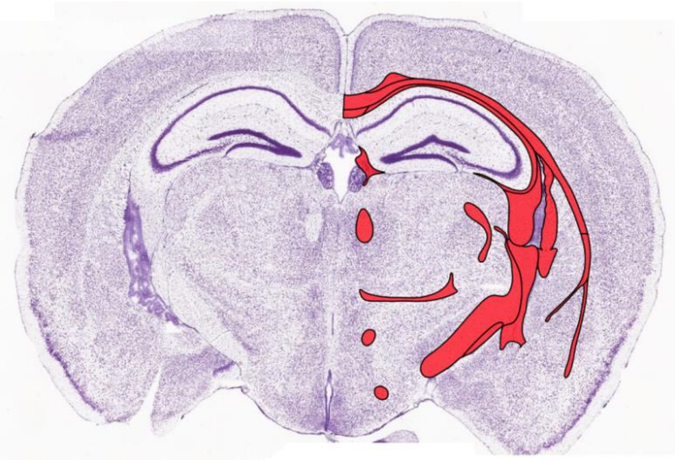
Spatial characteristics of the brain can be defined / described using an atlas.

What are the parts of the brain, and where are they (in relation to other features)?

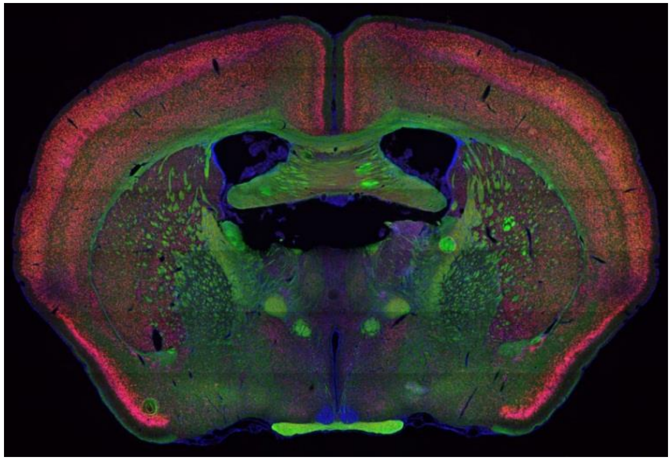
Diverse types of data used to draw anatomical atlases

- Cytoarchitectonics (e.g. nissl)
- Chemoarchitectonics (e.g. antibody staining or classic stains for AchE)
- Axon degeneration and tracing studies
- Gene expression (ISH)
- Functional studies (electrophysiological mapping)

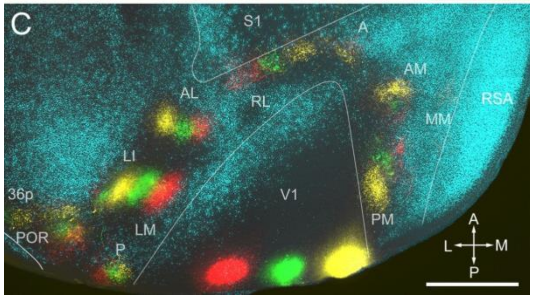
Nissl



NeuN / NF-160



Anterograde tracing



Wang & Burkhalter, 2007

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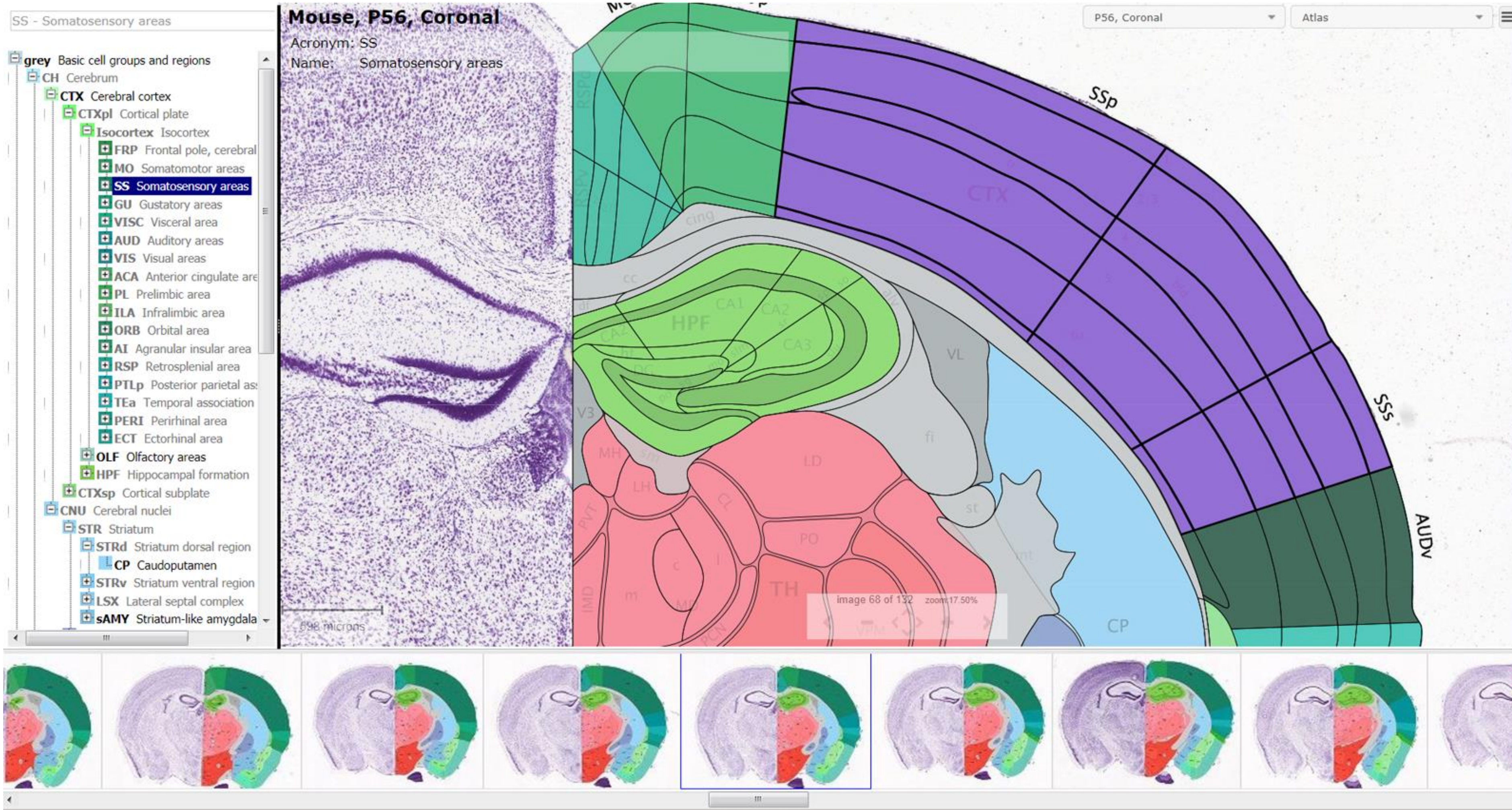
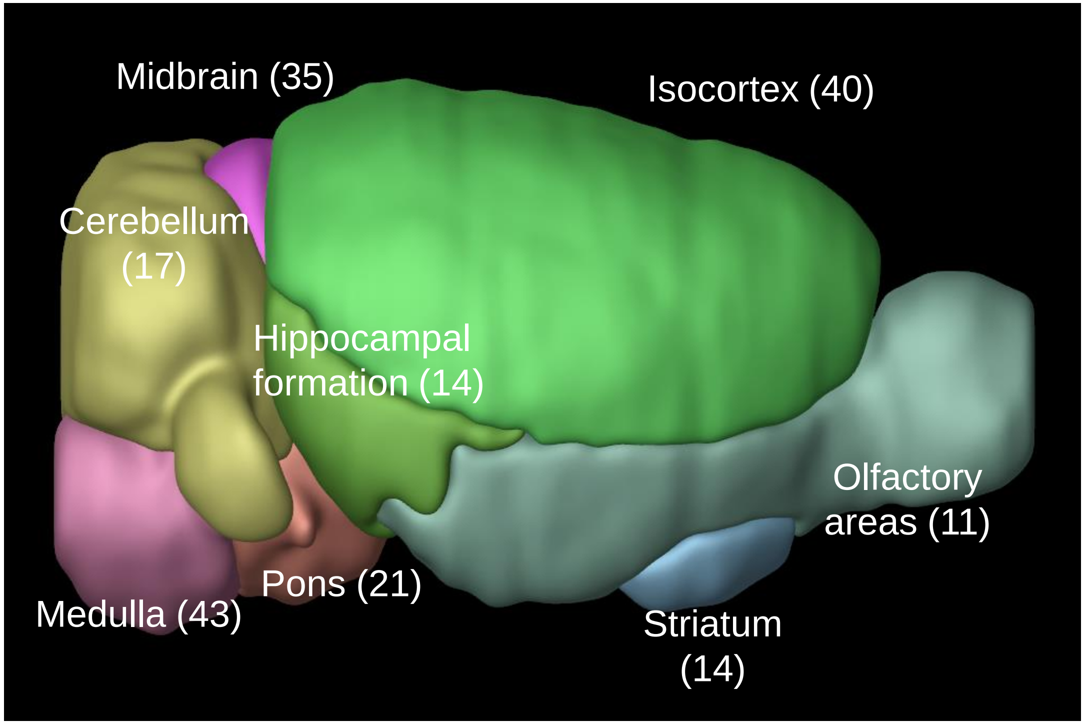
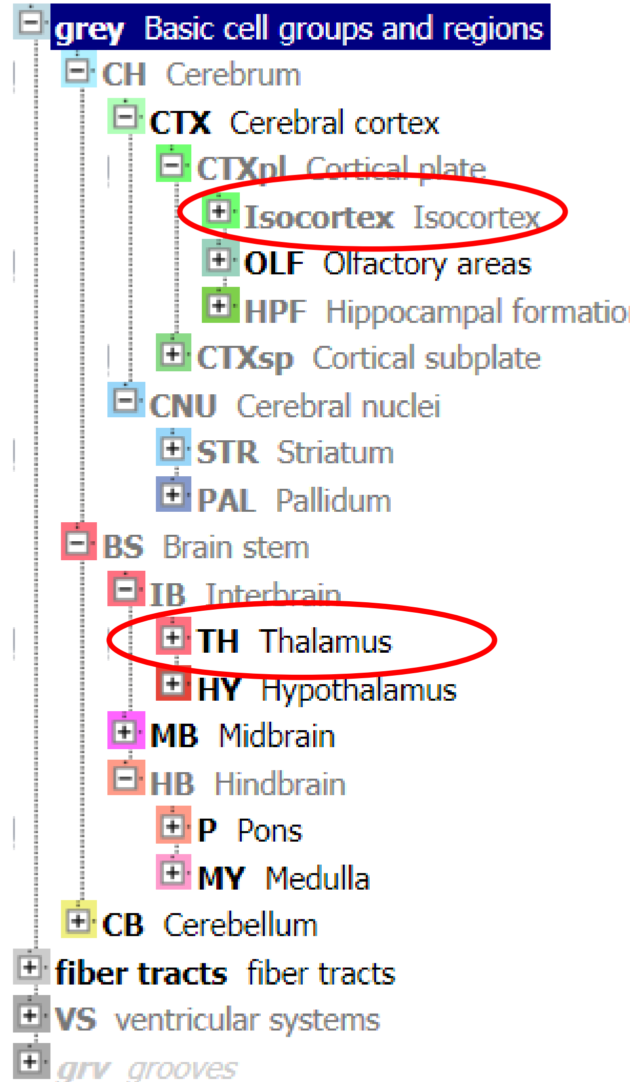
Allen Reference Atlas



Allen Reference Atlas Divisions and Nuclei





Cortical subplate (7)
Pallidum (9)
Thalamus (43)
Hypothalamus (41)

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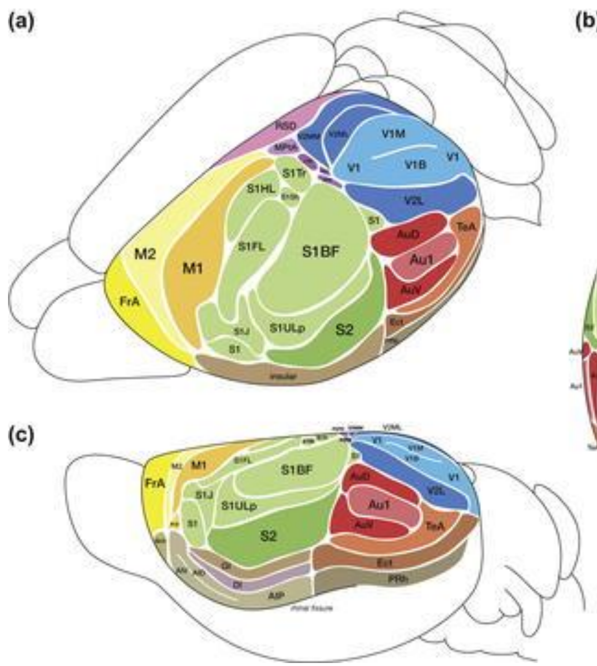
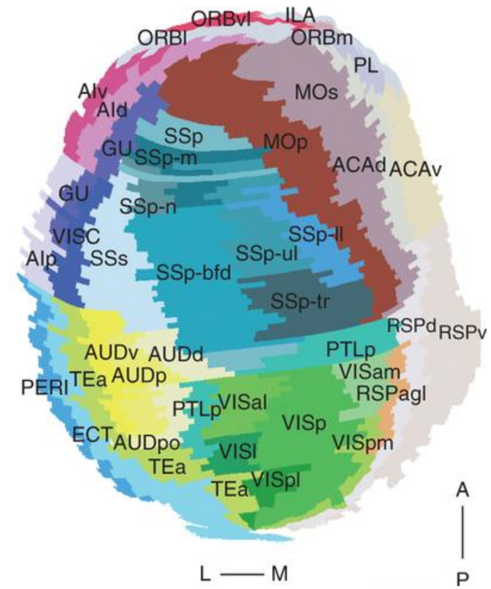
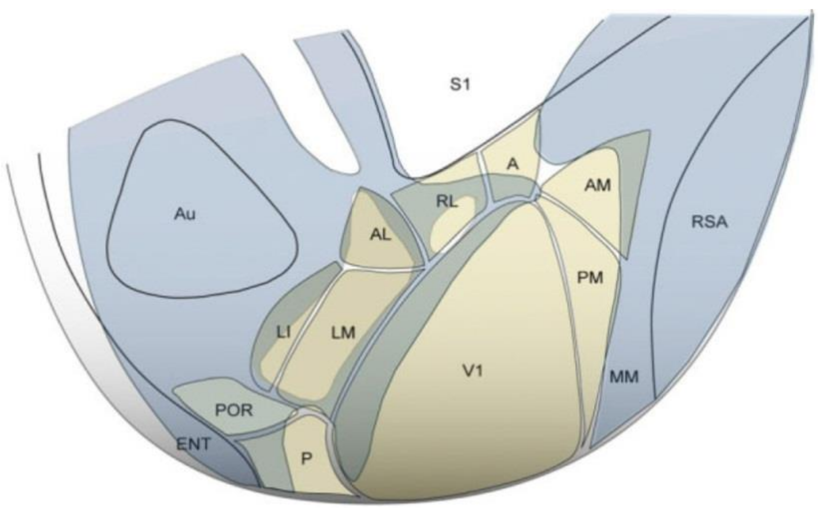
General Cortical Organization

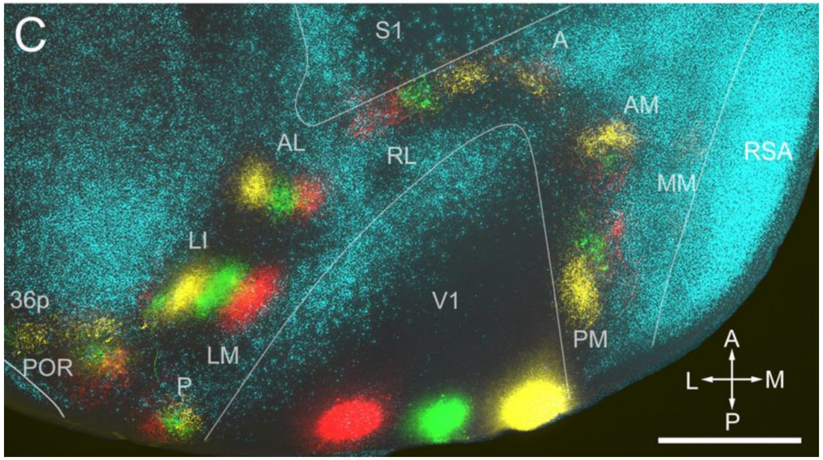
- Regions organized based on function
- Cortical regions can be divided into two types:
  - Primary areas:
    - Receive sensory input directly from thalamus (for most senses).
      - Primary visual, primary auditory, primary somatosensory, primary gustatory
    - Executing motor tasks
      - Primary motor
  - Association Areas:
    - Receive input from primary cortical areas (and other thalamic regions), involved in higher order processing
      - Unimodal (secondary visual, secondary somatosensory)
      - Multimodal (parietal and temporal association areas)

Multiple Cortical Parcellation Schemes



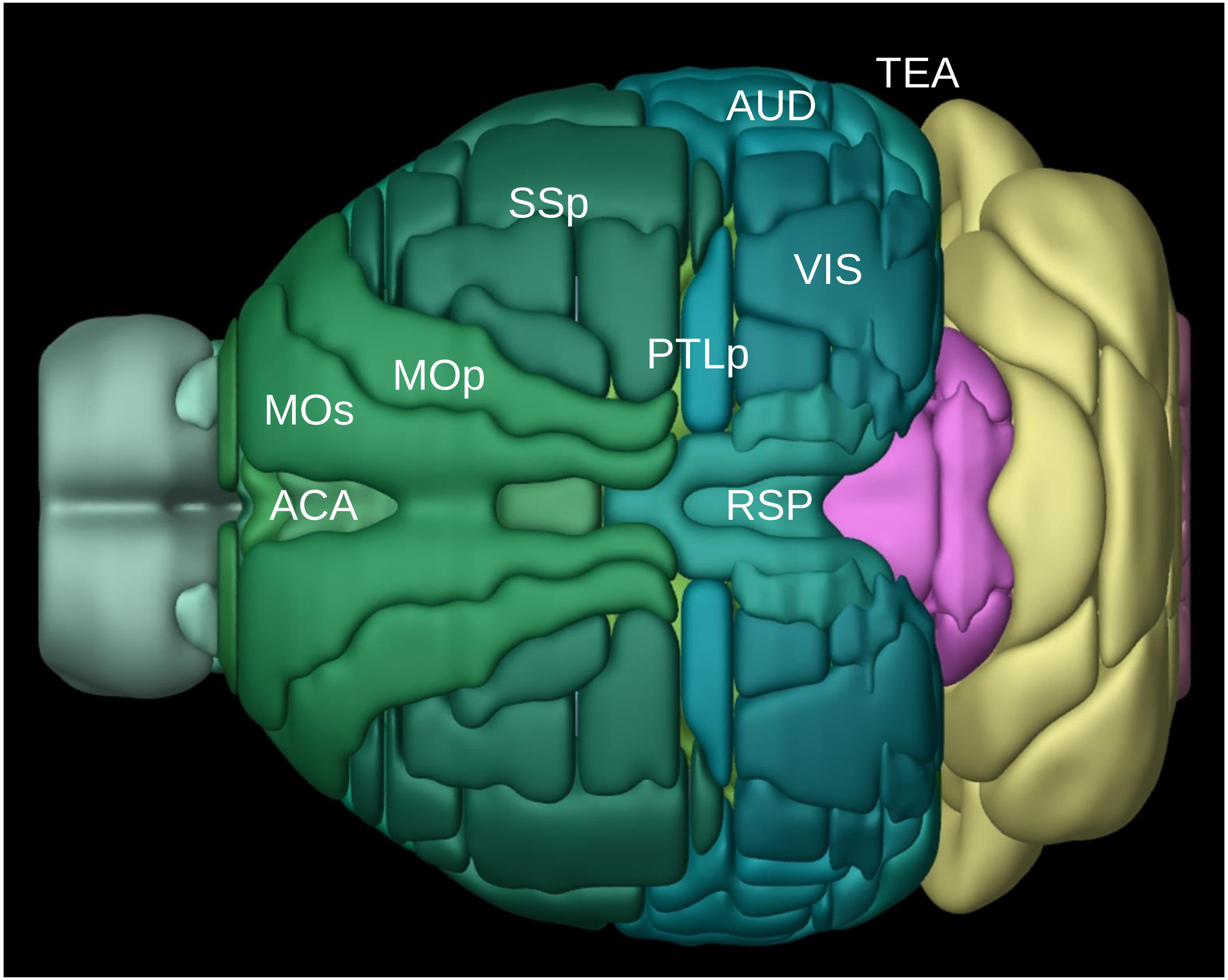


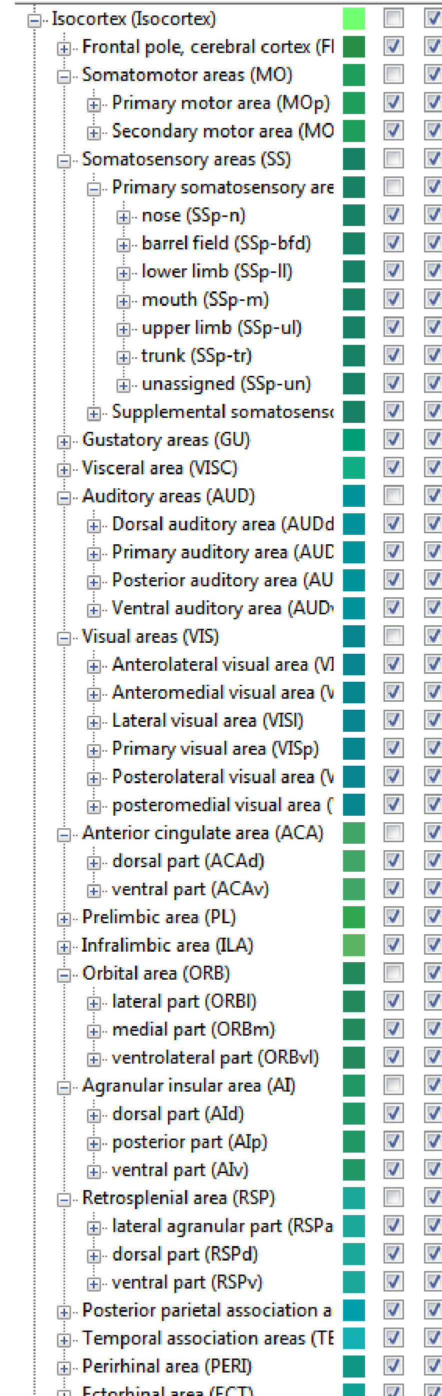




Wang & Burkhalter, 2007

~ 40 Regions in the Mouse Isocortex





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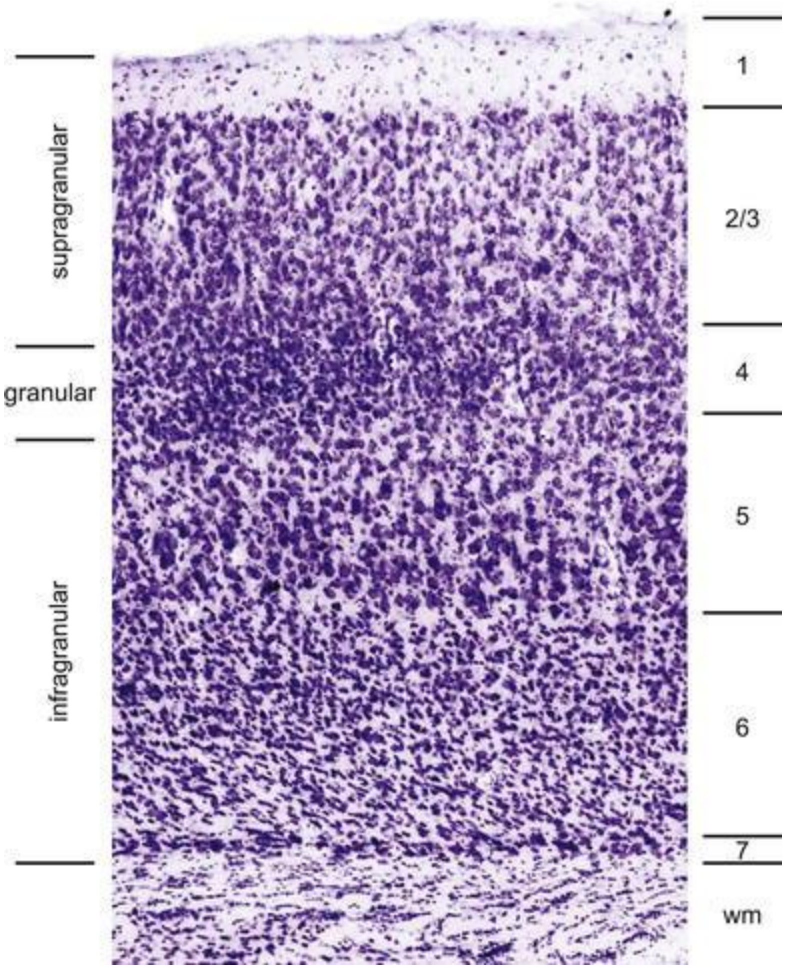
Isocortex – Uniform, *layered* Structure



Few neurons, most inhibitory, largely axons parallel to surface

Mostly pyramidal cells, local and corticocortical connectivity

Small cells, prominent in sensory regions, primary target of thalamic fibers

Largest pyramidal neurons, projections to variety of targets, including subcortical structures. Often subdivided based on these targets (5A = corticostriatal, 5B = corticospinal)

Mostly corticothalamic output, also strong thalamic input. Some cortical and callosal projections.

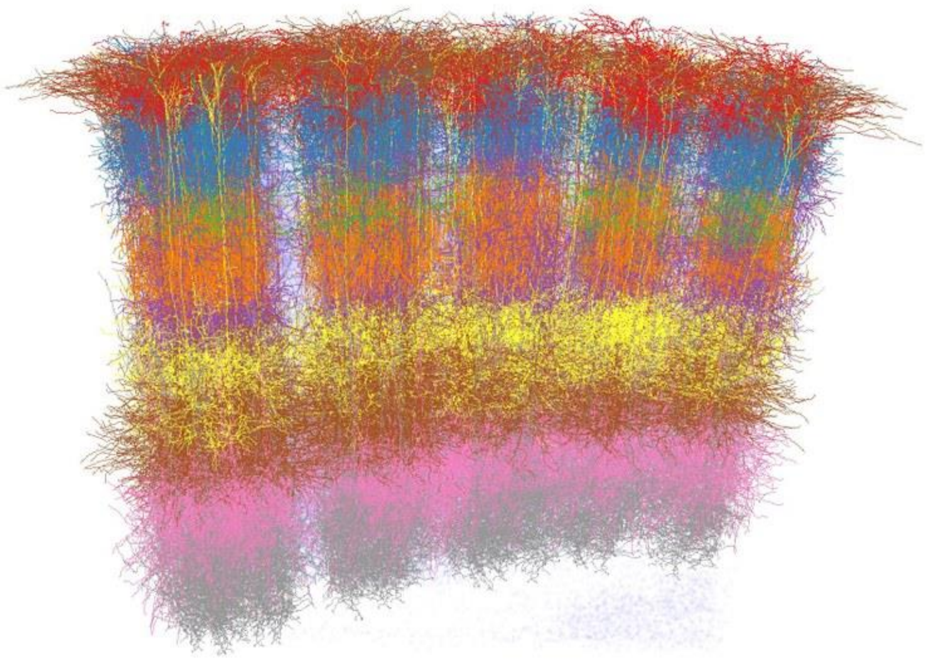
Also called 6b, cortical subplate neurons with projections distinct from layer 6 (layer 1 and callosal)

Columns or modular structure

- Columns described in other species using functional recordings (ie neurons along a single tract have similar response properties, Mountcastle, Hubel and Wiesel).

- Little anatomical evidence for this being widespread in mice (or other species), although most dendrites extend radially in relation to white matter.

- In mouse, most striking example of repeated units ("columns" are in barrel cortex.



Marcel Oberlaender et al
Rat barrel cortex, cell type specific reconstruction.

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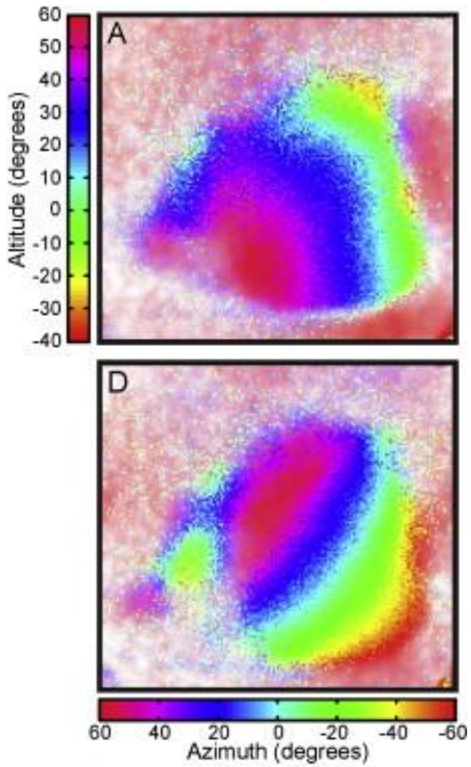
Topography

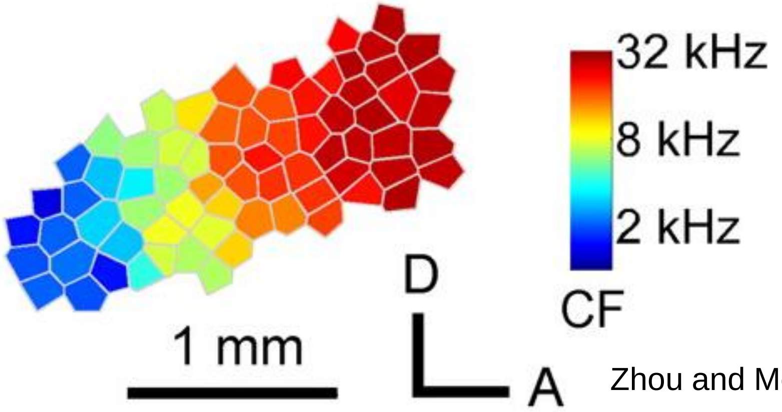
- Sensory regions often defined as containing a complete topographic representation of the sensory surface.

Retinotopy



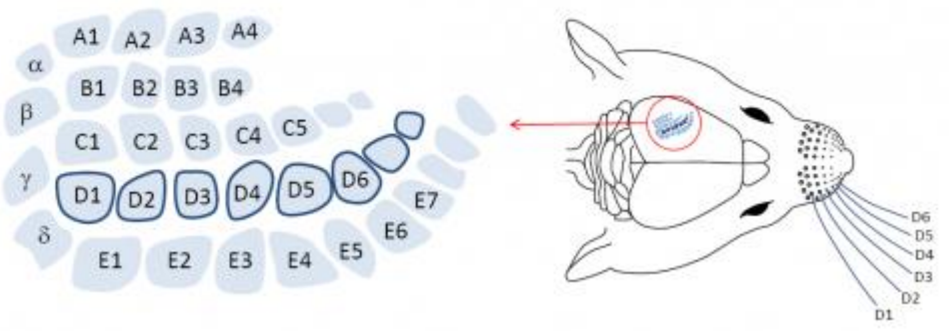
Marshel et al. 2011

Tonotopy



Zhou and Merzenich, 2007

Somatotopy



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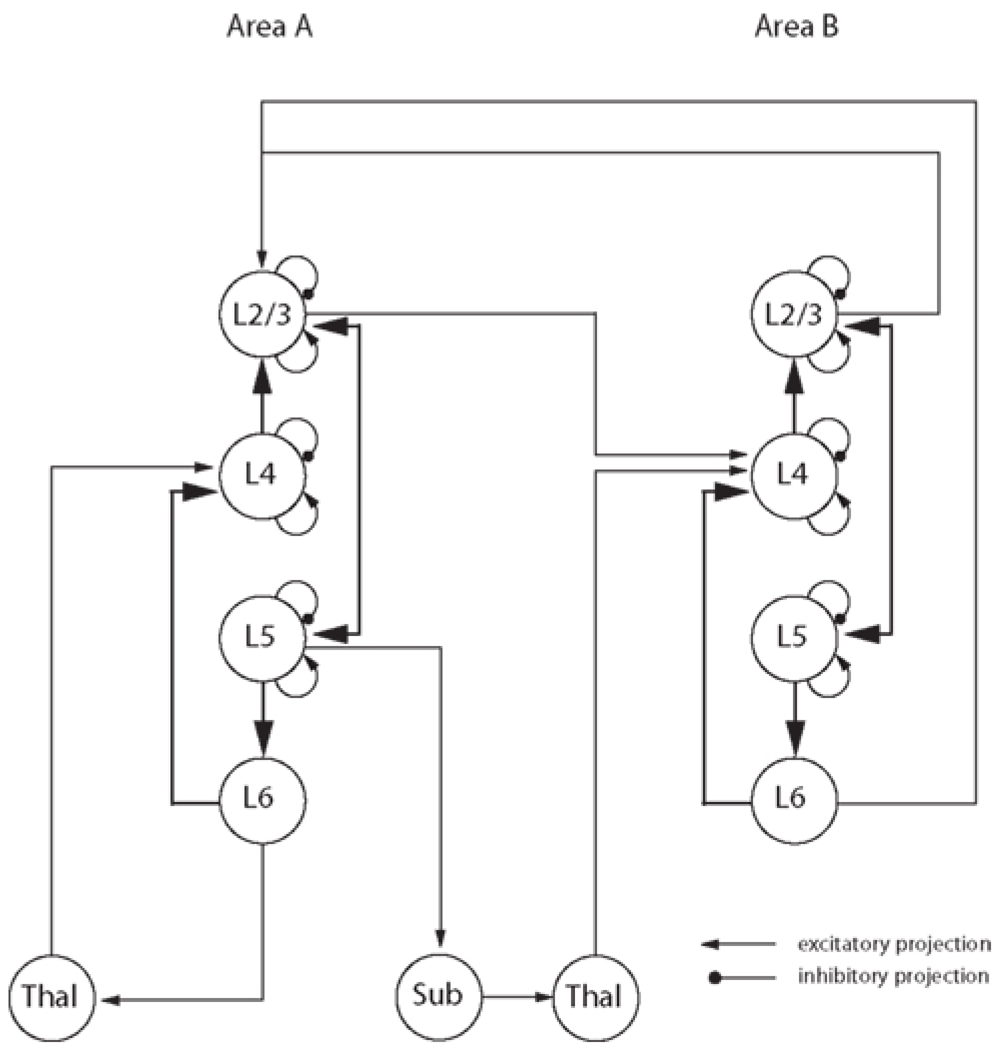
Local structure / canonical cortical circuit



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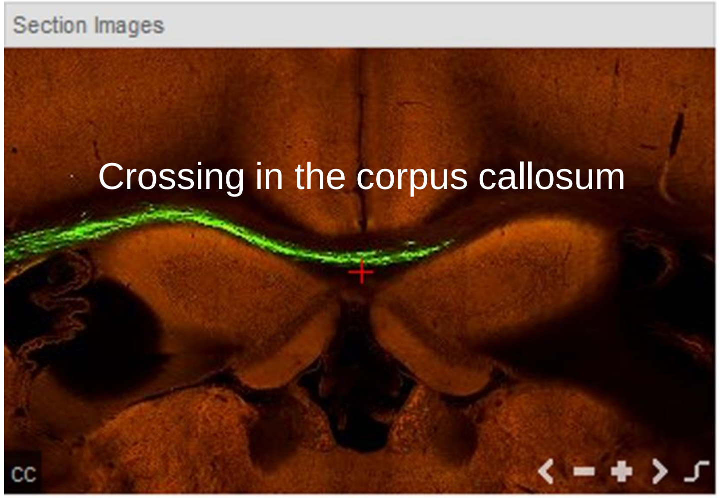
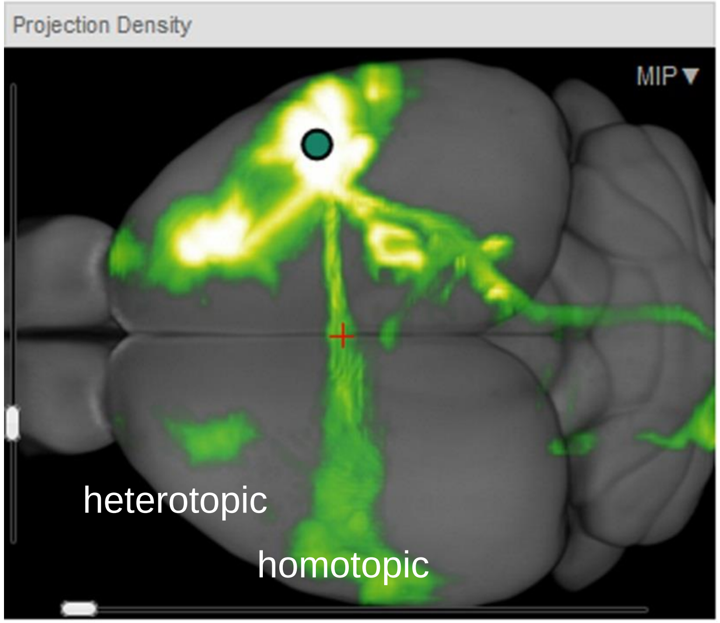
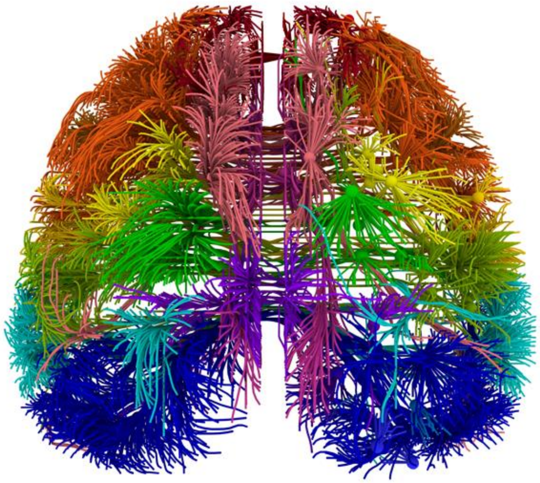
Nuno da Costa and Kevan Martin 2010

Interareal Corticocortical Connectivity

- Ipsilateral: Majority intracortical projections originate from pyramidal neurons in layers 2/3, 5, and 6
  - Axons travel both through white matter and grey matter.
- Callosal: **Homotopic** and **Heterotopic** connections to the contralateral hemisphere
  - Most axons travel through the large white matter tract, the corpus callosum. Majority originate from pyramidal neurons in layers 2/3 and 5.







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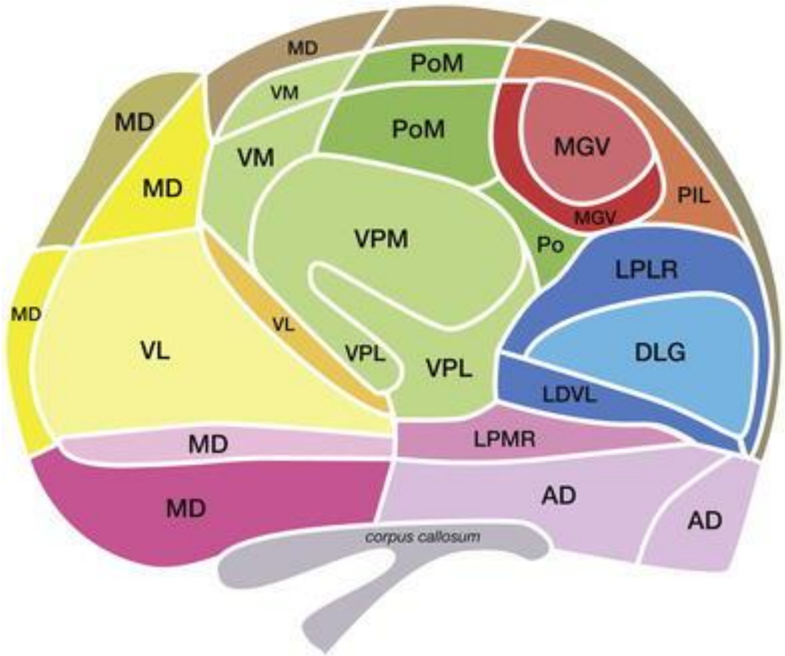
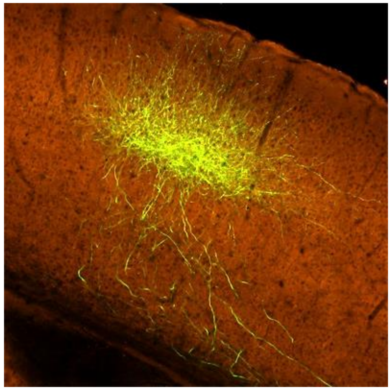
Cortex and Thalamus Connectivity

- Input (*thalamocortical*)
  - Primary input to cortex (all senses except olfaction)
  - Majority are ipsilateral
  - **Specific projections**
    - target a single or cluster of areas
    - terminals across layers, but concentrated in layer 4
  - **Non-specific projections**
    - projections to widespread regions
    - terminals in layer 1 (apical dendrites of layer 2/3-5 pyramidal cells)
    - may modulate attention or arousal levels

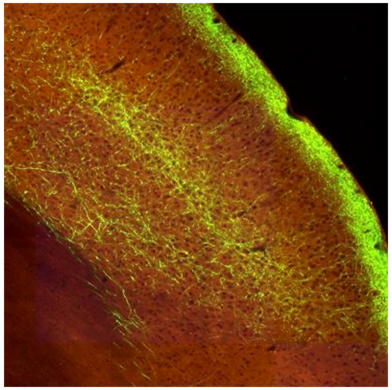
- Output (*corticothalamic*)
  - Distinct populations of neurons in layer 5 and 6, with different targets
  - Layer 6 typically reciprocal back to primary thalamic region, Layer 5 to secondary or non-specific thalamic regions).



Specific (LGd to V1)

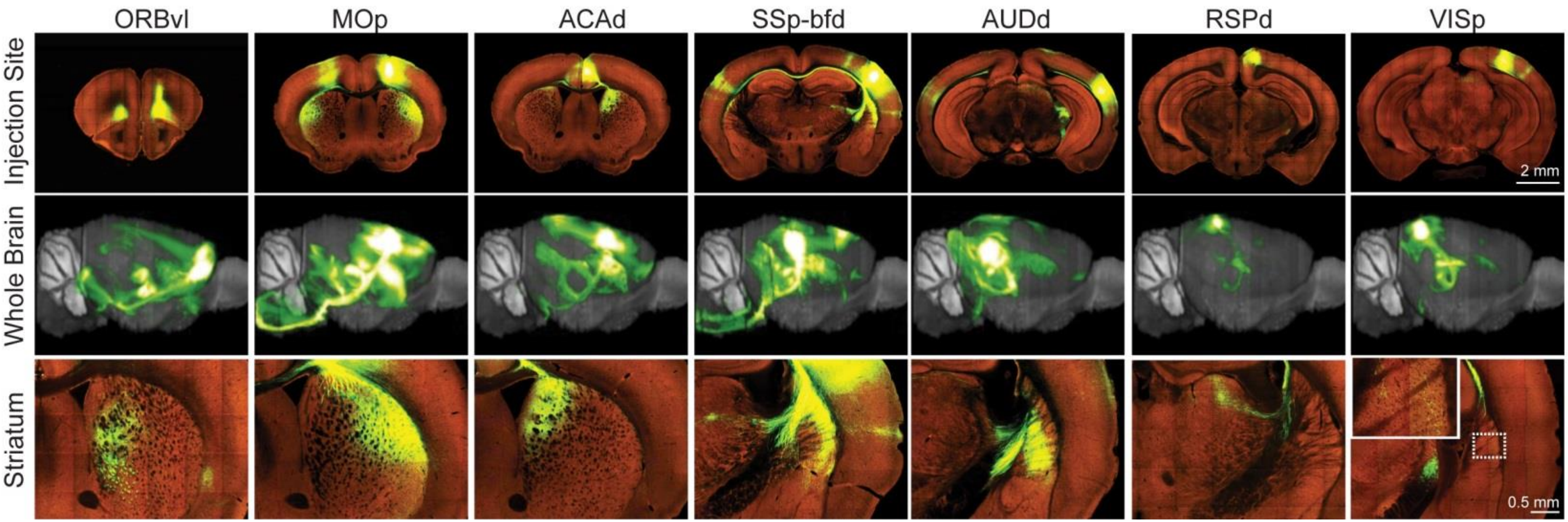


Non-specific (LP to VISl)



Cortex and Striatum Connectivity

- All cortical regions project to striatum
- No direct input from striatum to cortex
- Cortical projection is contralateral (usually stronger ipsilateral).
- Layer 2/3 and 5 pyramidal neurons contribute to this projection, perhaps in different ways (patch-matrix organization).



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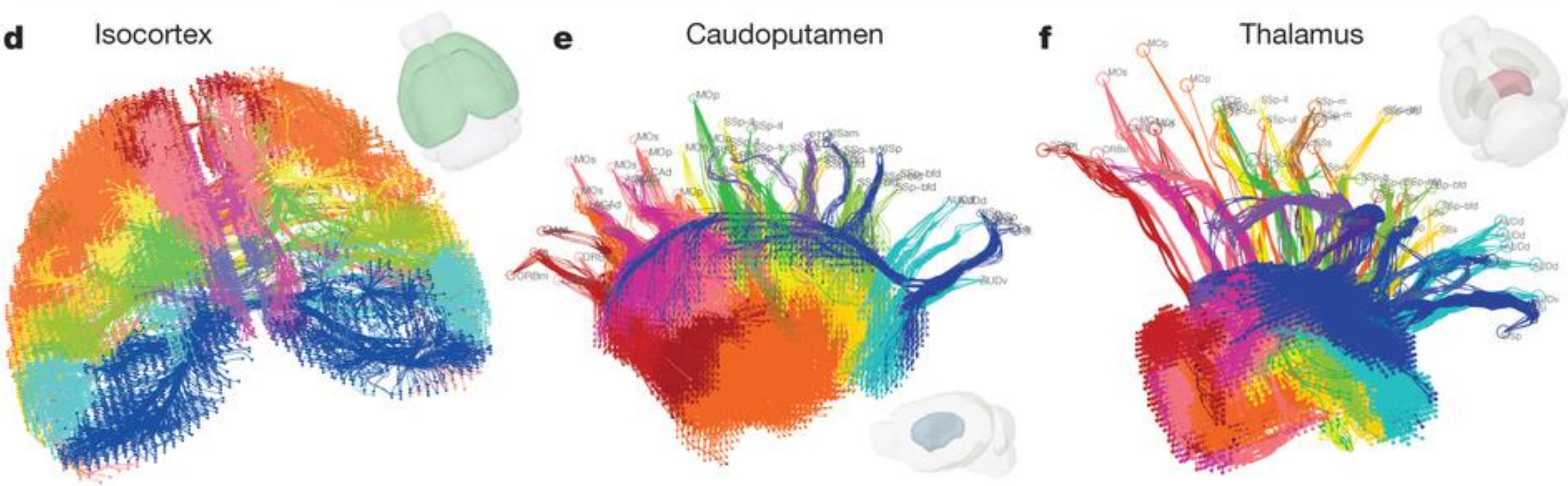
Topography relationships maintained in output



Homotopic relationship

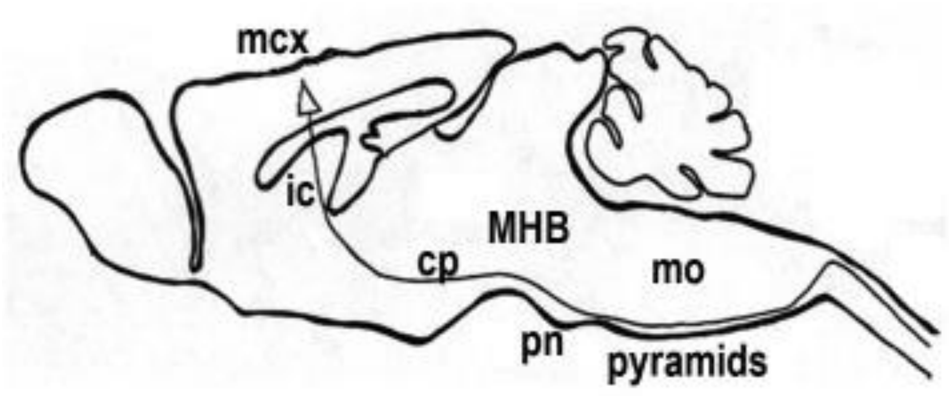
Rostral-caudal = rostral-caudal

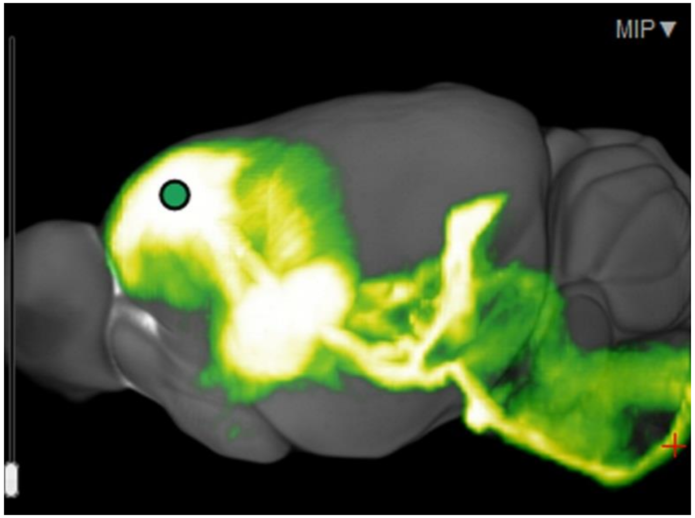
Rostral-caudal = medial-lateral

Oh et al, 2014

Cortex and connectivity with other regions

- Amygdala and Hippocampus: extensively and reciprocally connected
- Claustrum: a small nucleus close to the cortical subplate and deep white matter. All isocortex sends bilateral projections and receives ipsilateral input.
- Midbrain: specific connections depending on region of origin (e.g. auditory cortex contacts inferior colliculus).
- Hindbrain: major effector pathways for direct cortical regulation of behavior and physiology (motor n. for cranial nerves).
  - Pontine nuclei relay activity to cerebellum.
- Spinal cord: Motor cortex and adjacent somatosensory cortex layer 5 neurons send axons along the corticospinal tract (pyramidal tract)





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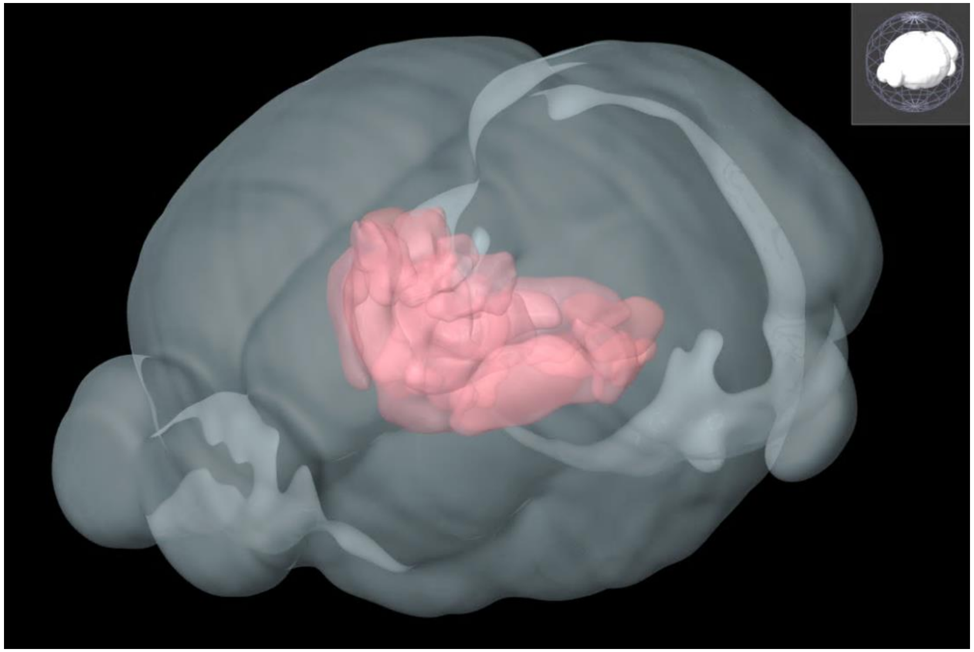
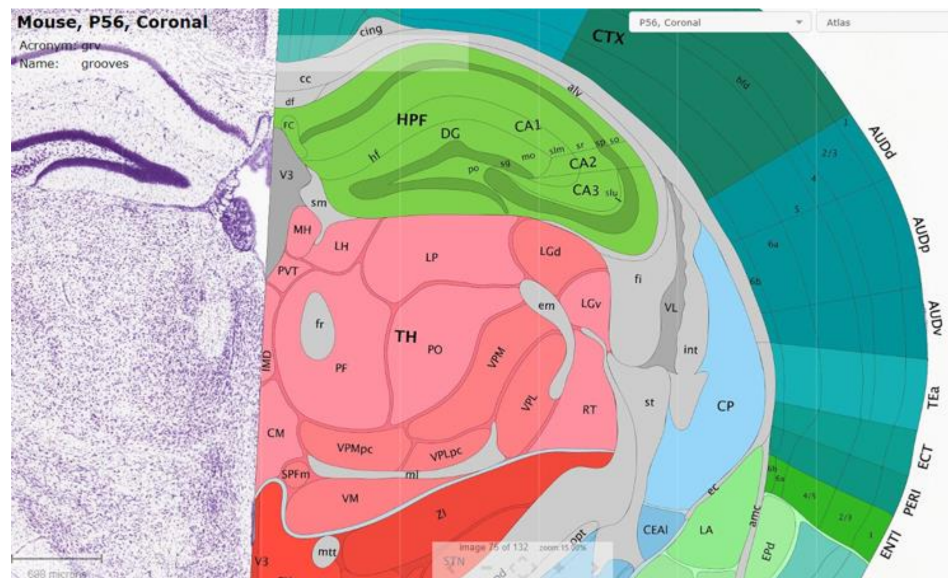
Organization of Thalamus



- Thalamic nuclei projections to cortex are highly organized.
- All sensory pathways (except olfactory) end in specific thalamic nuclei.
- Many thalamic nuclei are part of higher-order associative circuitry (not primary sensory recipients)

- Boundaries of many thalamic nuclei can be defined with nissl or histochemistry.
- ~ 45-50 distinct nuclei
- Classified into ~ 12 anatomical groups:
  - ventral, dorsal, lateral, anterior, medial, midline, intralaminar, epithalamus, dorsal and ventral geniculate, as well as subparafascicular and reticular nucleus.



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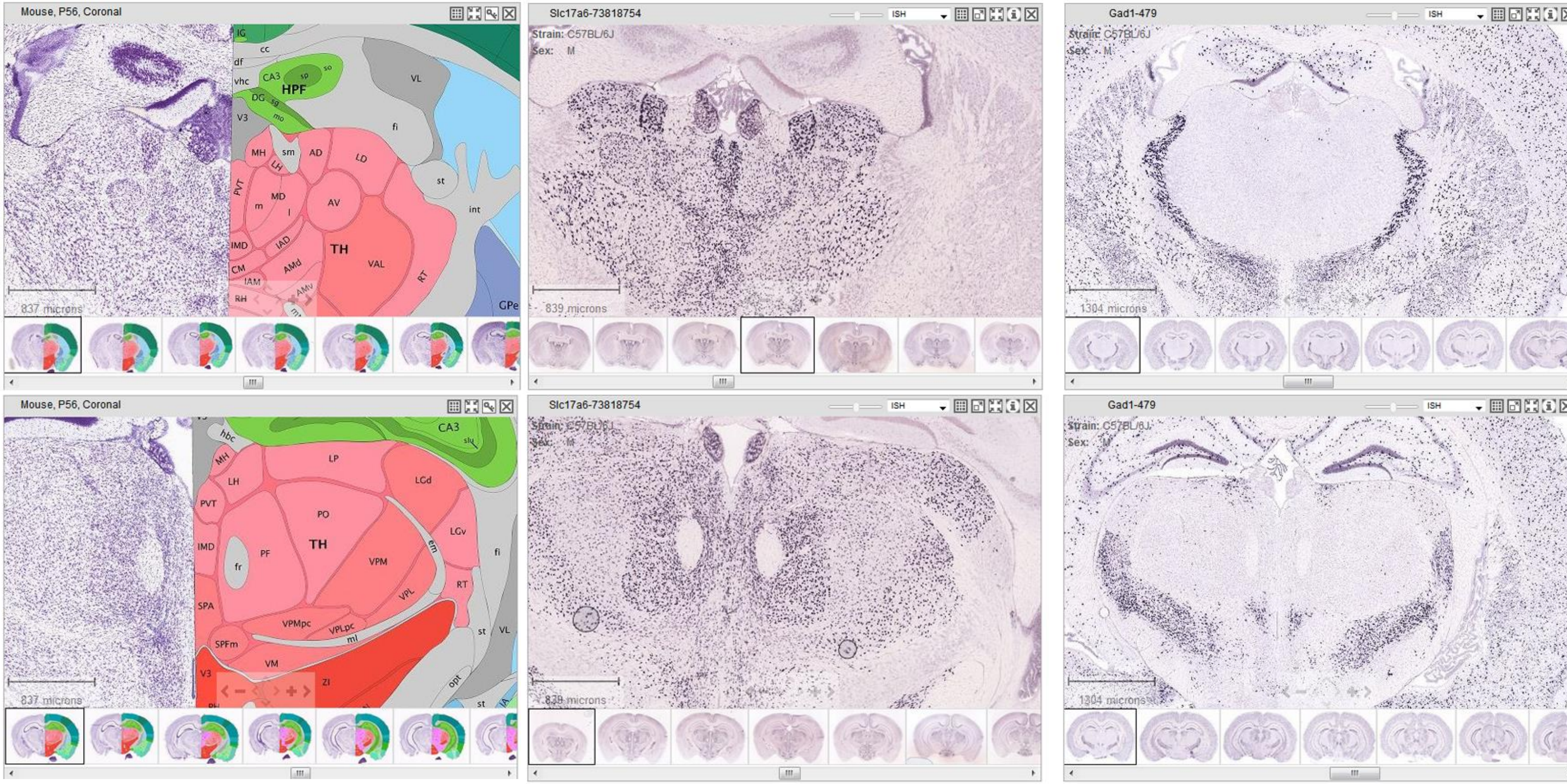
Organization of Thalamus

- Majority of nuclei are glutamatergic, only a few (e.g. RT, LGd) have GABAergic neurons.



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Thalamocortical connectivity

- Specific (majority) vs non-specific classification
- Core-Matrix organization
  - Core: specific projections to single cortical area, usually layer 4
  - Matrix: diffuse (non-specific) projections, usually layer 1
  - Some nuclei are one or the other, some mixed.
- Jones (2007) principles:
  - Every thalamic nucleus projects to the cerebral cortex
  - All cortical areas receive a thalamic input
  - The pattern of cortical projection varies from nucleus to nucleus, and even within a nucleus
  - While some thalamic nuclei project only to the cortex, others project to both cortex and striatum
    - Reticular nucleus (prethalamus) does not project to cortex, but receives a major input from cortex and sends GABAergic projections to other thalamic nuclei to exert profound inhibitory effects on thalamic projection neurons.
- Every nucleus receives reciprocal input, although not always symmetrical (cortical projections cover area larger than source of thalamocortical projections and some have very diffuse projections to thalamus).

Thalamocortical connectivity – Allen Atlas

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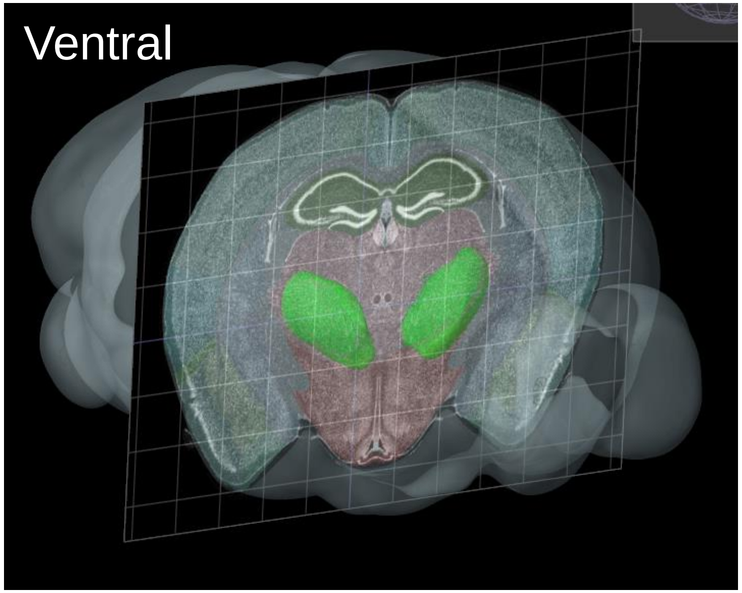
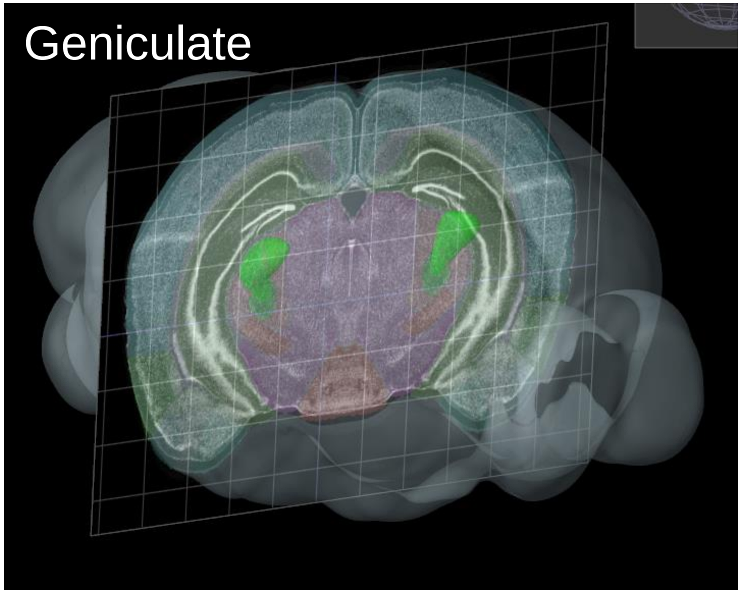
Oh et al, 2014

Ventral and dorsal geniculate groups





- VAL / VM
  - Inputs: pallidum, cerebellum, red nucleus, substantia nigra
  - Cortical outputs: motor cortex

- VPL / VPM
  - Inputs: somatosensory inputs from face (VPM) and limbs/trunk (VPL)
  - Cortical outputs: somatosensory cortex
  - VPLpc and VPMpc continuous, but have different inputs/outputs.

- MG (d, v, m)
  - Inputs: auditory from midbrain (inferior colliculus) and hindbrain
  - Cortical outputs: : auditory cortex

- LGd
  - Inputs: retinothalamic fibers (retinotopic organization)
  - LGd also contains some gabaergic neurons.
  - Cortical outputs: visual cortex

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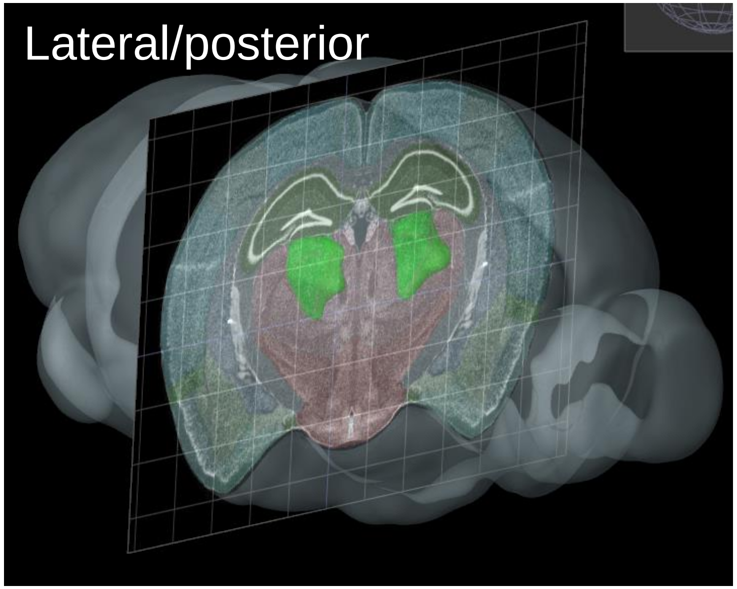
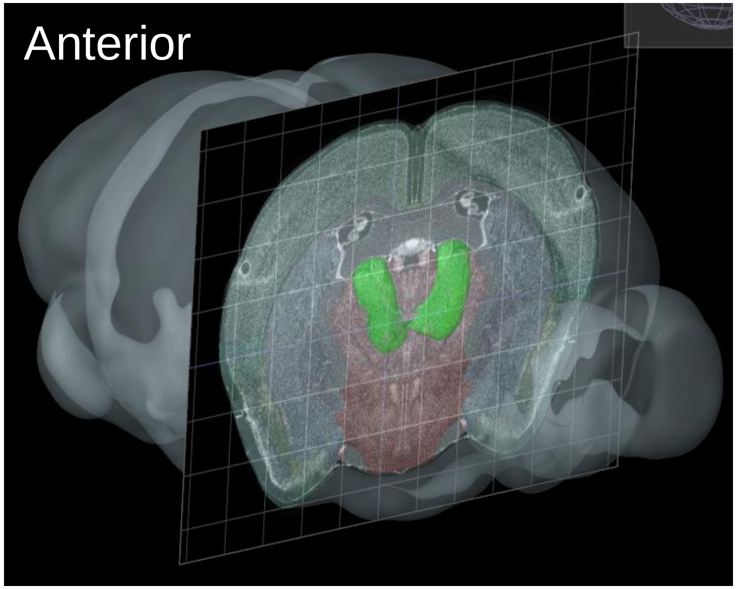
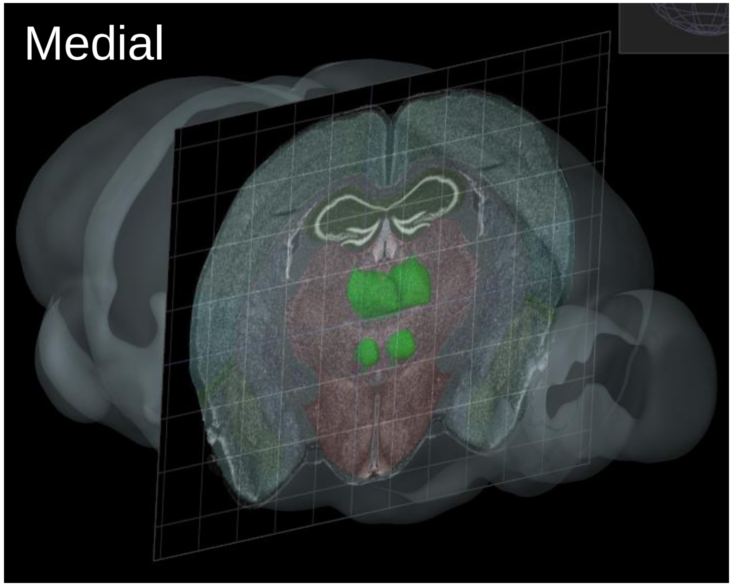
Lateral (posterior) and anterior groups

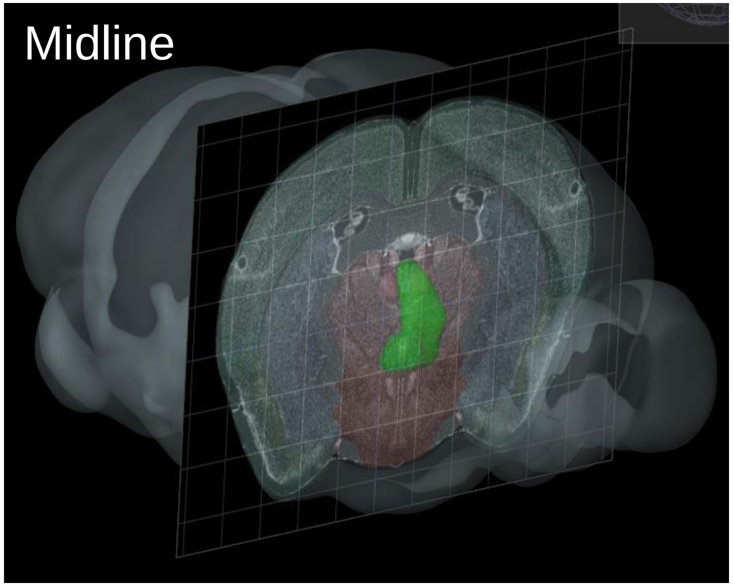




- PO
  - Inputs: somatosensory input from spinal cord and trigeminal nuclei in hindbrain
  - Cortical outputs: sensory cortex (mostly somatosensory)
- POL
  - Inputs: auditory from inferior colliculus
- LP
  - Inputs: superficial superior colliculus
  - Outputs: visual cortex (to layer 1 in primary V1)
- AV / AM / AD:
  - Inputs: mamillary bodies (which in turn receive input from hippocampus), subiculum and entorhinal cortex
  - Cortical outputs: Cingulate and retrosplenial cortex
- LD
  - Inputs: somatosensory input from trigeminal nuclei in hindbrain, others.
  - Cortical outputs: cingulate cortex

Medial and Midline Groups

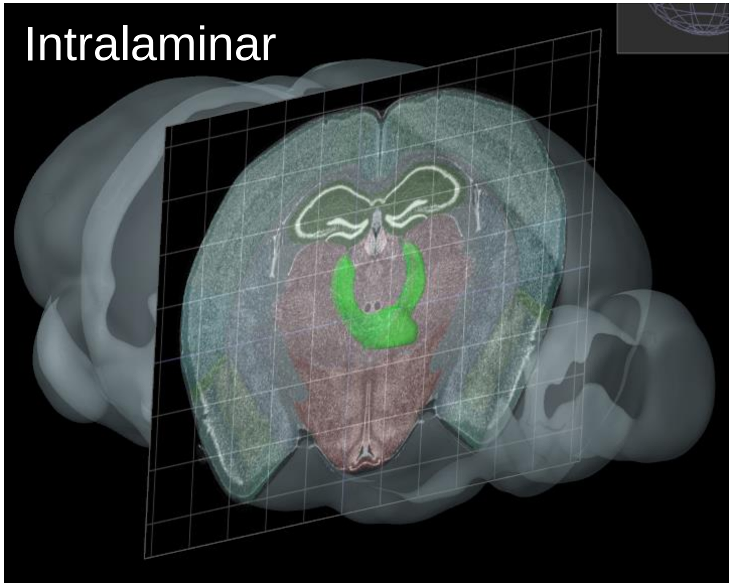


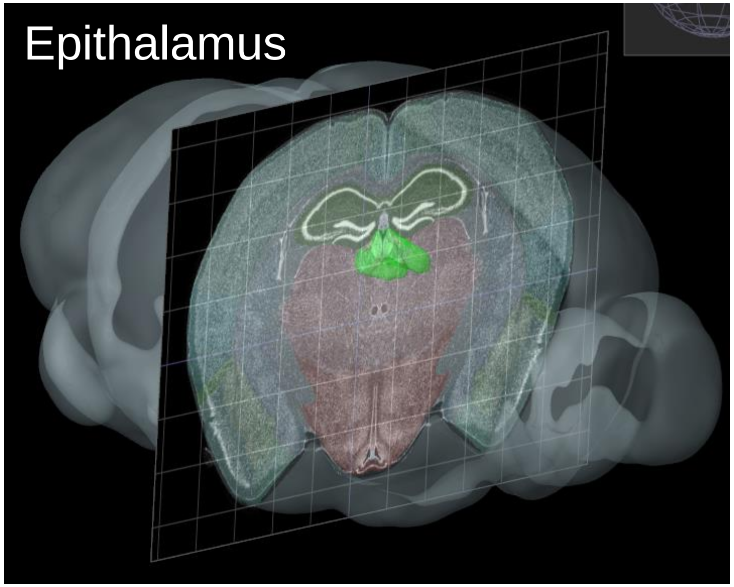


- MD (c, l, m)
  - Inputs: olfactory cortex (c), superior colliculus (l), substantia nigra (l), amygdala and entorhinal cortex
  - Cortical outputs: medial frontal cortex (m), anterior cingulate

- PVT
  - Inputs: hypothalamus, basal forebrain, hindbrain cholinergic and monoaminergic nuclei
  - Cortical outputs: medial frontal and entorhinal cortex
- RE
  - Cortical outputs: frontal cortex

Intralaminar and Epithalamus





- All have prominent projections to striatum
- CM
  - Cortical outputs: frontal cortex
- PCN
  - Cortical outputs: primary motor, somatosensory and parietal cortex
- CL
  - Cortical outputs: medial frontal, entorhinal cortex
- PF
  - Cortical outputs: frontal cortex (?)

- MH
  - Inputs: septum
  - Outputs: (not cortical) interpeduncular nucleus
- LH
  - Inputs: basal forebrain, preoptic area, lateral hypothalamus
  - Outputs: (not cortical) VTA, SNc, and raphe nuclei.

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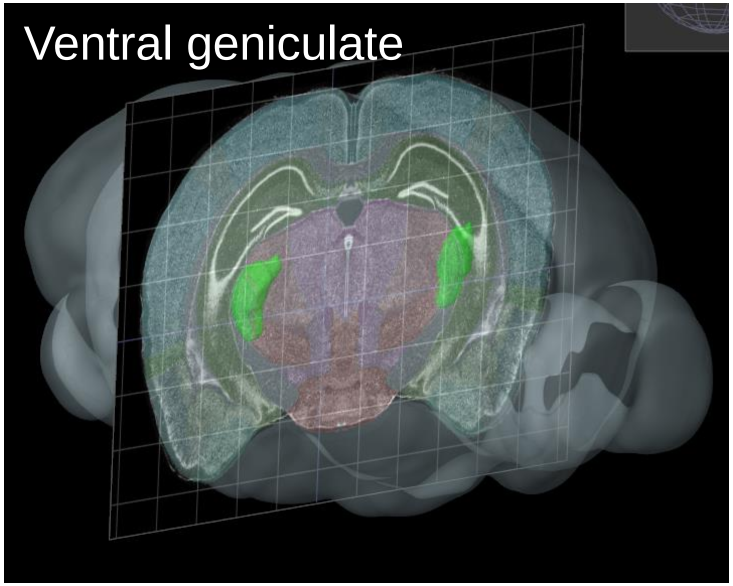
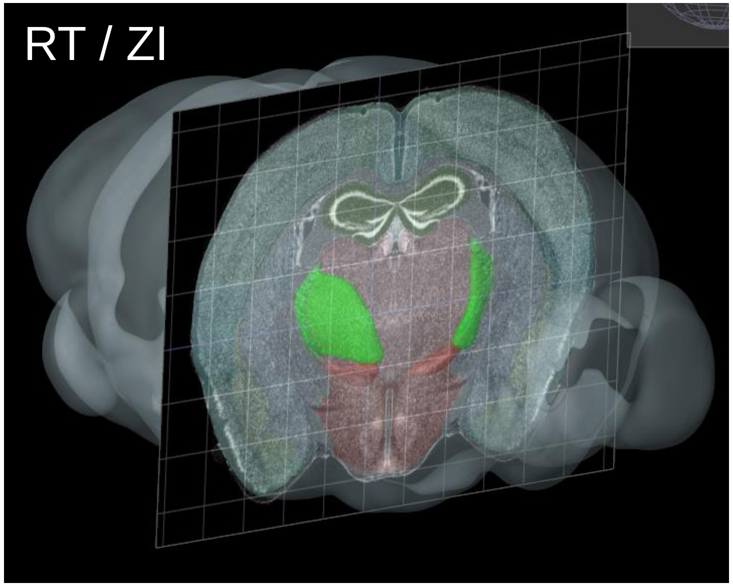
Ventral geniculate, Reticular Nucleus and Zona Incerta





- IGL
  - Inputs: bilateral retinal input
  - Outputs (not cortical): suprachiasmatic nucleus and contralateral IGL
- LGv
  - Inputs: retina and superior colliculus
  - Outputs (not cortical)
- SubG
  - Inputs: retinal
  - Outputs: superior colliculus

- Both populated by GABAergic cells
- RT:
  - Inputs: all cortical areas
  - Outputs: thalamic regions

- ZI:
  - Inputs: all cortical areas and spinal cord
  - Outputs: parts of the thalamus

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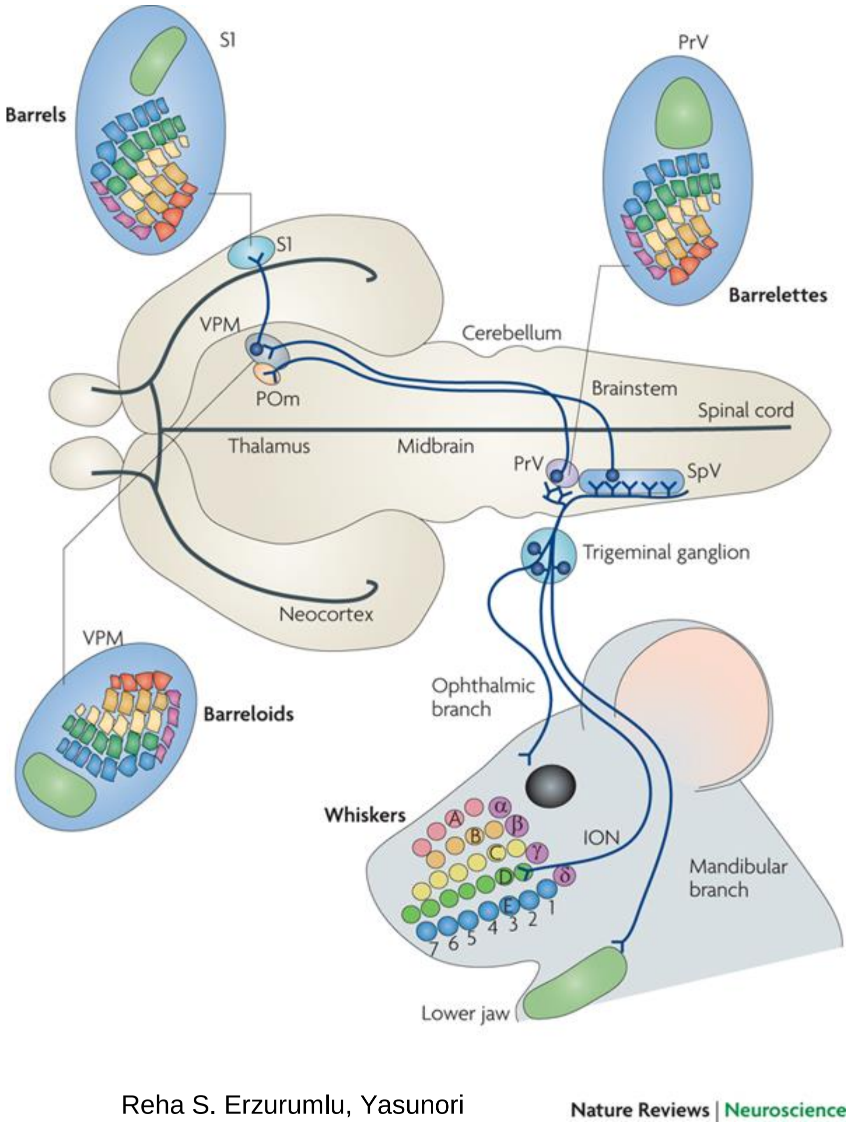
Summary of Thalamocortical Outputs

TABLE 9.2 Projections of Individual Thalamic Nuclei to the Cortex and Subcortex

Thalamic nuclear group	Thalamic nucleus	Cortical target	Subcortical target
Anterior	AV	medial frontal	
	AM	cingulate, retrosplenial, subiculum	
	AD	cingulate, retrosplenial	
Medial	MD	'frontal', orbitofrontal, olfactory areas	
	Re	hippocampus	
	PT	medial frontal, entorhinal, olfactory areas	striatum
Intralaminar parafascicular	CL	diffuse to M1, S1, Pt	striatum
	CM	'frontal'	
	PF	'frontal'	
Ventral	VA	diffuse to frontal and cingulate	
	VL	motor	
	VM	diffuse to frontal	
	VPM, VPL	S1	
	VPPC	S1, S2, insula	
	Sub	medial frontal	
Lateral	LP	V1, V2	
	LD	cingulate, retrosplenial	
Posterior	Po (PoM)	S1 BF	
	PoT (PoL)	peri-auditory	amygdala
Geniculate nuclei	MGV	A1	
	MGD	peri-auditory	
	MGM (MGMC)	diffuse to insula and temporal areas	amygdala
	DLG	V1	

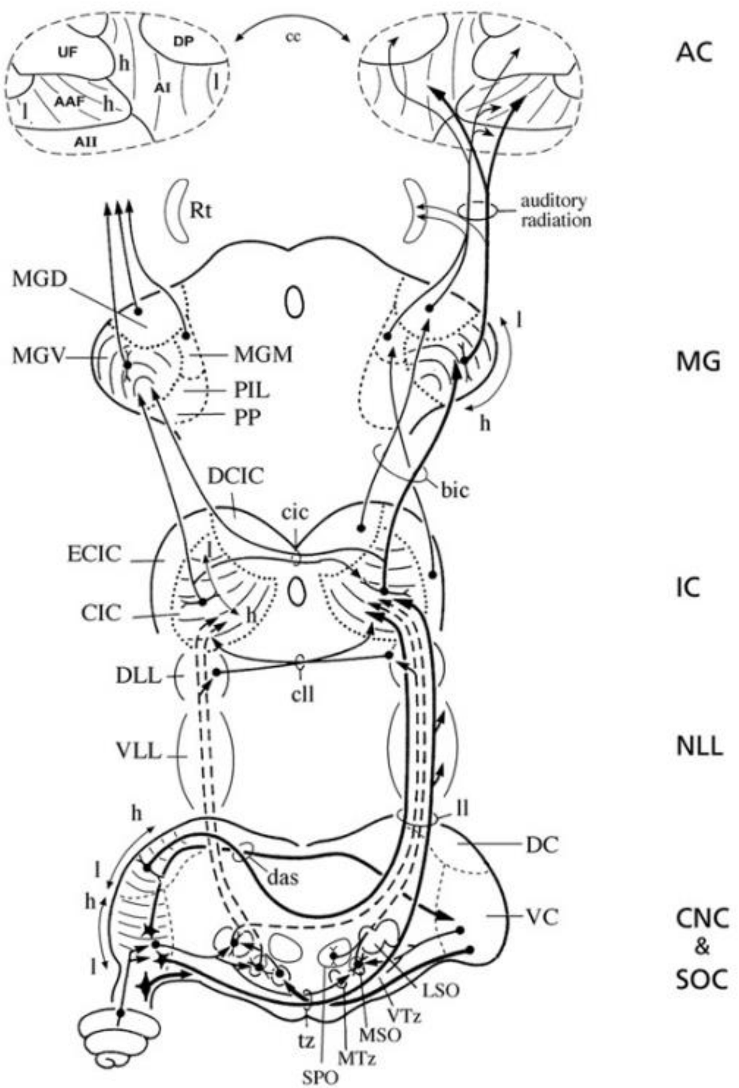
Somatosensory System

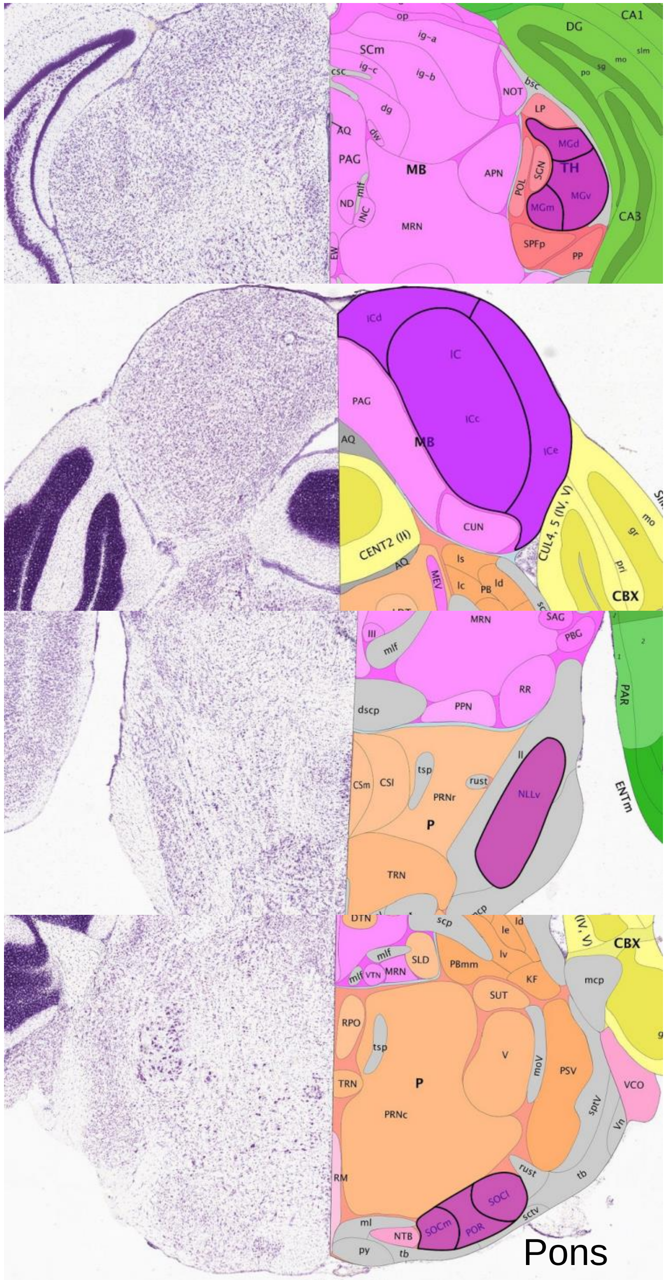
- Multiple ascending sensory tracts
  - **Dorsal column nuclei / medial lemniscus**: Cuneate and gracile (input from upper and lower limbs, torso, shoulder, neck, ear via spinal cord). Projections cross midline to VPL in thalamus.
  - **Trigeminal system:** Brainstem trigeminal nuclei, SPVO, SPVI, SPVC and the principal trigeminal nucleus (PSV) in pons (input from face, e.g. whiskers). Projections to VPM in thalamus.



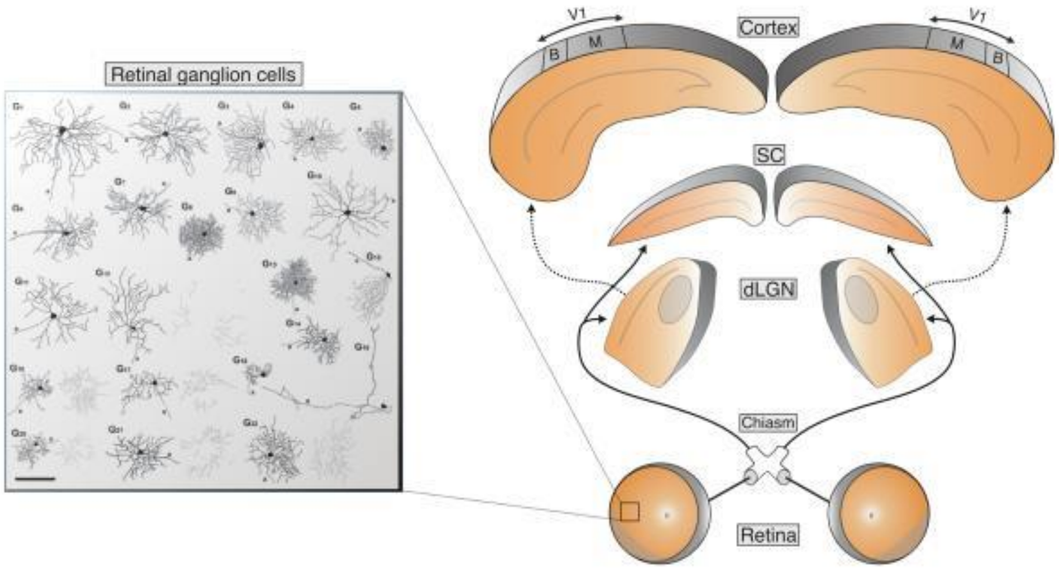
Reha S. Erzurumlu, Yasunori Murakami & Filippo M. Rijli (2010)

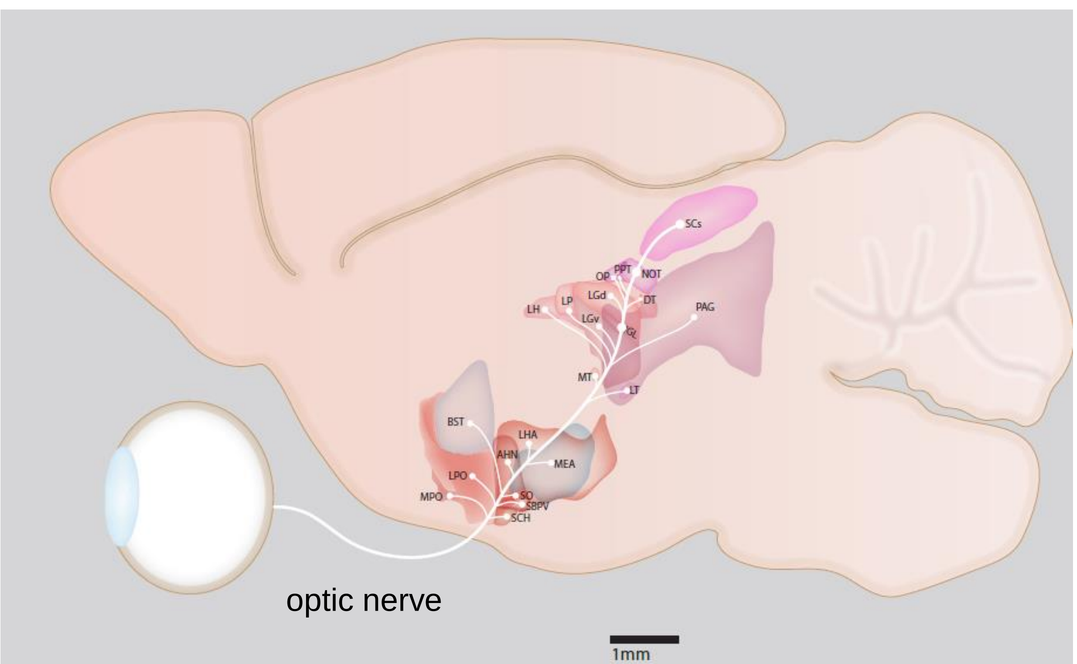
Auditory System





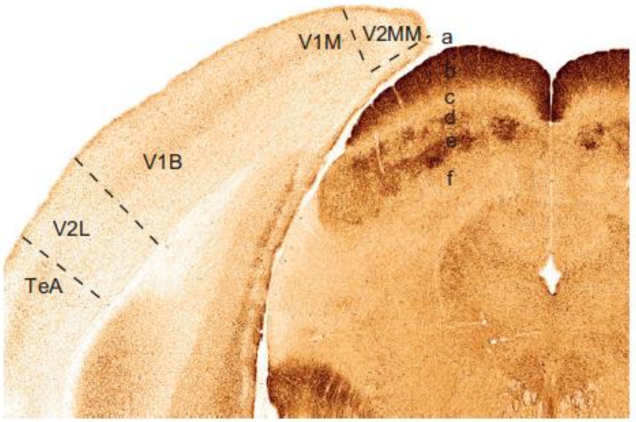
Visual System

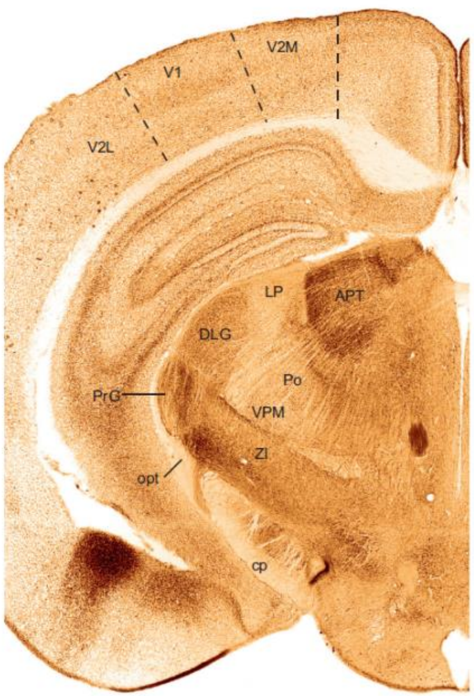




- Retinal ganglion cells output of the retina that transmit visual information to the brain.
- Primary thalamic target is dorsal lateral geniculate nucleus (LGN or LGd), provides input to visual cortex.
- All axons to LGN are collaterals of those headed to superior colliculus.
- >22 retinorecipient areas in mouse brain
- Multiple regions in hypothalamus, thalamus, and midbrain

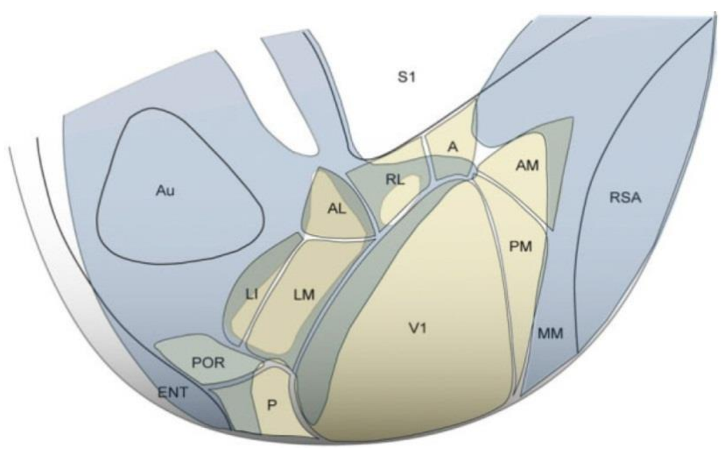
Visual System

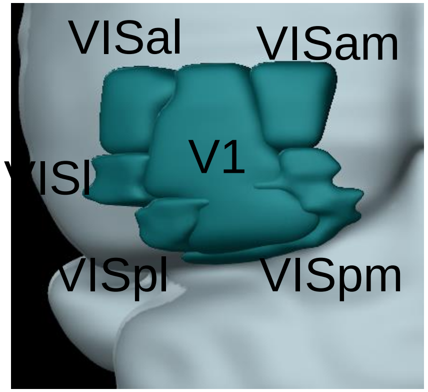




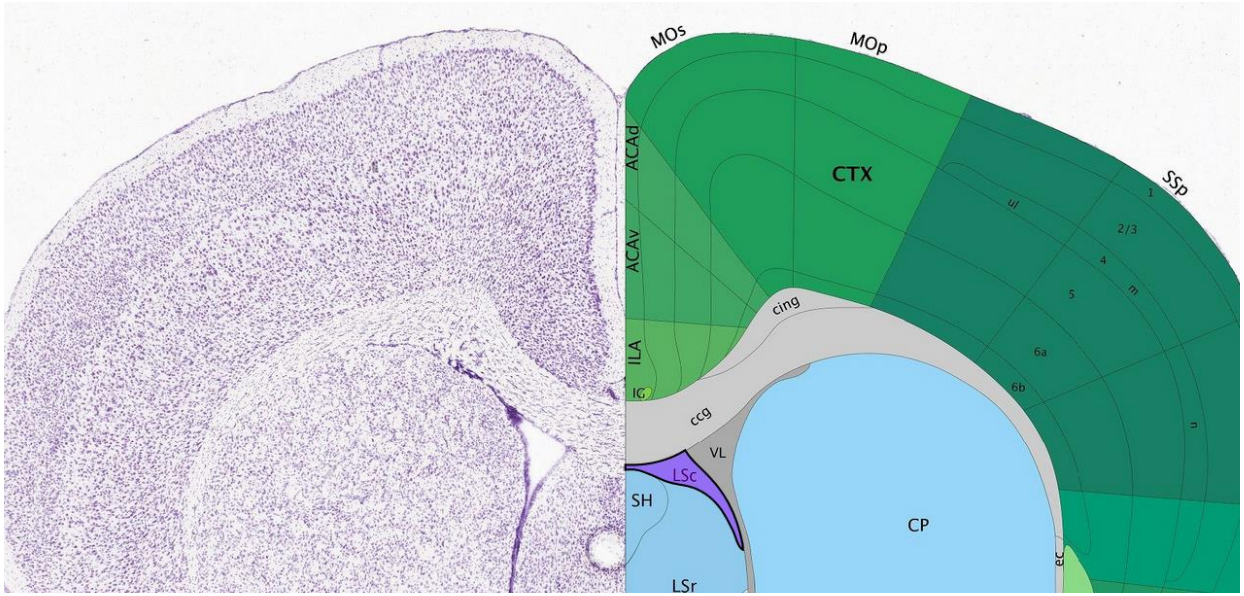
- **Superior colliculus** is a layered structure.
- Retinal input terminates in superficial layers.
- Retinotopic organization (e.g. upper to lower visual field = medial to lateral)
- Receives input from ipsilateral visual cortex.

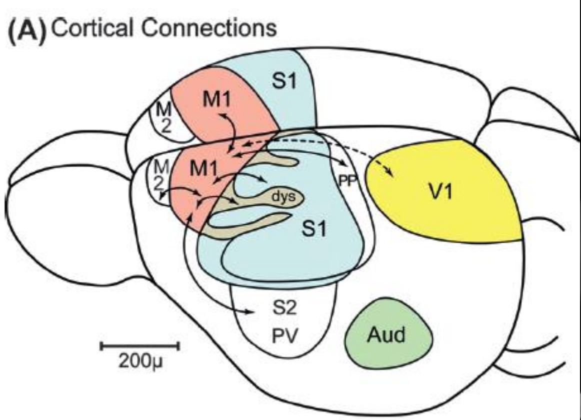
- **LGd** projects to visual cortex.
- Retinotopic organization (e.g. temporal to nasal visual field = rostrolateral to caudal).
- Visual cortex likely consists of 10 areas (Allen Reference Atlas only has 6 currently)

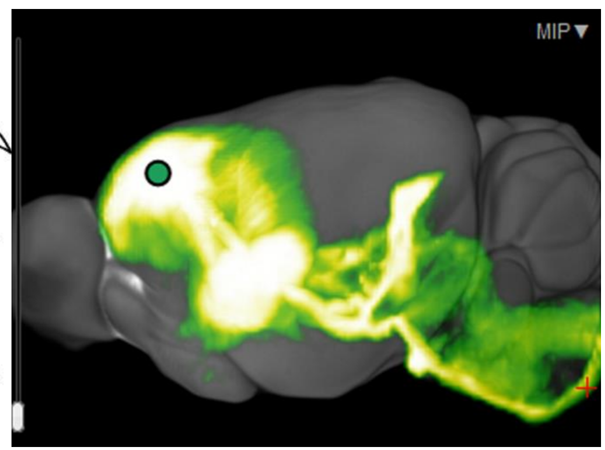




Motor Systems







- Primary motor cortex is agranular (no layer 4)
- Systematic representation of the movements of contralateral body parts.
- Connectivity:
  - Corticocortical: reciprocal and topographical connectivity with somatosensory cortex.
  - Corticospinal: most projections to spinal cord motor neurons start in MOp layer 5

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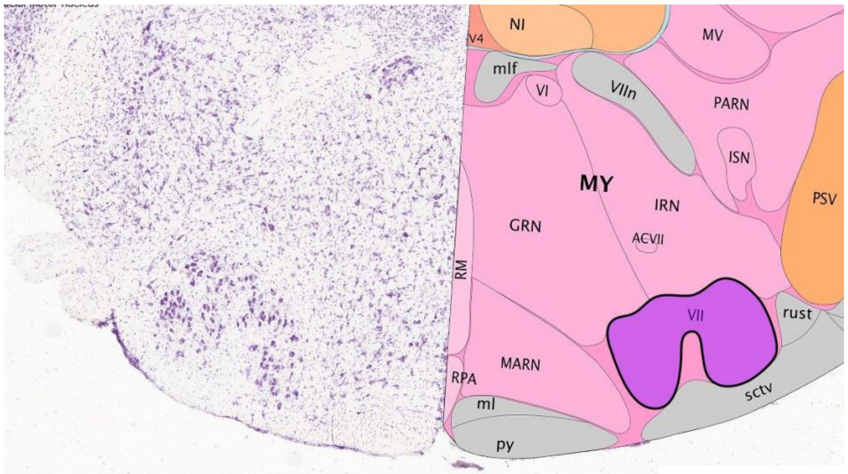
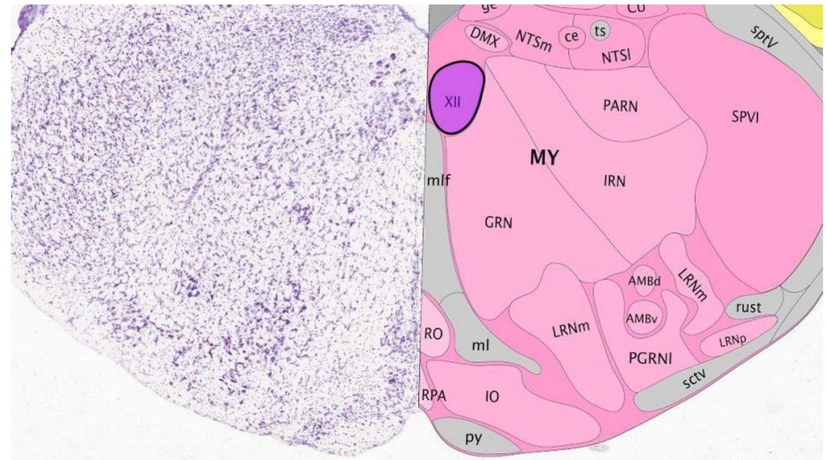
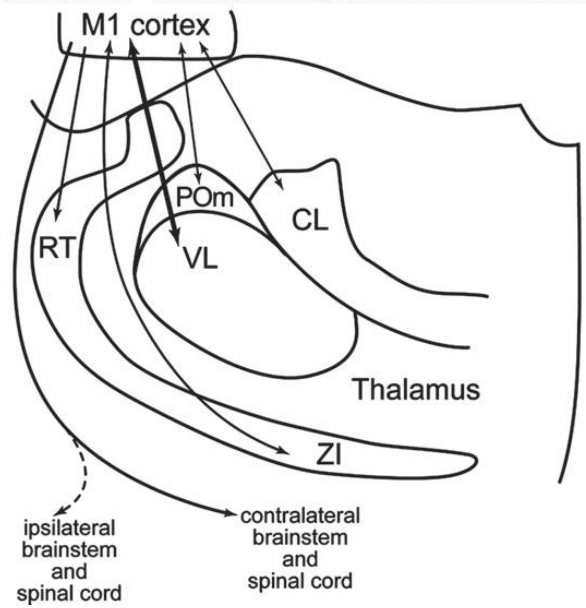
Motor Systems

Corticobulbar: Direct projections to cranial motor nuclei in hindbrain (e.g. EW, oculomotor (III), trochlear (IV), motor trigeminal (V), abducens (VI), facial (VII), ISN, AMB, dorsal motor nucleus of vagus nerve (X), hypoglossal (XII).





Corticothalamic:



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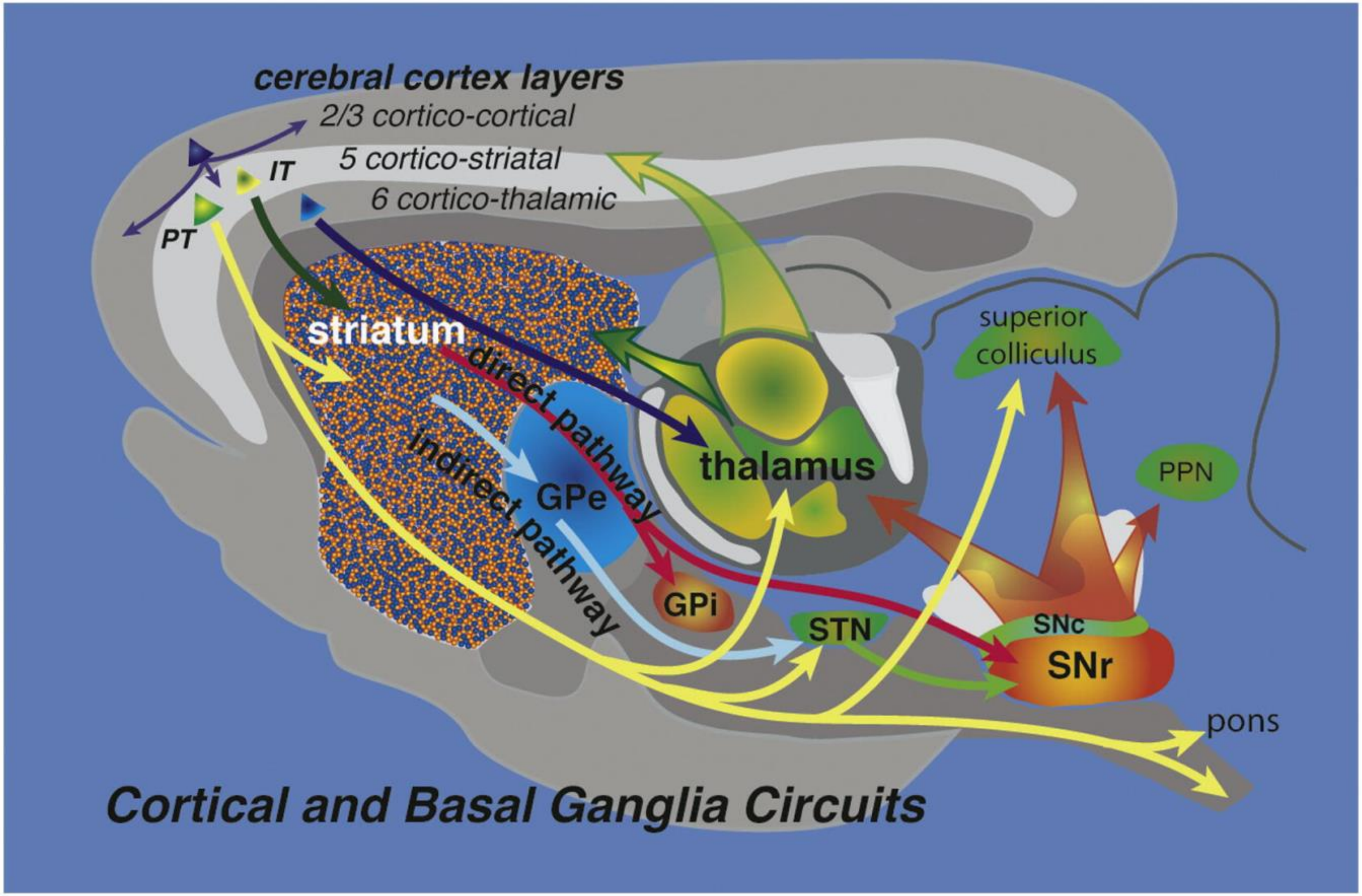
Basal Ganglia (motor functions, dorsal)



Gerfen and Heintz, 2014

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Neuroanatomy - Observations

- Most current mouse anatomical atlases built using cyto- and chemo-architecture, comparison with brains of other species.

- Piecemeal data on whole-brain connectivity.

- Relatively little information on location and connectivity of specific cell types within delineated regions.

- Mapping connectomes is critical in order to describe and understand the brains' anatomy and how it relates to function in a comprehensive way.

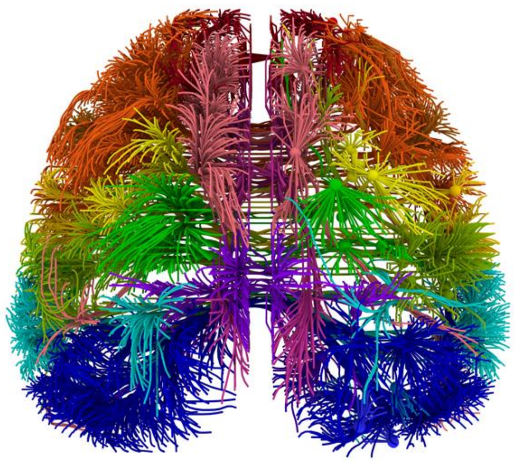
Anatomical Connectomes: Types and Scales

Macroscale

Inter-areal
Resolution: mm
MRI, DTI
Humans

Human Connectome Project

Mesoscale

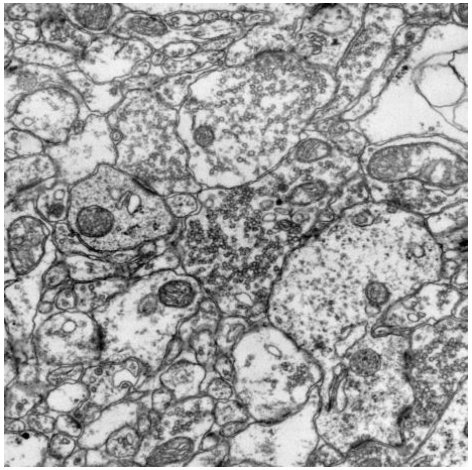


Cell populations, inter-and intra-areal
Resolution: μms
Tracers; Light Microscopy
Animal Models

Allen Mouse Connectivity Atlas

(Oh S*, Harris JA*, Ng L* et al. 2014, Nature)

Microscale



Single cell
Resolution: submicron
Electron Microscopy
Animal Models

Open Connectome Project

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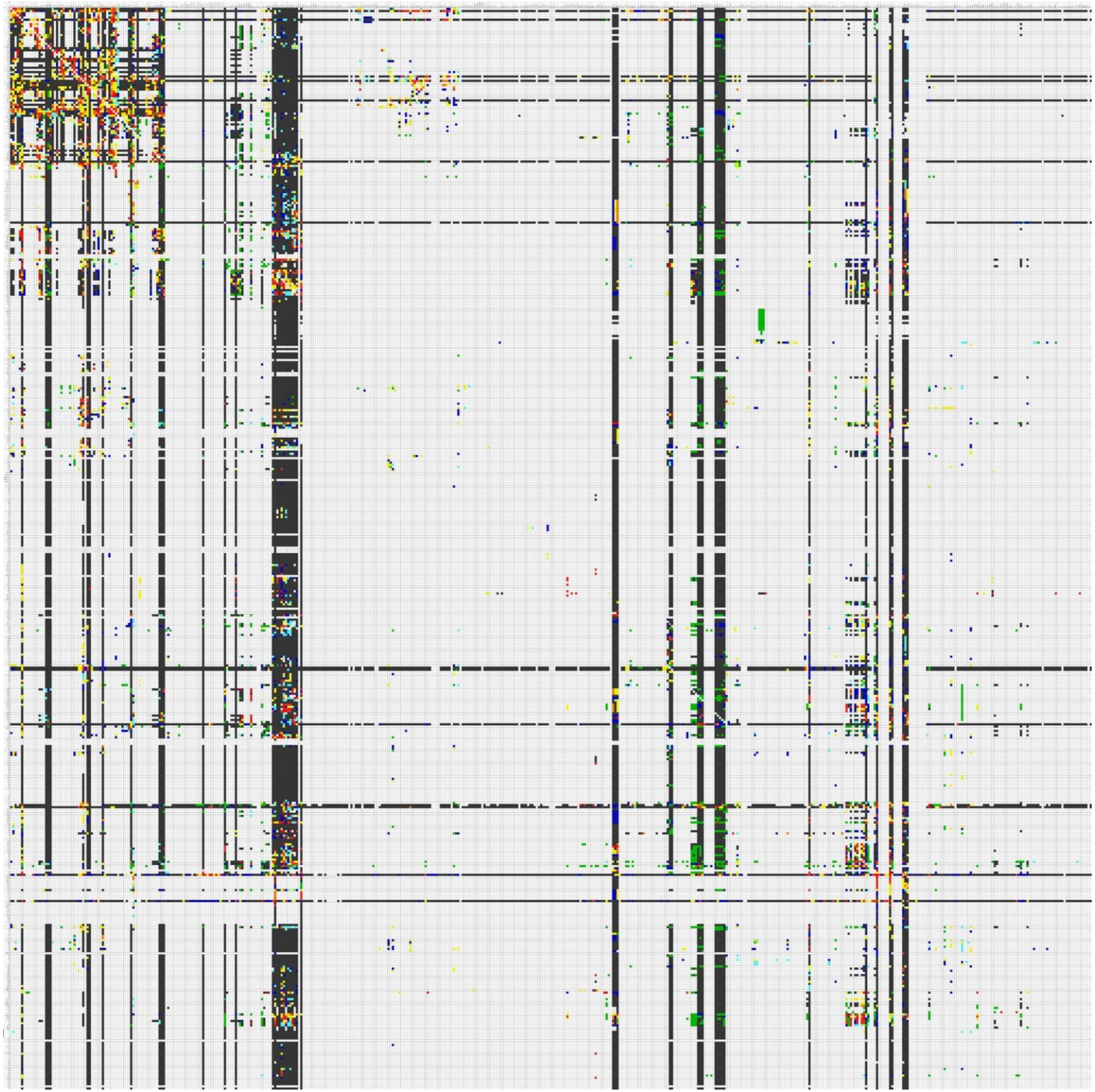
Post-hoc collation of literature: Brain Architecture Management System



Jan 2013
Whole Brain: 13% coverage
Cortico-Cortico: 39% coverage

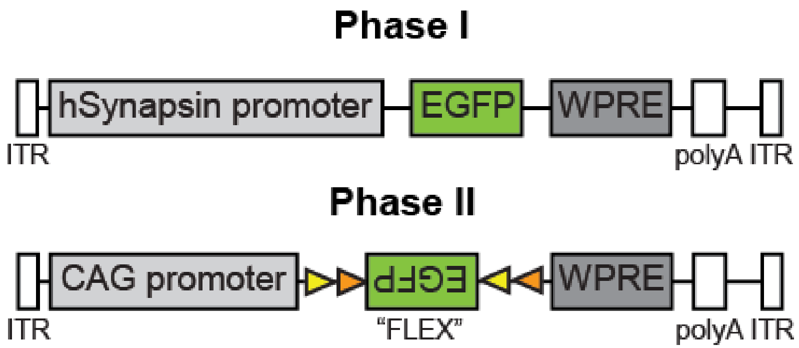
Allen Mouse Brain Connectivity Atlas Scope

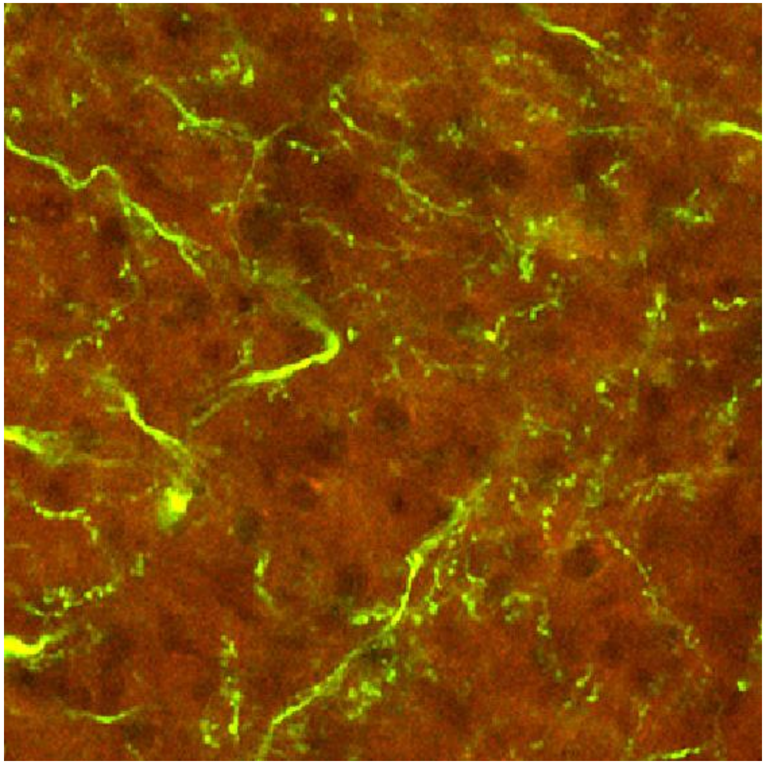
Goals: Build a 3-D atlas of inter-areal neural PROJECTIONS in the adult mouse brain in TWO Phases:

1) Map axonal projections from over **300 anatomically-defined** brain areas in P56 male C57Bl/6 mice (Phase I).

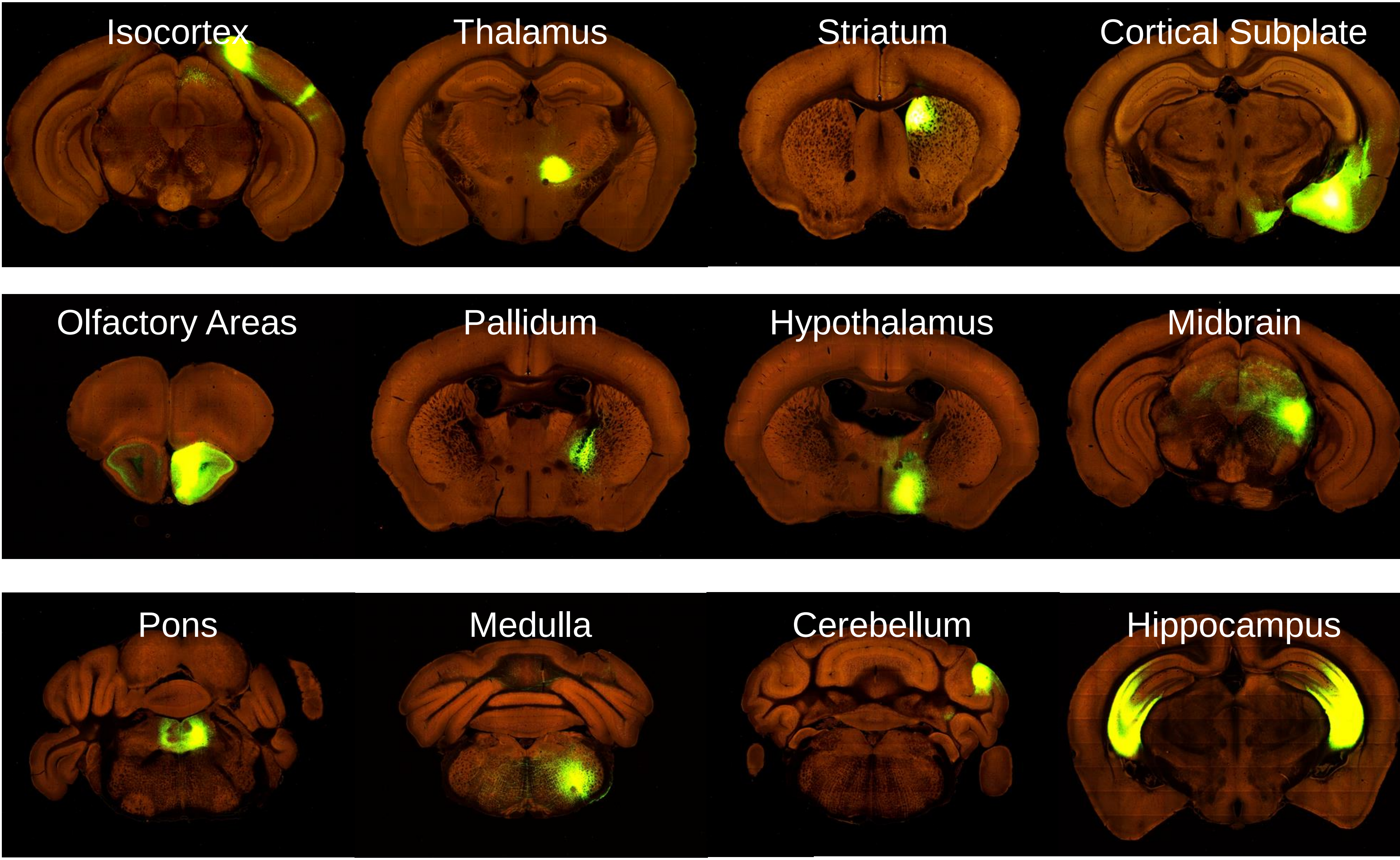
2) Map axonal projections from **genetically-defined** cell types in subcortical areas and **laminar-specific** projections in cortical areas using P56 Cre driver mice. (Phase II).

Anterograde tracers: rAAVs





Phase I: rAAV2.1 infection in all major brain areas



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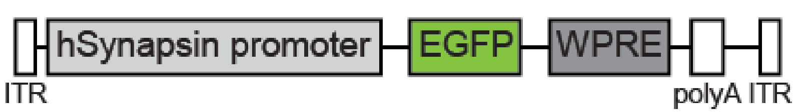
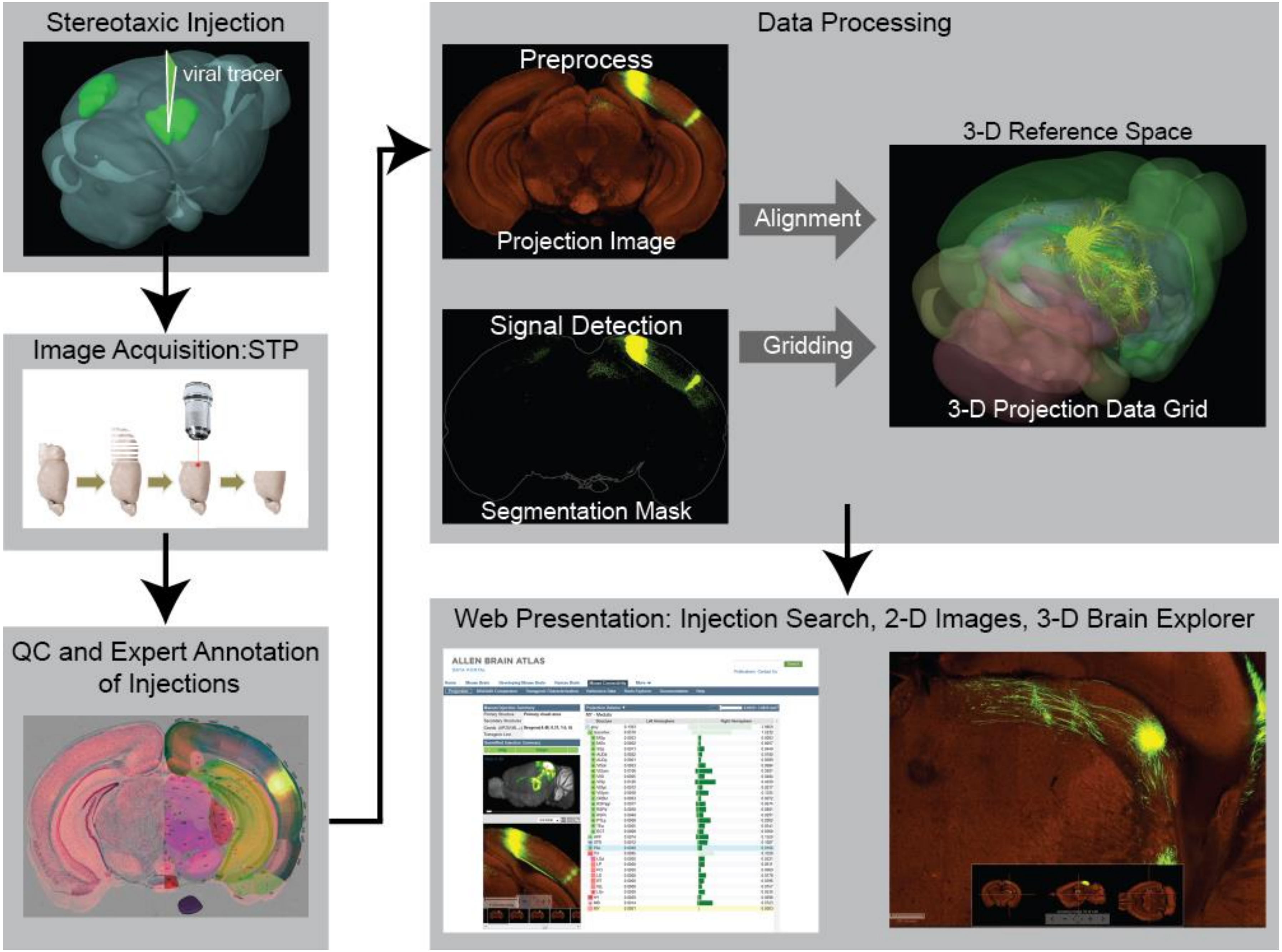
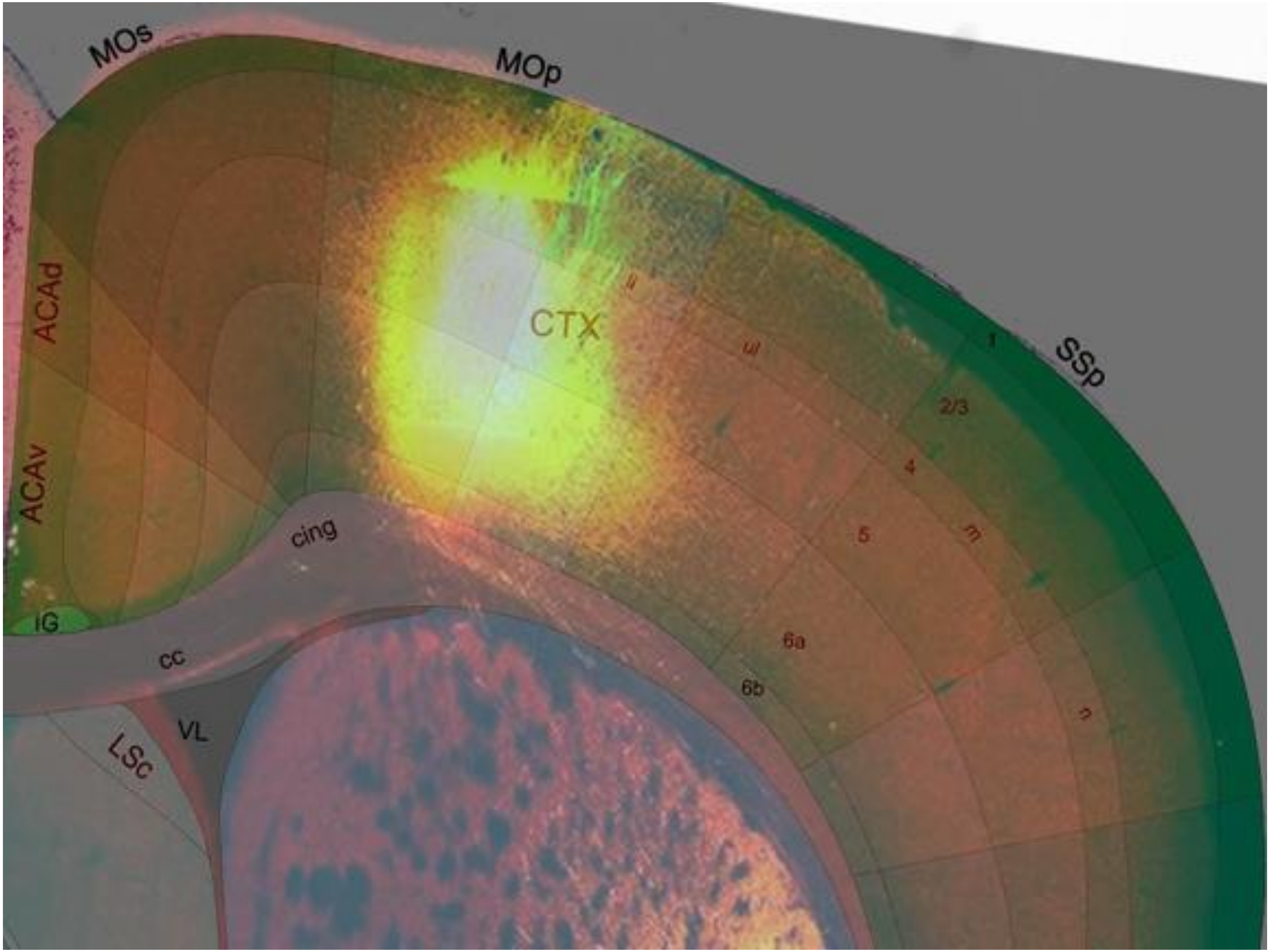


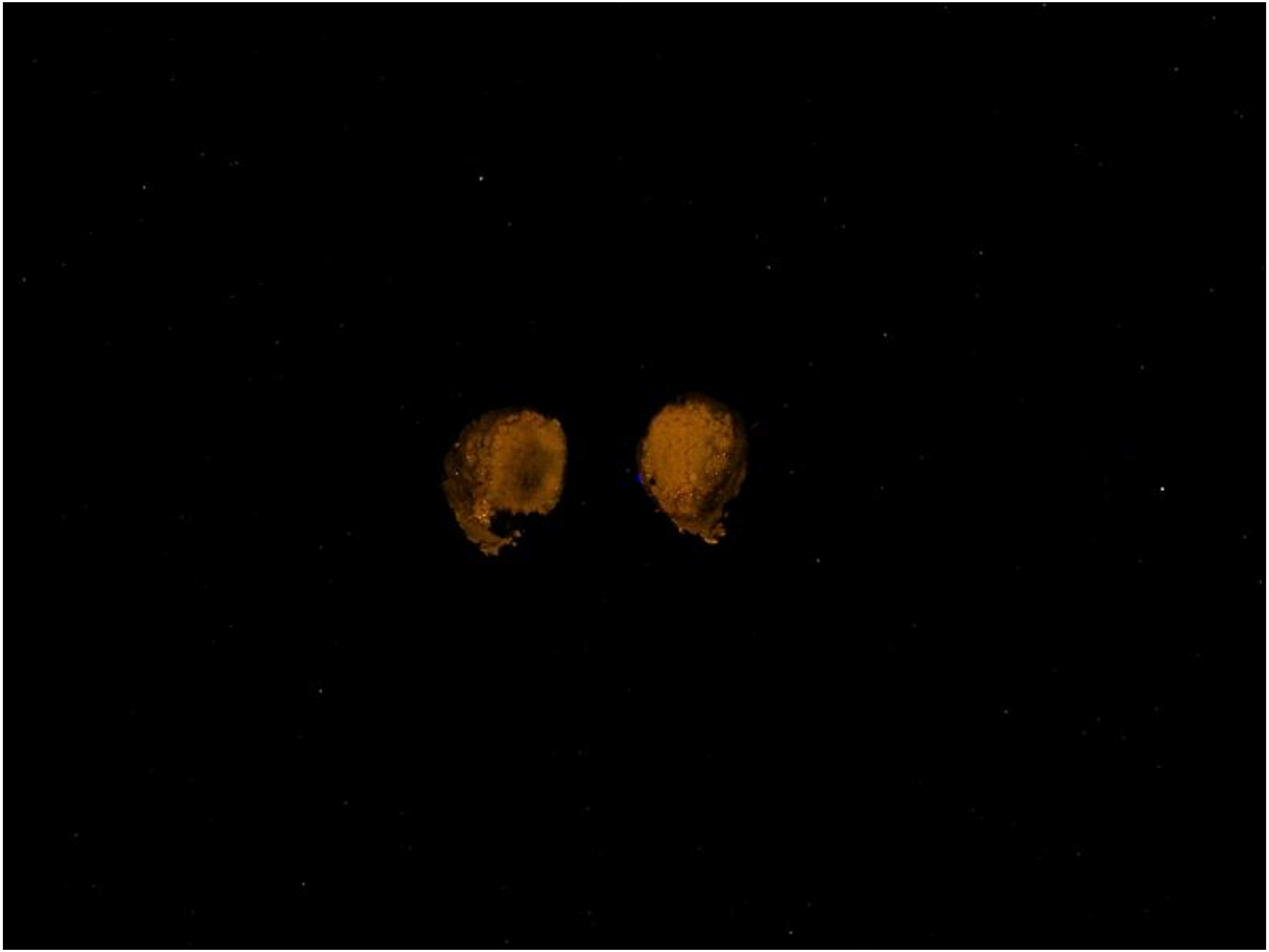
Pipeline


Stereotaxic Injection
viral tracer
Image Acquisition:STP
QC and Expert Annotation of Injections
Data Processing
Preprocess
Projection Image
Alignment
3-D Reference Space
Signal Detection
Segmentation Mask
Gridding
3-D Projection Data Grid
Web Presentation: Injection Search, 2-D Images, 3-D Brain Explorer
ALLEN BRAIN ATLAS

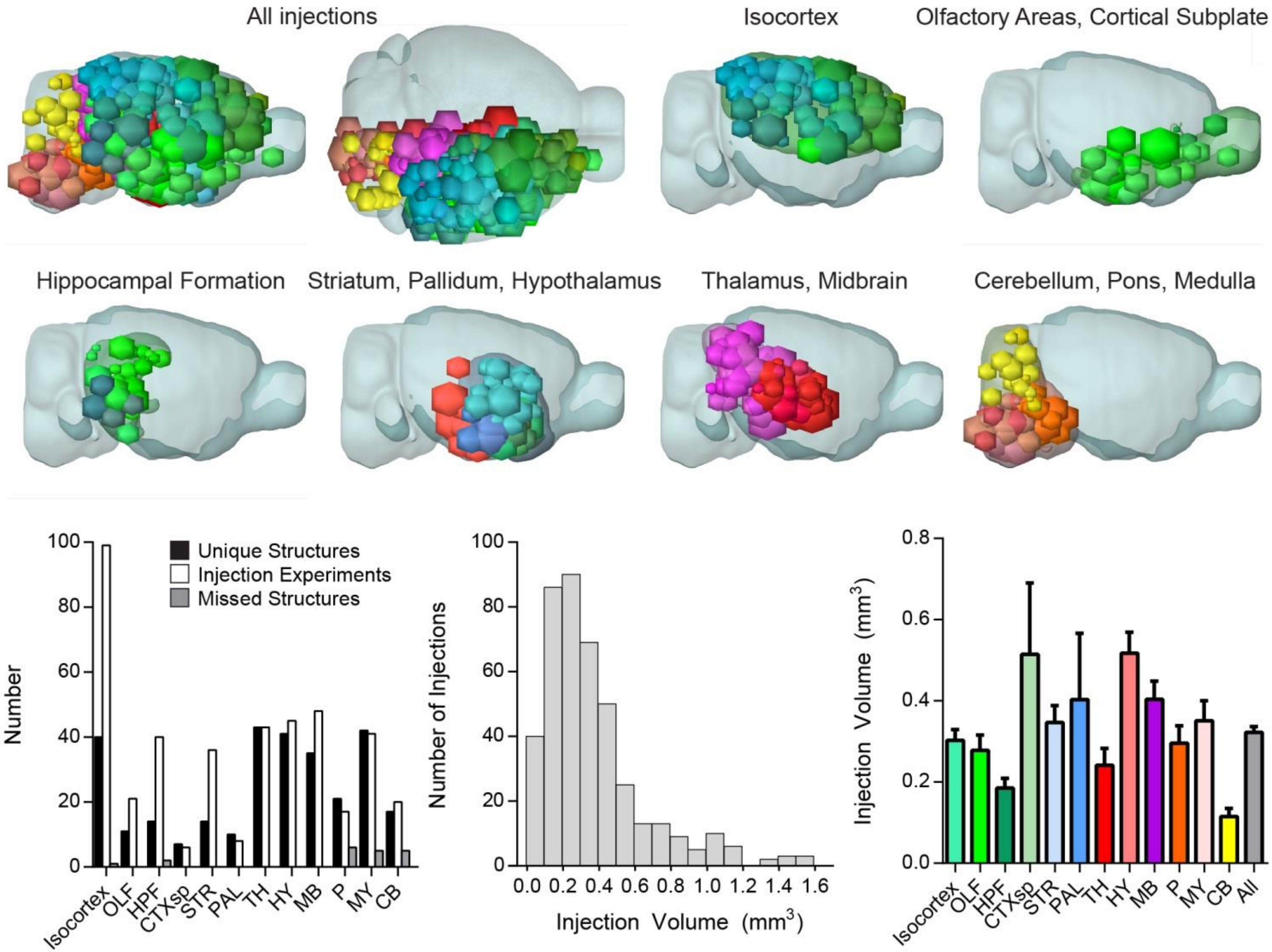
rAAV2.1-hSyn-EGFP-WPRE into Primary Motor Cortex (MOp)

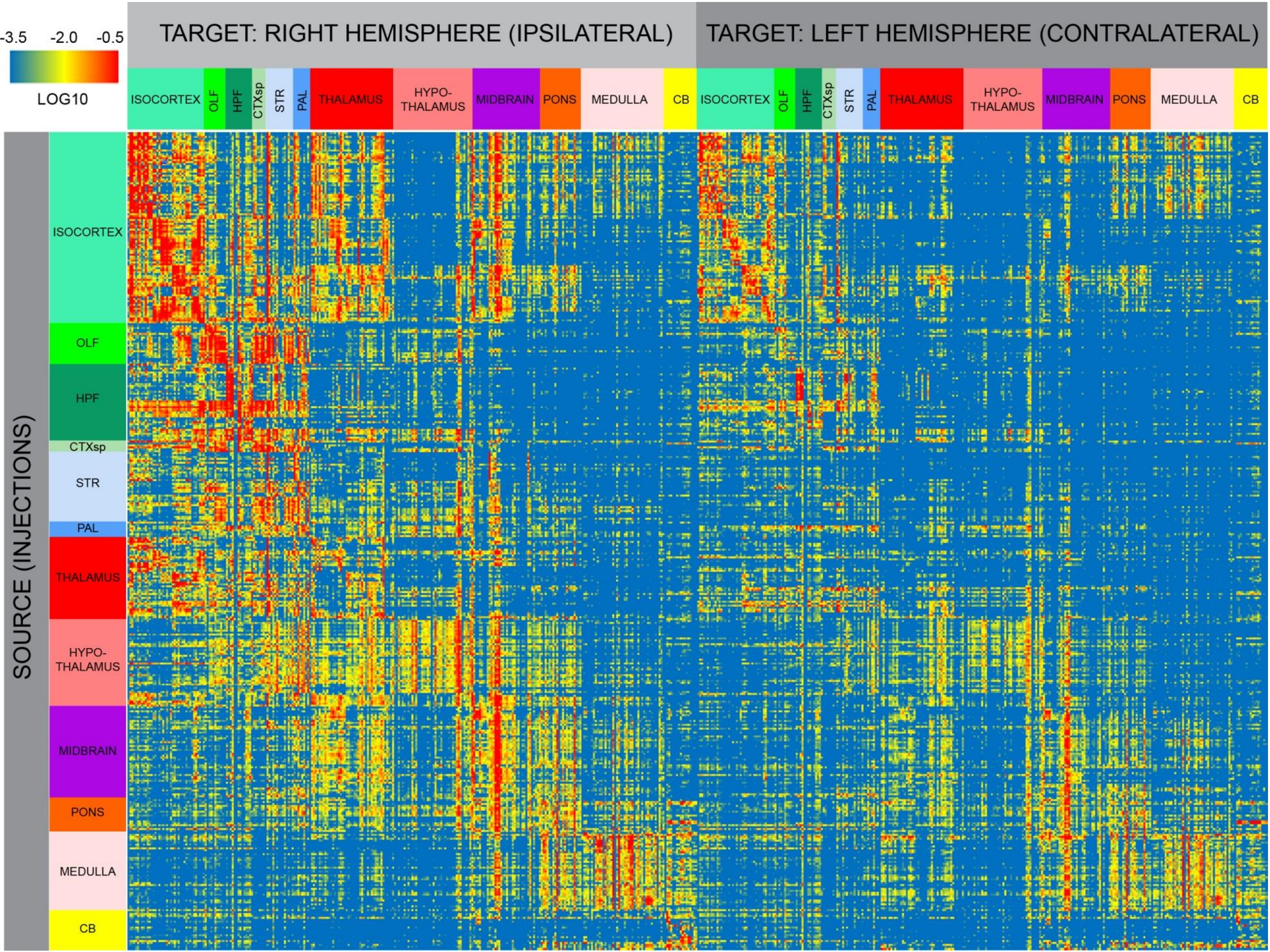


140 serial section images – MOp injection



Phase I: 424 Injection Sites and Whole Brain Coverage




-3.5 -2.0 -0.5
LOG10
TARGET: RIGHT HEMISPHERE (IPSILATERAL)
TARGET: LEFT HEMISPHERE (CONTRALATERAL)
ISOCORTEX
OLF
HPF
CTXsp
STR
PAL
THALAMUS
HYPO-THALAMUS
MIDBRAIN
PONS
MEDULLA
CB
SOURCE (INJECTIONS)

Phase II: Cre driver lines for projection mapping

GOAL: Map axonal projections from genetically-identified 1) **cell types** in subcortical areas and 2) **layer-specific cells** in cortical areas.

Scope:

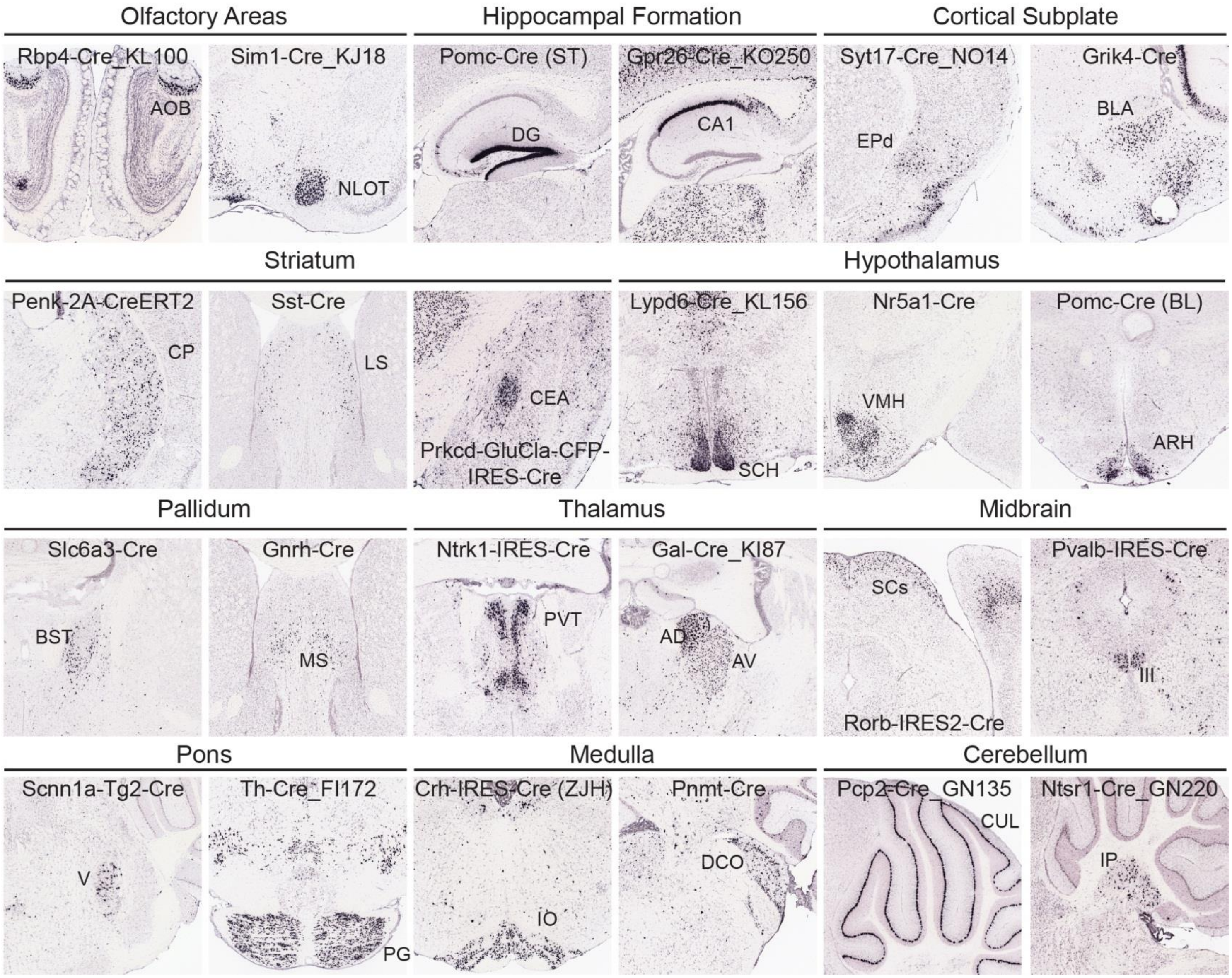
1) 800 subcortical injections, using most of ~ 100 Cre lines

2) 600 cortical injections, using 12 projection neuron + 10 interneuron lines Cre lines

Cre driver

Inject Cre-dependent virus to analyze projections

Cre lines for genetic access to brain areas or cell types



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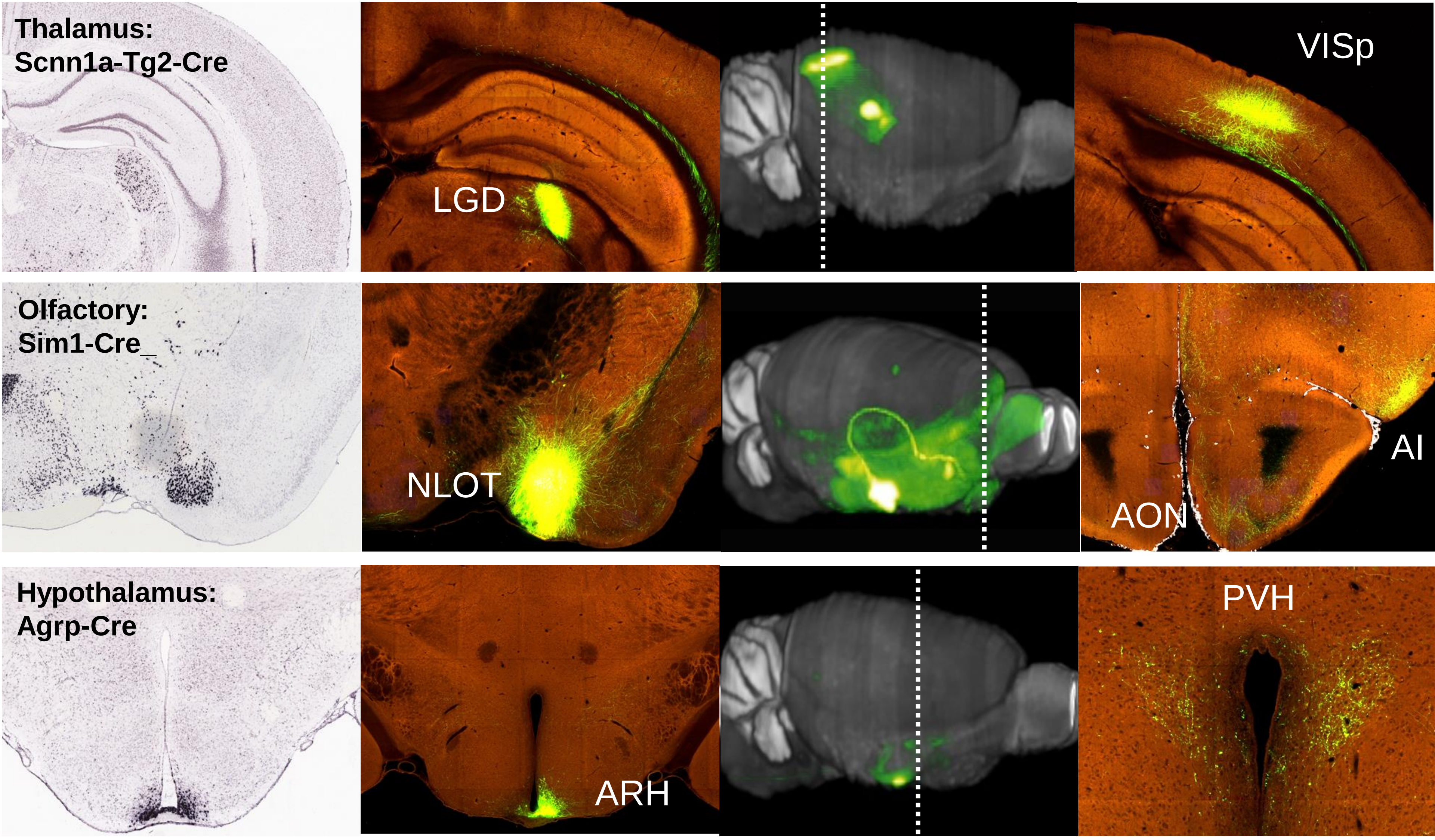
Harris et al. 2014

Subcortical Cre Lines Across Major Brain Divisions



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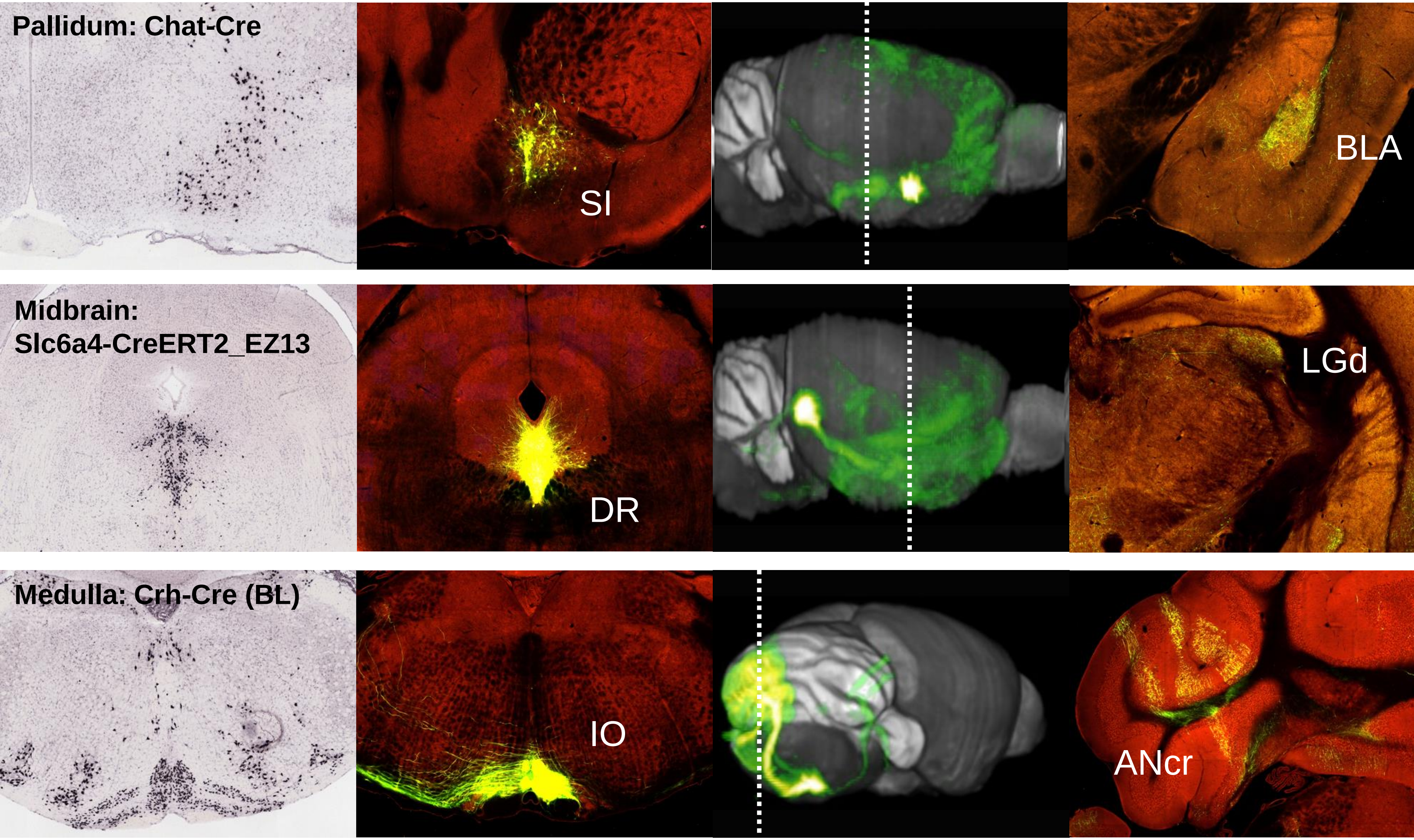
Subcortical Cre Lines Across Major Brain Divisions



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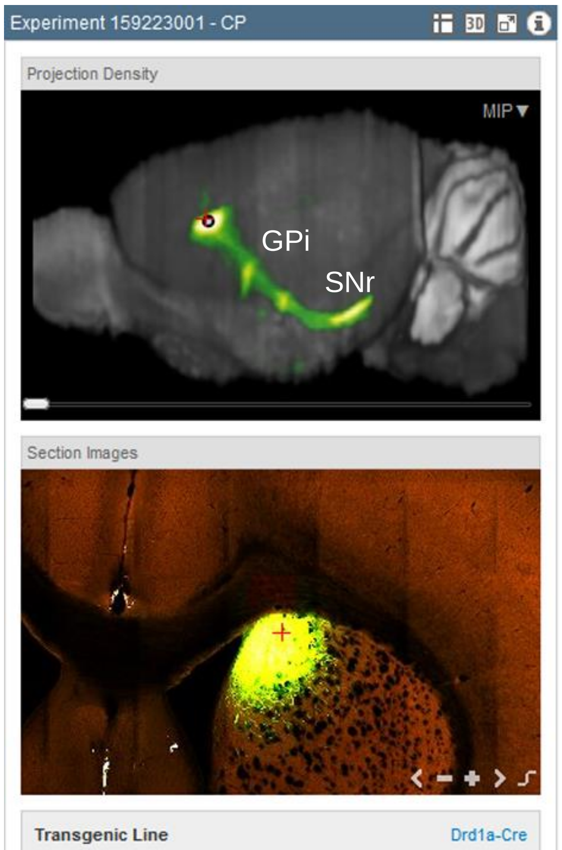
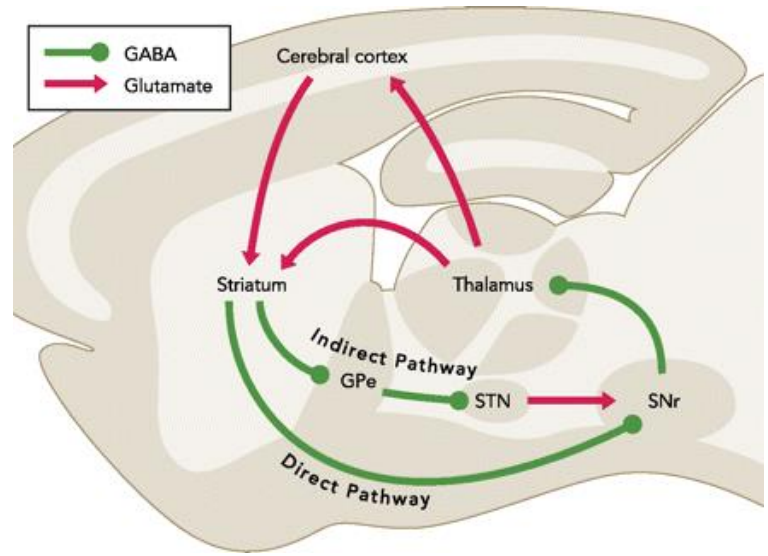
Cell type-specific long-range projections in subcortical areas

Confirmed differences in molecularly identified cell type projection neurons within circuits supporting distinct behaviors (D1 and D2 receptors in striatum).

Drd1a-Cre



Drd2-Cre_ER44



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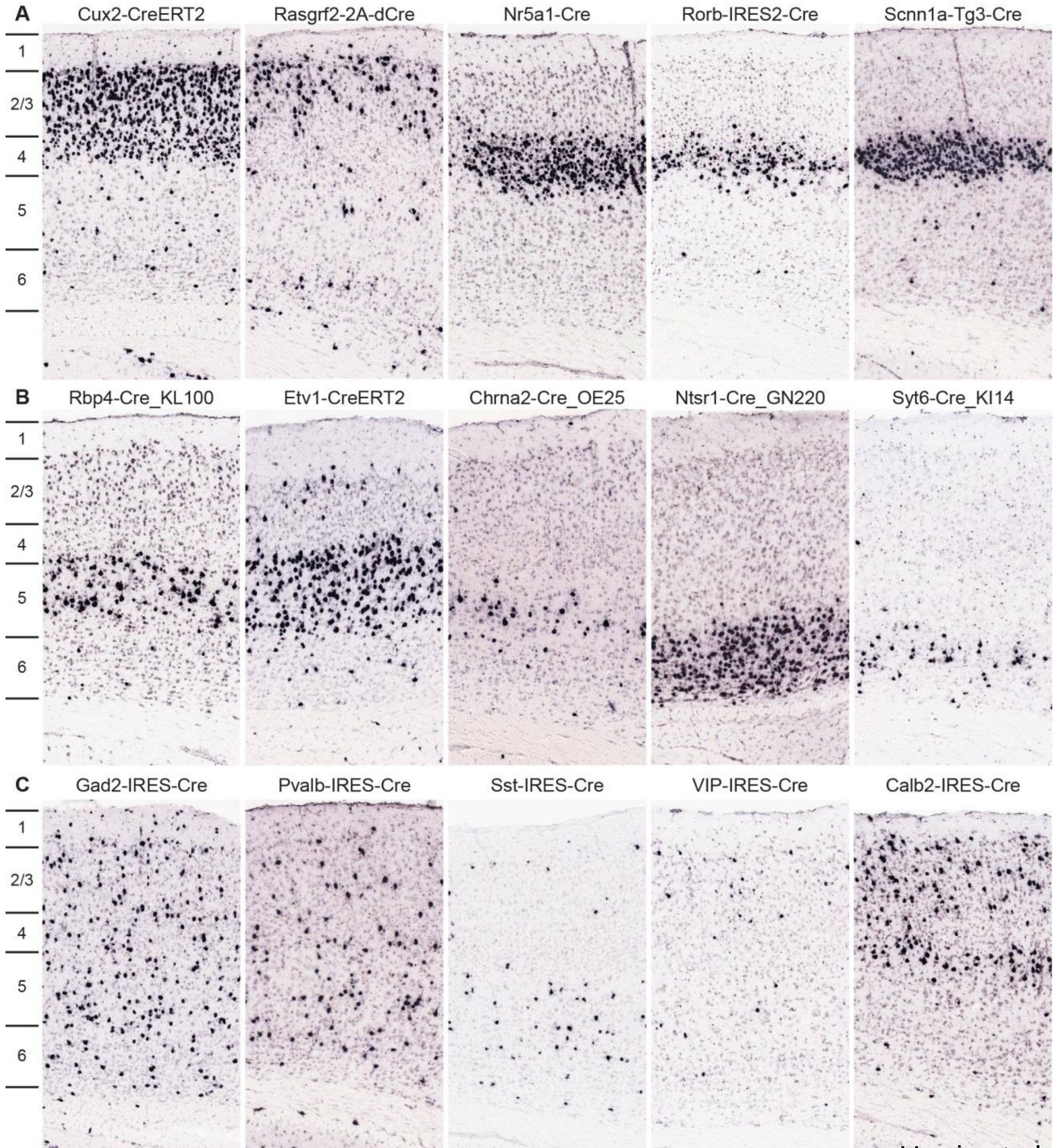
Cre Lines with Expression in Cortical Cell Types

- Mulitple Cre driver lines with expression restricted to cortical layers
- Cre driver lines with expression in interneurons



Harris et al. 2014

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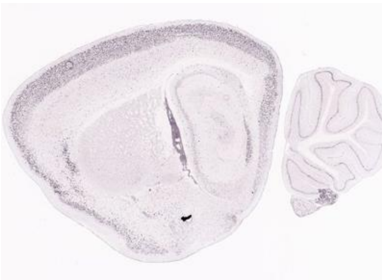
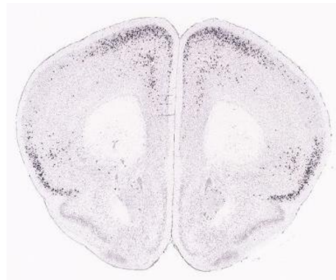
Superficial Cortical Projection Neuron Cre Lines in Pipeline

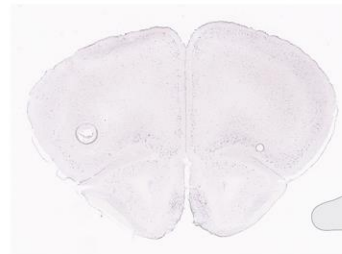
Layer2/3
Cux2-IRES-Cre



Layer 2/3, 5 (IT)
Grp-Cre_KH288



Layer 2 (PFC)
Grm2-Cre_MR90



Layer 2/3
Rasgrf2-2A-dCre

Layer 4 (SS)
Slc18a2-Cre_OZ14

Layer 4
Nr5a1-Cre

Layer 4
Rorb-IRES2-Cre

Layer 4/5
Scnn1a-Tg3-Cre

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Deep Layer Cortical Projection Neuron Cre Lines in Pipeline

Layer 5
Rbp4-Cre_KL100

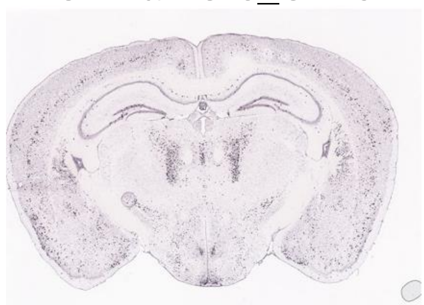
Layer 5
Gpr26-Cre_KO250

Layer 5
Etv1-CreERT2

Layer 5
Efr3a-Cre_NO108

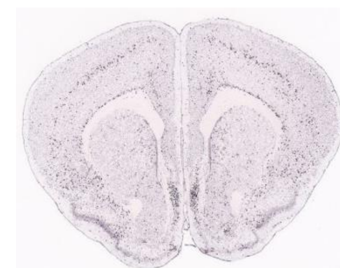
Layer 5
Trib2A-CreERT2

Layer 5 (PT)
Chrna2-Cre_OE25



Layer 5 (PT)
A93-Tg1-Cre

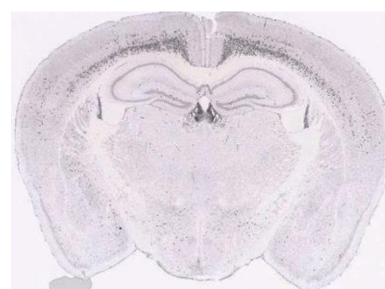
Layer 5 (MO)
Htr2a-Cre_KM207



Layer 5 (IT)
Tlx3-Cre

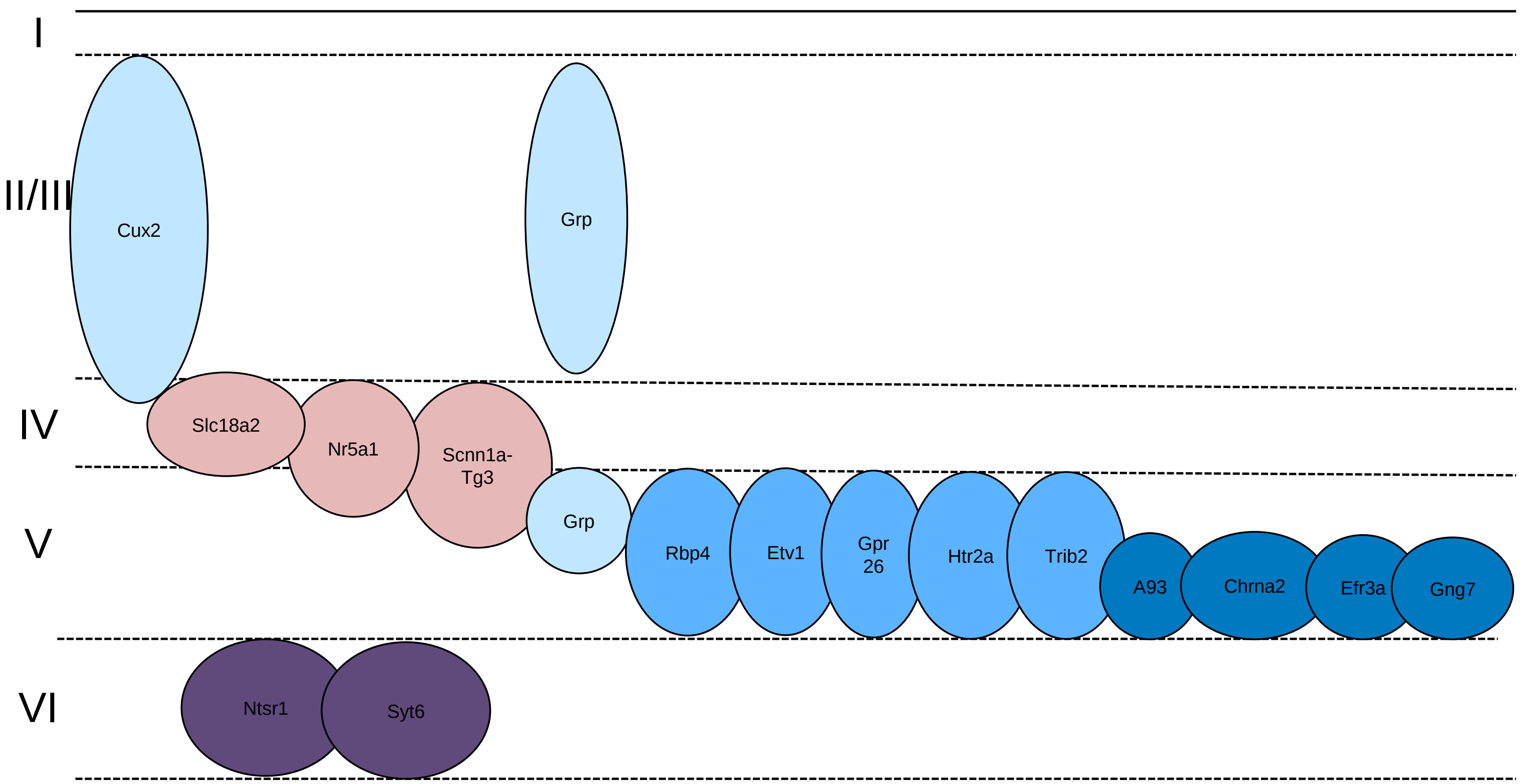
Layer 6
Ntsr1-Cre_GN220

Layer 6
Syt6-Cre_KI148



Layer 6b
Nxph4-2A-CreERT2

Building Cortical Layers out of Cre Lines



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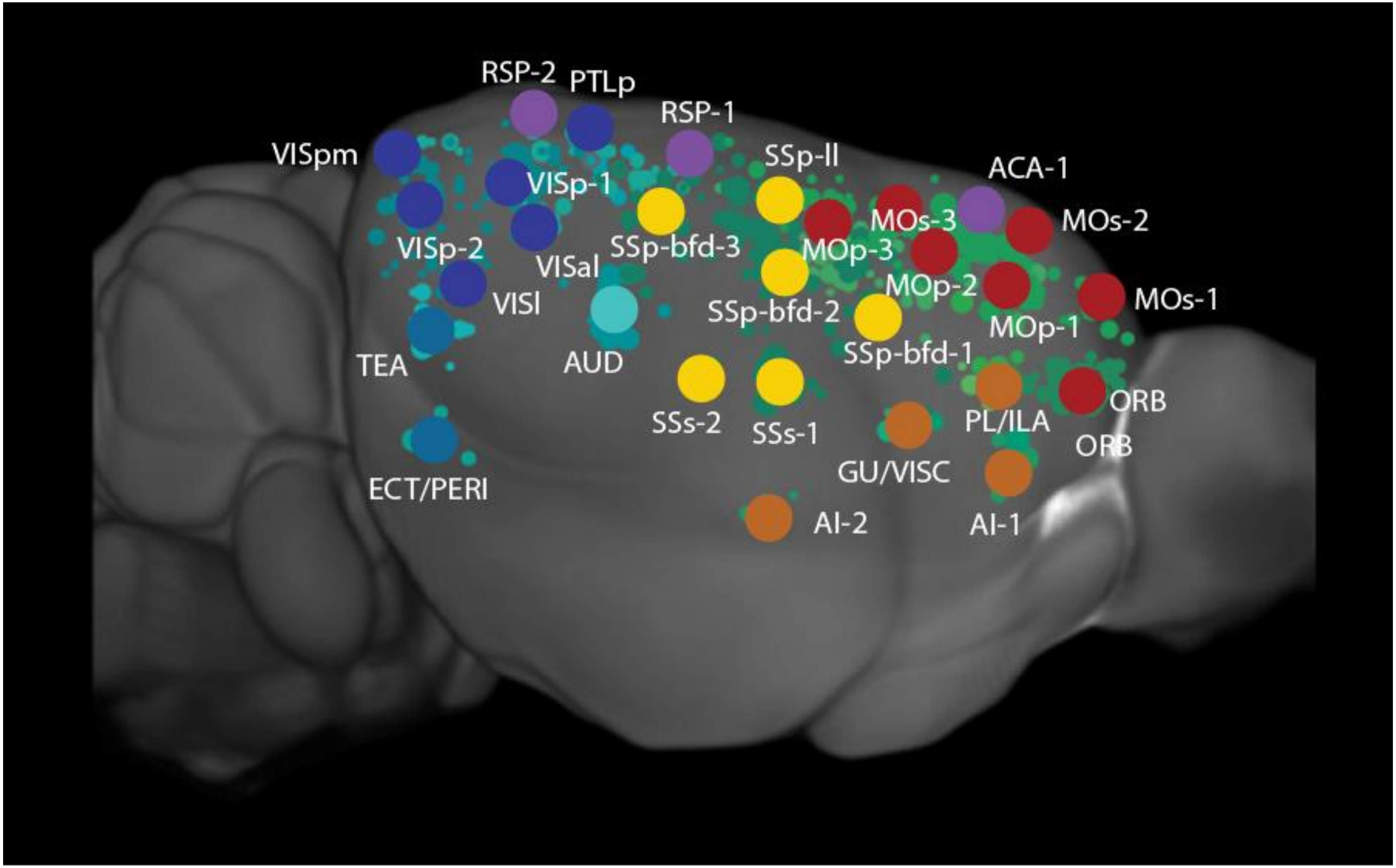
Cortical Cre Injection Sources (~ 30 total)



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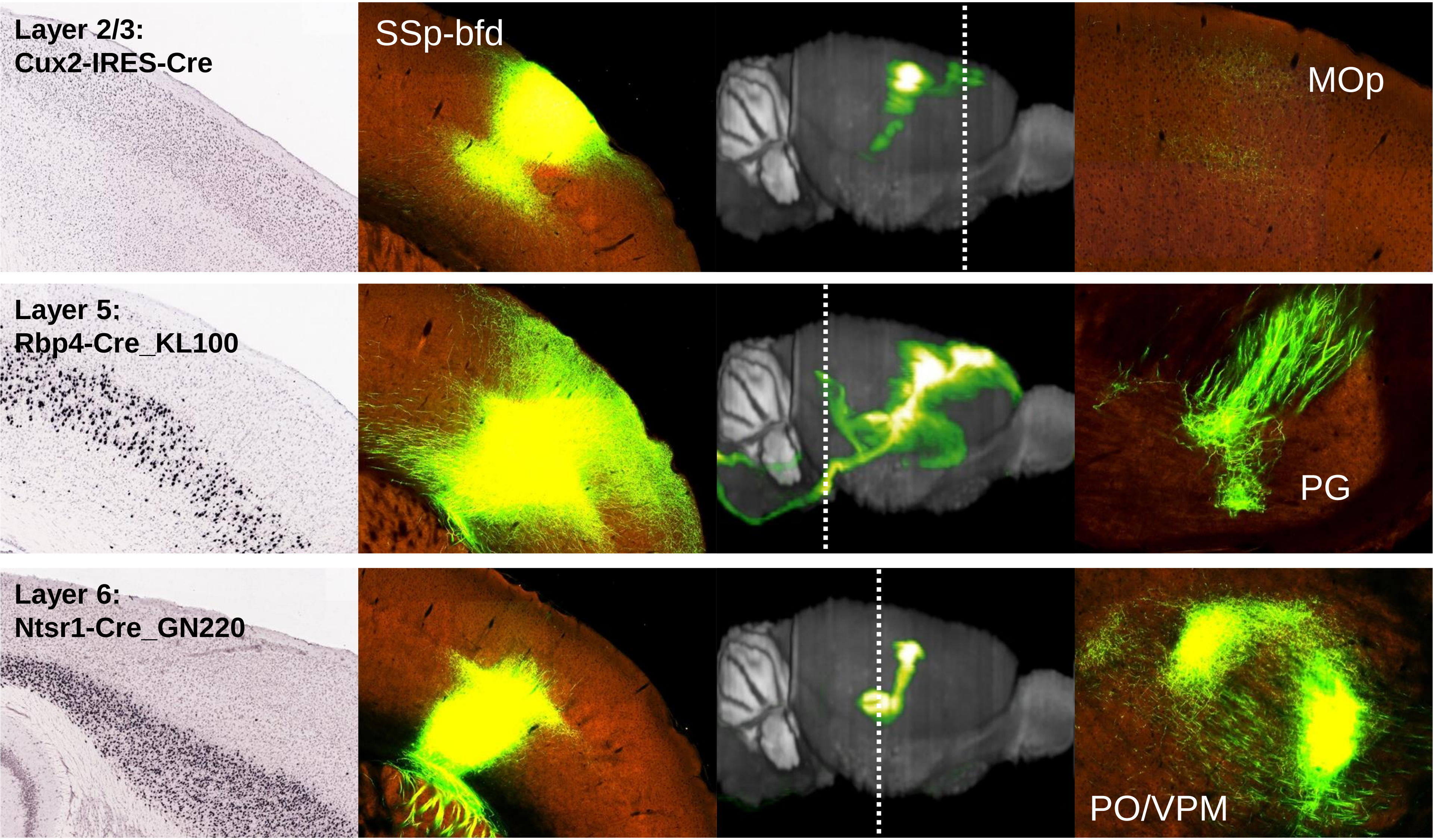
Cortical Cre Lines Representing Different Layers



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Connectivity Atlas – A Mesoscale Projectome

Features:

- Whole-brain coverage
- Single axon resolution
- High-precision co-registration of all datasets into a common 3D space
- Quantifiable
- Retaining realistic 3D spatial location and topography of projection targets as well as fiber tracts
- Including Cre-line based cell type specific projections (Phase II)

Enables:

- Computational network analysis: sub-networks, motifs, hubs, etc.
- More refined delineation of anatomical boundaries in 3D: improving traditional chemo- and cytoarchitecture based brain atlases
- Anterograde (from sources) and virtual retrograde (from targets) searches and comparisons
- Mapping anatomical network connectivity alterations in disease models.

alleninstitute.org
brain-map.org

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