New technologies for the identification and functional study of neuronal networks

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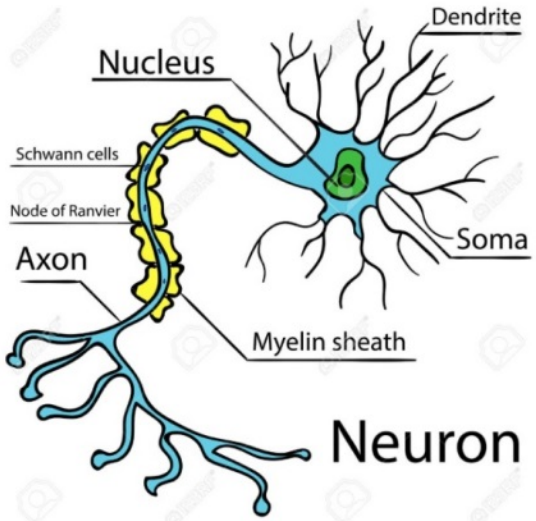
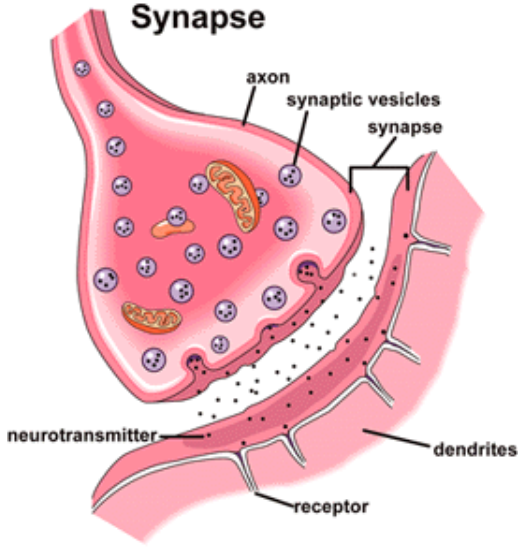
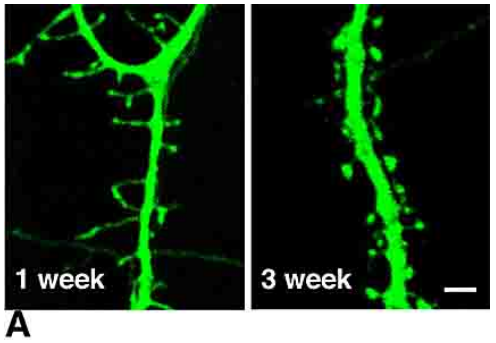
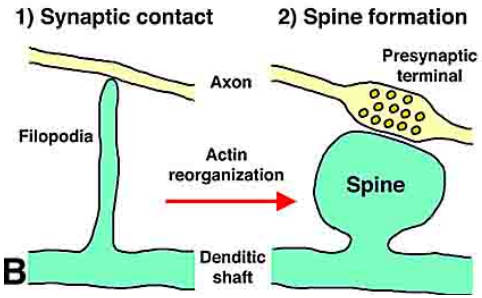
- History of the functional study of the brain
- Whole-brain mapping of neuronal networks responsible for specific brain functions
- Methods for functional study of neural networks

Neural circuit is the basis for brain fucntions









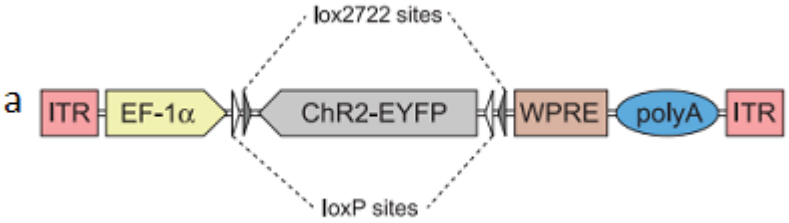
Early functional studies of the brain

(1) Selective surgical ablation of parts of the brain of animals;

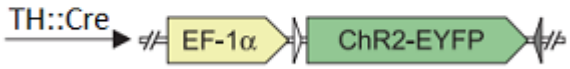
(2) Faradic and galvanic (i.e., steady or pulsed electrical) stimulation of the brain of animals and humans;

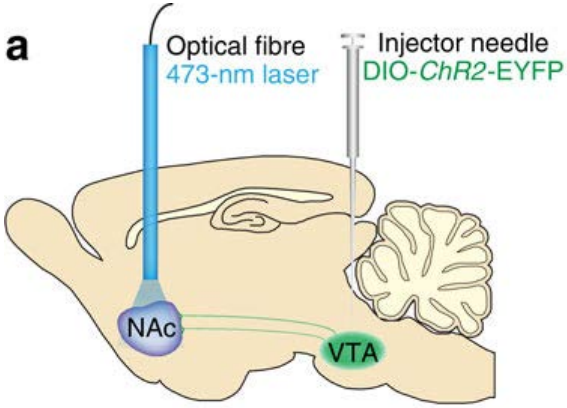
Cell-type specific ablation and functional manipulations

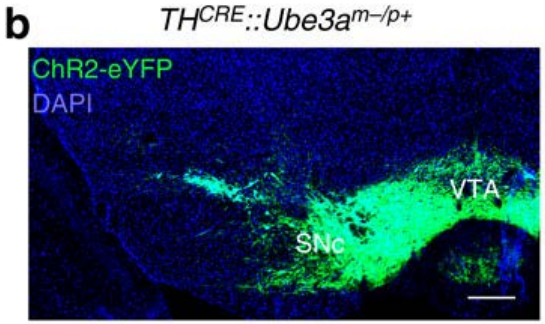
iDTR
hM3Dq



iDTR
hM3Dq

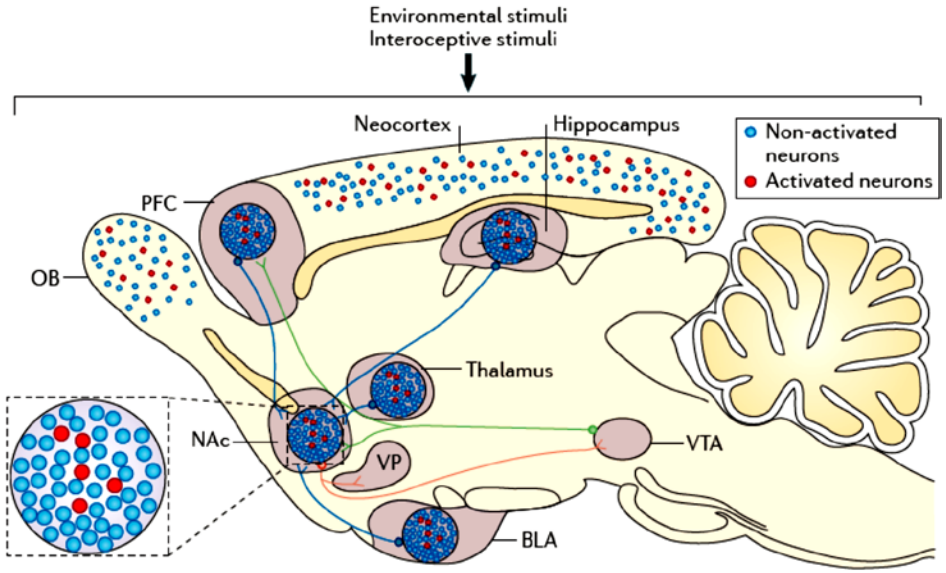






Modified from
Tsai HC and Deisseroth K et al., 2009, Science
Janet Berrios, Garret D. Stuber & Benjamin D. Philpot et al., 2015, Nature Communications

Neuronal ensembles in brian activities



Mesocorticolimbic dopamine reward system

Neuronal Ensembles

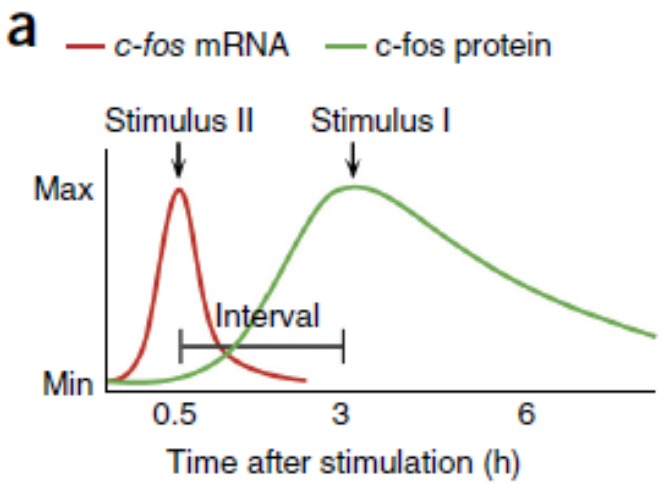
A group of neurons that show spatiotemporal co-activation.

Fabio C. Cruz and Bruce T. Hope et al., 2013, Nature Reviews Neuroscience

Immediate early genes (IEGs) as faithful surrogates of neuronal activation

Table 1
Summary of activity-regulated, neuronal immediate-early genes.

Category	Gene
Transcription factors	*c-fos*
	fos B
	c-jun
	junB
	*zif*268/egr1/*krox*24/*NGFI-A* *egr2*/*krox*20 egr3/pilot nur-77/*NGFI-B*
Postsynaptic proteins	*Arc*/*arg*3.1 *homer*1a/*vesl*1s
Intracellular signaling	Rheb
	RSG2
	SNK/Plk2 Cox-2
Secretory factors	BDNF
	Activin β A
	Narp
	Tissue-plasminogen activator (tPA)
Membrane proteins	Arcadin CPG15/neuritin



Hiroyuki Okuno, 2011, Neuroscience Research
Jianbo Xiu, Qi Zhang & Hailan Hu, 2014, Nature Neuroscience

Mapping of Brain Activity by Automated Volume Analysis of Immediate Early Genes

Cell

Nicolas Renier,[1,7] Eliza L. Adams,[1,7] Christoph Kirst,[2,7] Zhuhao Wu,[1,7] Ricardo Azevedo,[1] Johannes Kohl,[3] Anita E. Autry,[3] Lolahon Kadiri,[5] Kannan Umadevi Venkataraju,[4,5] Yu Zhou,[6] Victoria X. Wang,[6] Cheuk Y. Tang,[6] Olav Olsen,[1] Catherine Dulac,[3] Pavel Osten,[4] and Marc Tessier-Lavigne[1,*]

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[7]Co-first author
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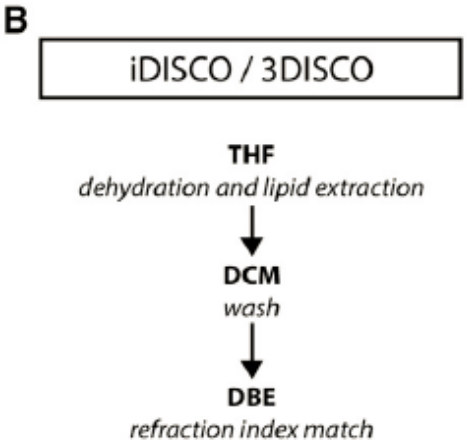
Whole-mount tissue staining

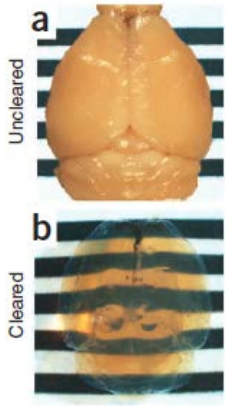
Whole-mount tissue clearing

Method[1]	Year
ScaleA2	2011
ClearT2	2012
3DISCO	2012
CLARITY	2013
SeeDB	2013
CUBIC	2014

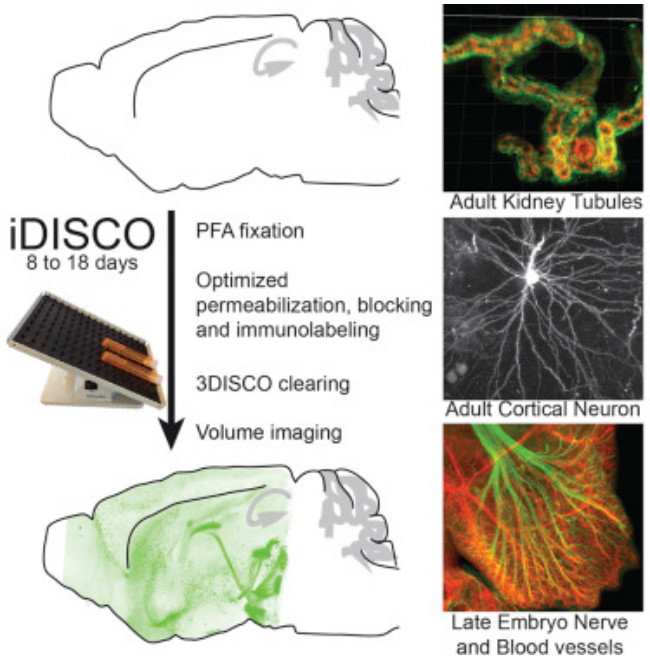
Method[1]	Year	Main clearing & refractive index matching agents	Cost[2]	Clearing capability	Ease of implementation	Time to clear	Compatibility with immunostaining
ScaleA2	2011	Urea	Inexpensive	Good	Very easy	*Months*	*May be limited*[3]
ClearT2	2012	Formamide	Inexpensive	*Moderate*	Very easy	1 day	Compatible
3DISCO	2012	Benzyl Ether	Inexpensive	Very good	Very easy	1 day	Compatible
CLARITY	2013	SDS & Focusclear	*More expensive*[4]	Good	*More difficult*[4]	*Weeks*[4]	*May be limited*[3]
SeeDB	2013	Fructose	Inexpensive	*Moderate*	*Moderate*[5]	*Several days*	Compatible
CUBIC	2014	Multiple Compounds	Inexpensive	Not fully tested	*Moderate*[5]	*Weeks*	Not fully tested



THF: tetrahydrofuran
DCM: Dichloromethane
DBE: DiBenzyl Ether

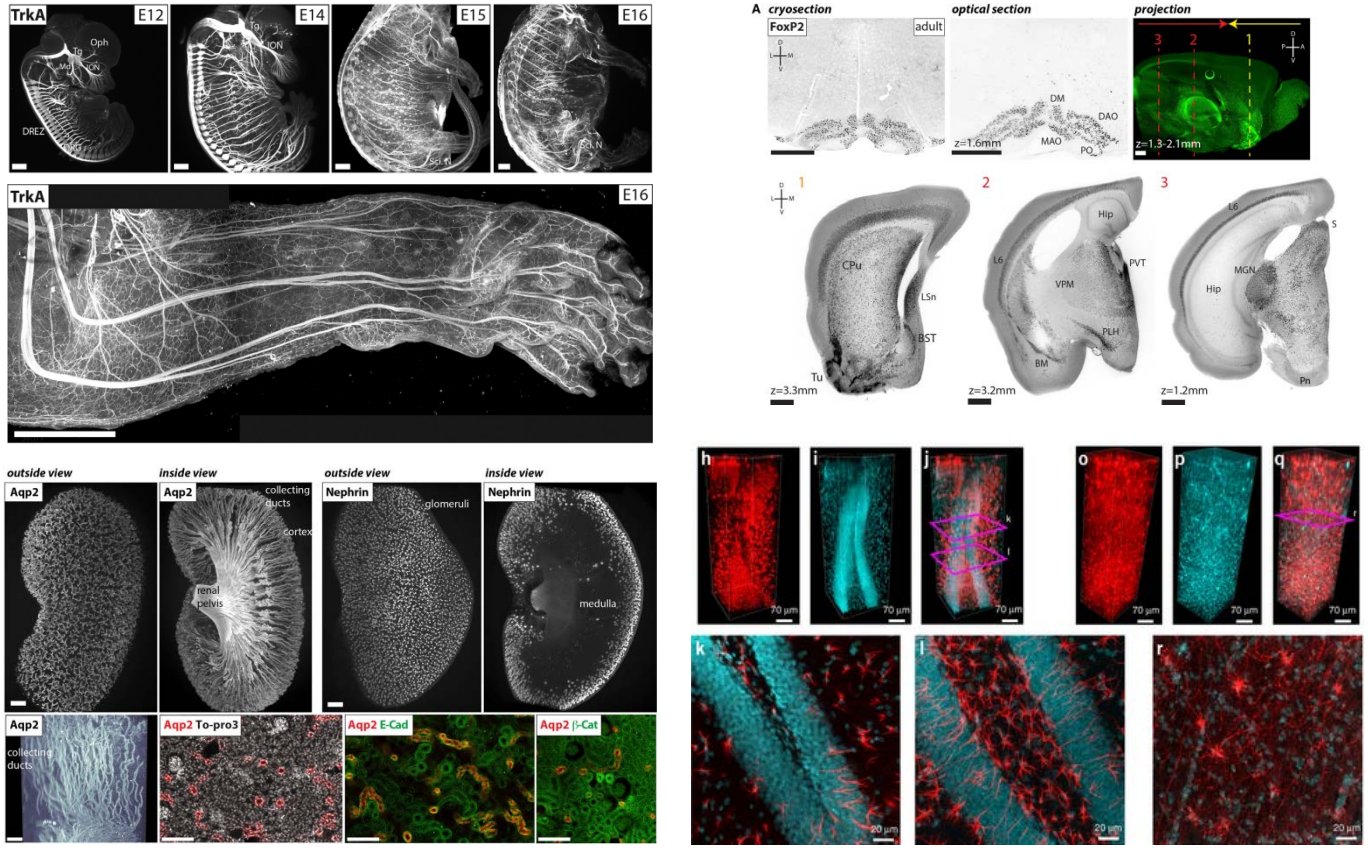


iDISCO: A Simple, Rapid Method to Immunolabel Large Tissue Samples for Volume Imaging



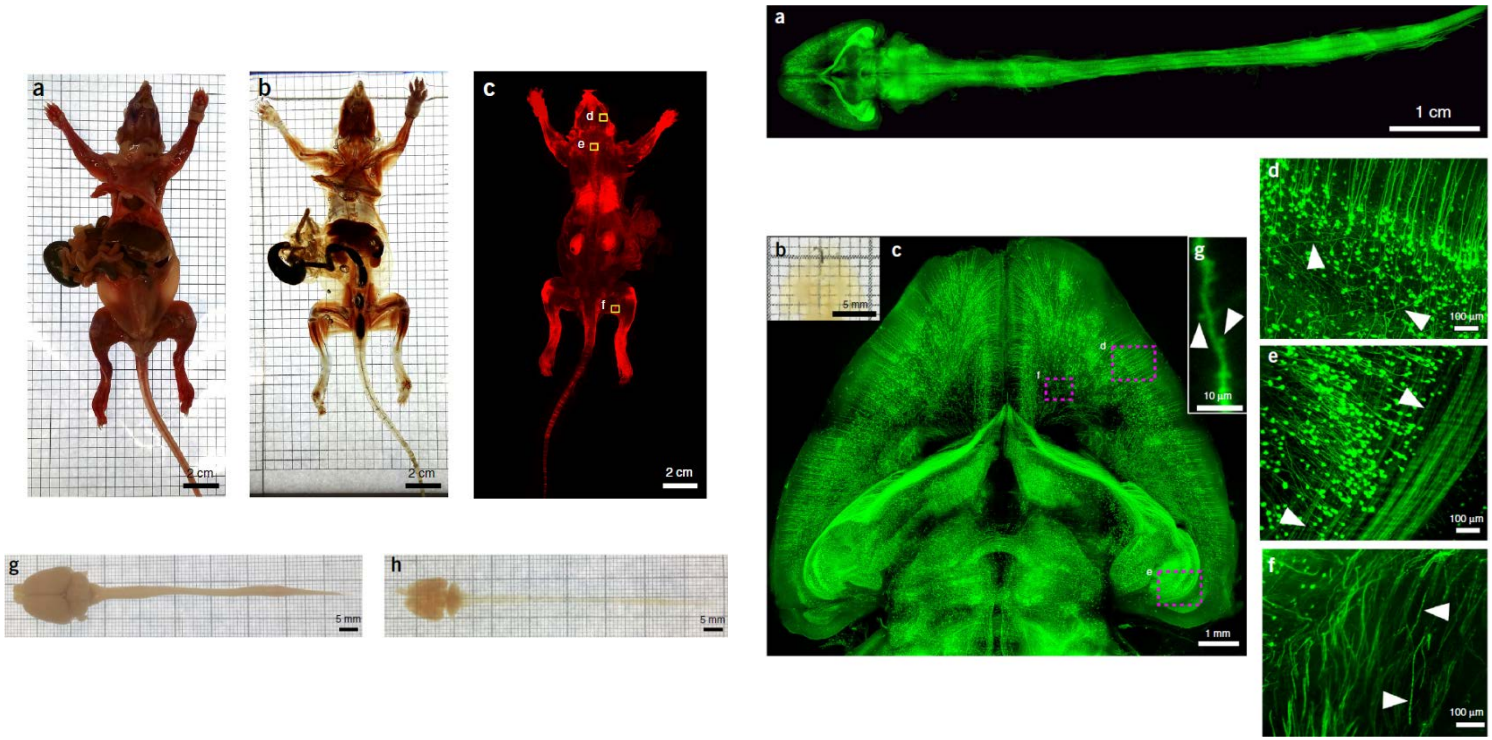
Highlights

- iDISCO is a method for immunolabeling and volume imaging of large biological samples
- Could be used in large mouse embryos and adult organs
- Easy to implement, inexpensive, fast, and reliable



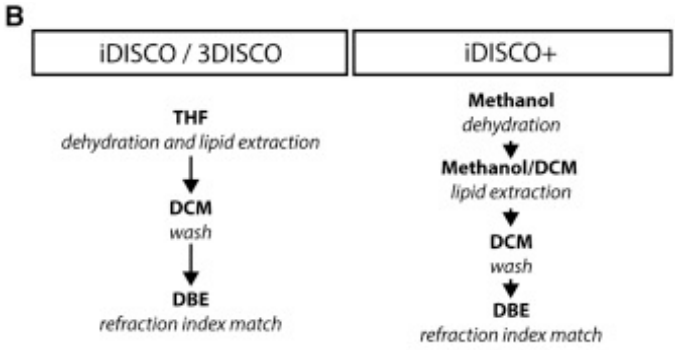
Nicolas Renier, Zhuhao Wu and Marc Tessier-Lavigne et al., 2014, Cell
Chenchen Pan, Ruiyao Cai, Francesca Paola Quacquarelli & Ali Ertürk et al., 2016, Nature Methods

Shrinkage-mediated imaging of entire organs and organisms using uDISCO



Chenchen Pan, Ruiyao Cai, Francesca Paola Quacquarelli & Ali Ertürk et al., 2016, Nature Methods

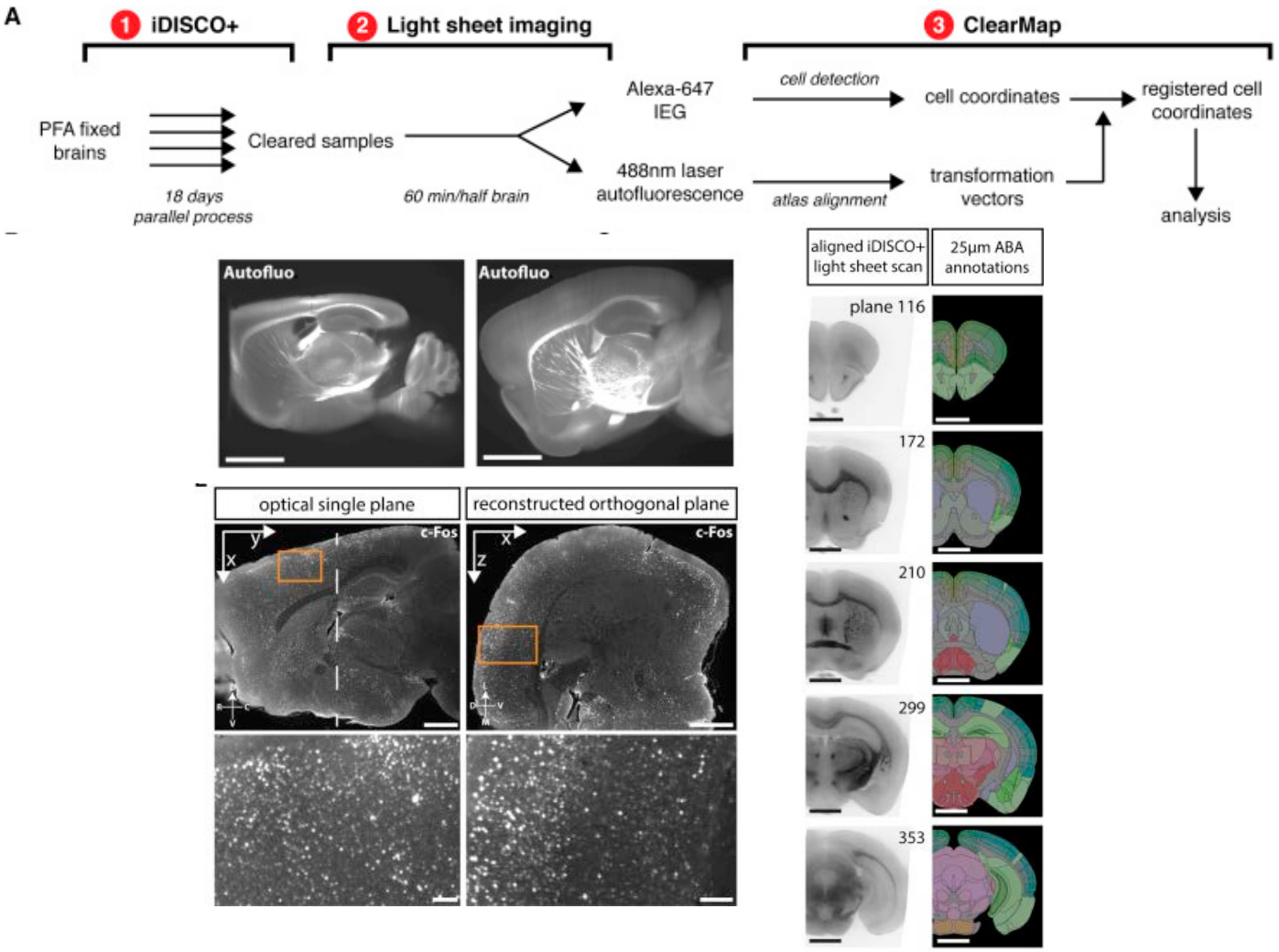
iDISCO+ and ClearMap: A Pipeline for Cell Detection, Registration, and Mapping in Intact Samples Using Light Sheet Microscopy

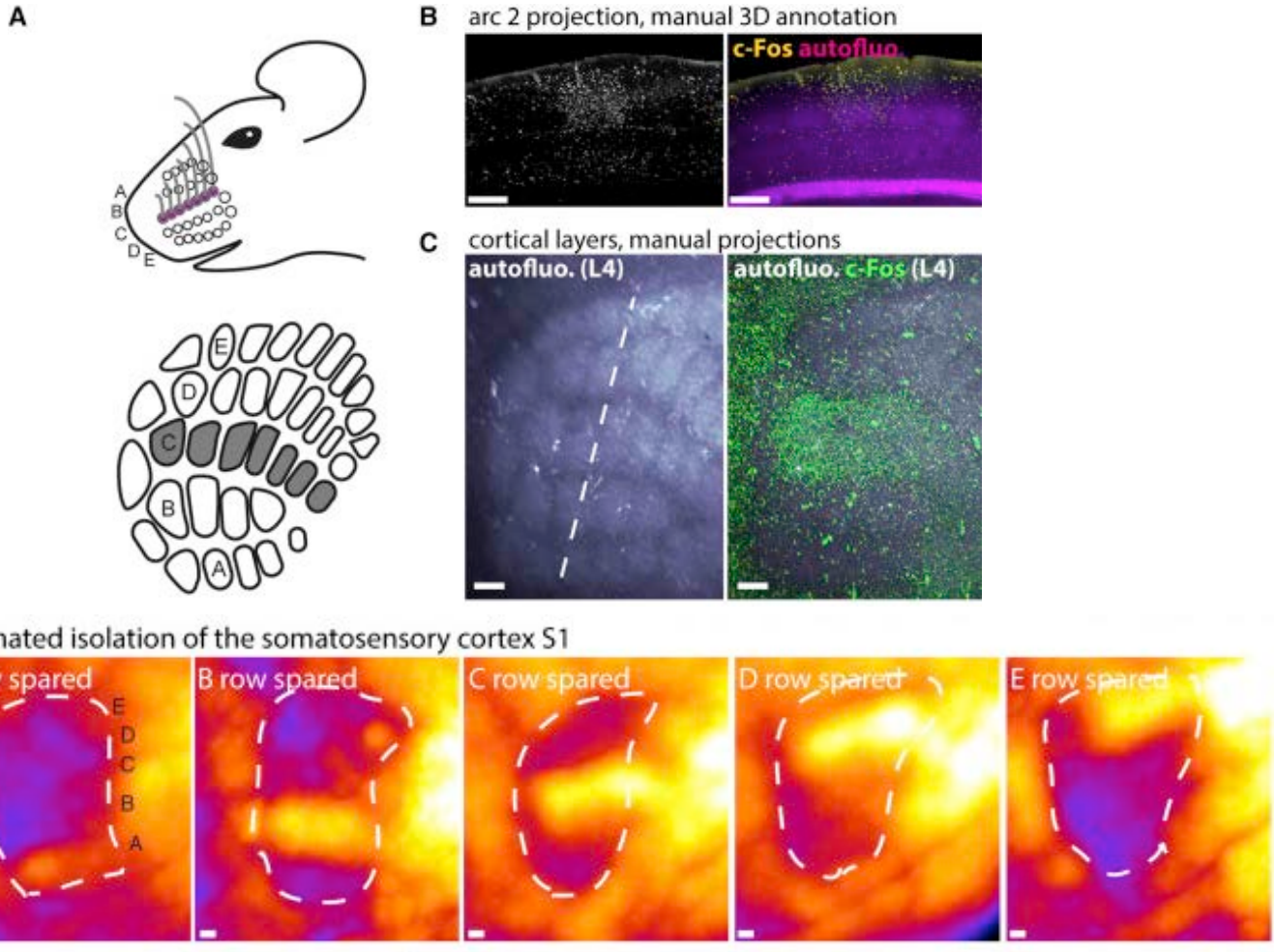


THF: tetrahydrofuran
DCM: Dichloromethane
DBE: DiBenzyl Ether

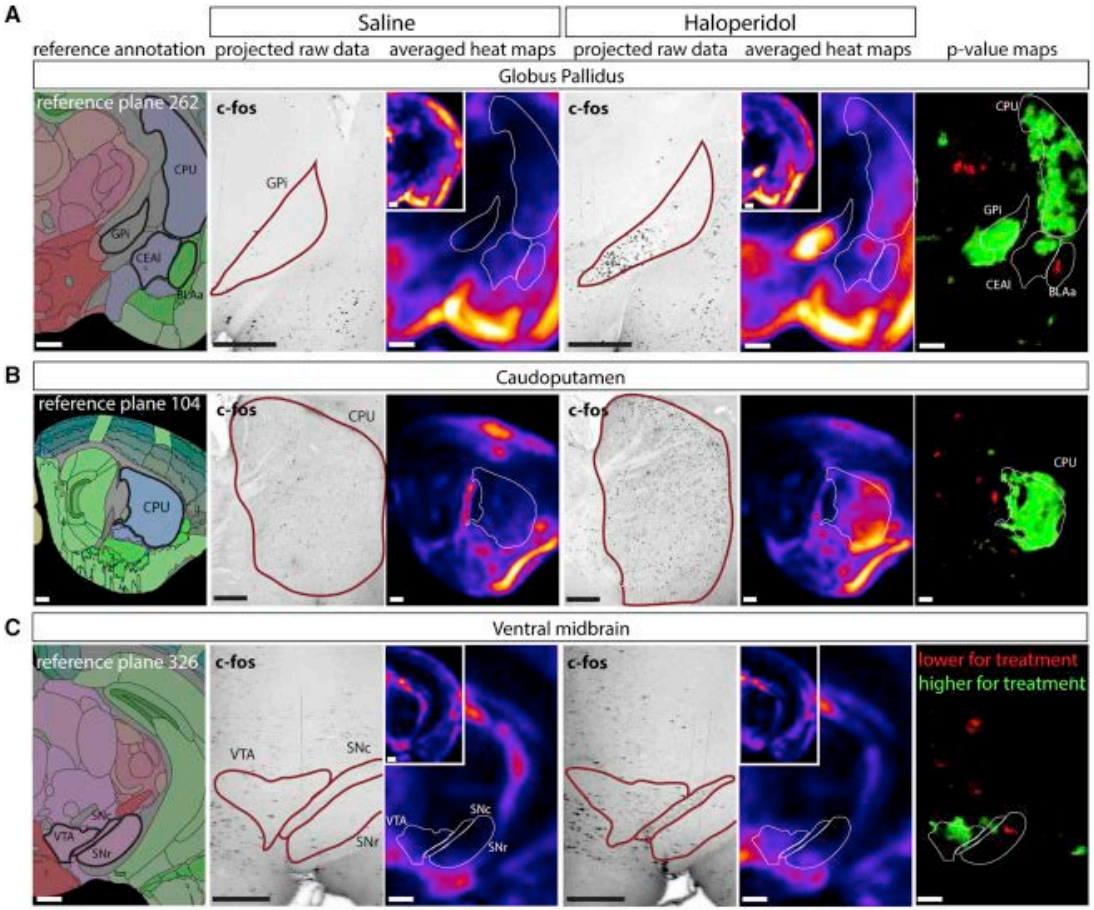
• **iDISCO+**
Improved version of the iDISCO protocol to reduce sample shrinkage and better preserve brain morphology, thereby enabling automated registration of the LSFM-imaged sample onto a reference brain atlas for automated comparisons

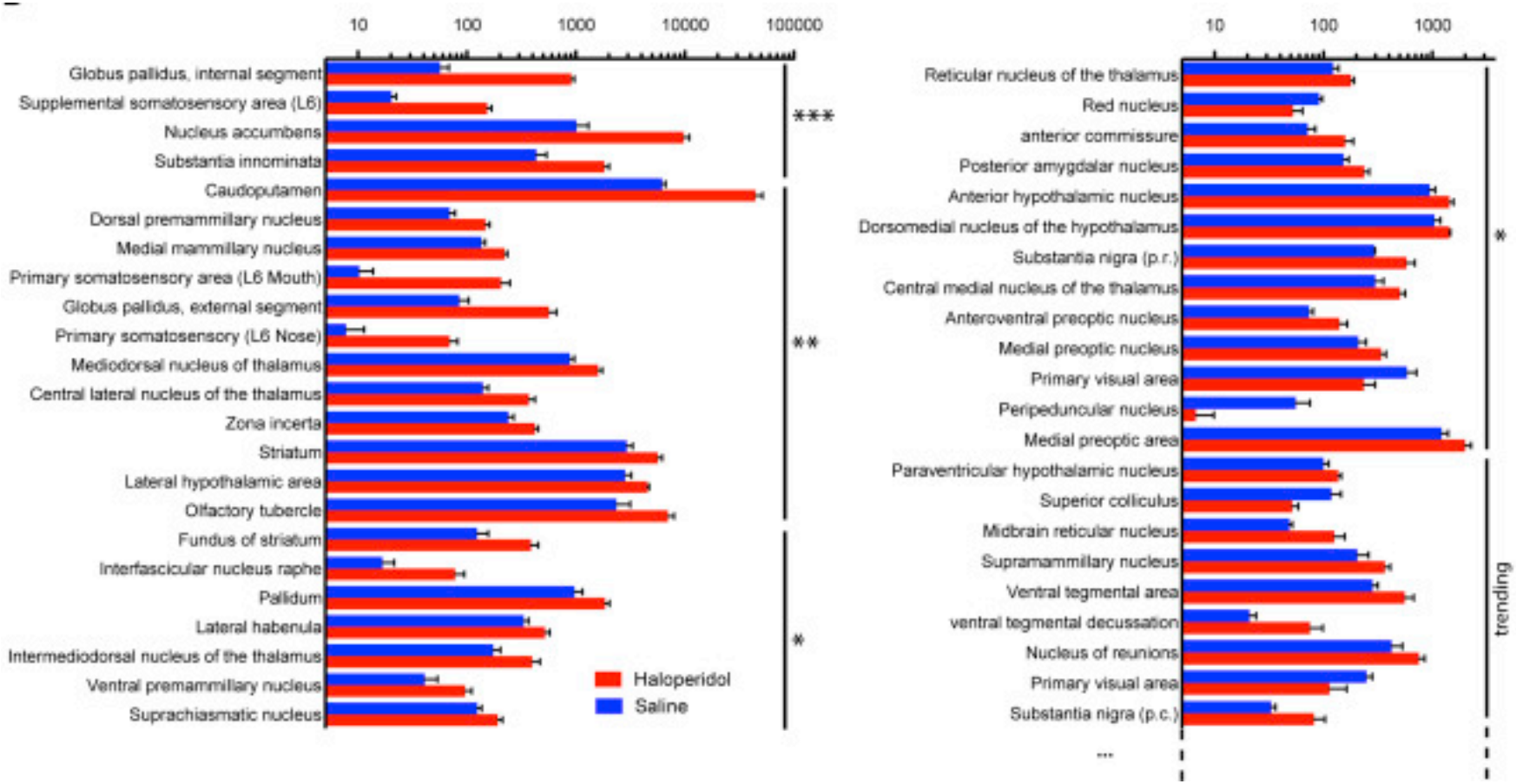
•ClearMap
A computer program based solely on open-source components and compatible with a desktop workstation that is applied to the imaging data to count cells in 3D, registers them onto a reference atlas, and generates distribution maps and statistical analysis of intact mouse brains in <1 hr per sample.


A
1 iDISCO+
2 Light sheet imaging
3 ClearMap
PFA fixed brains
18 days parallel process
Cleared samples
60 min/half brain
Alexa-647 IEG
488nm laser autofluorescence
cell detection
cell coordinates
registered cell coordinates
atlas alignment
transformation vectors
analysis
Autofluo
Autofluo
optical single plane
reconstructed orthogonal plane
c-Fos
c-Fos
aligned iDISCO+ light sheet scan
25µm ABA annotations
plane 116
172
210
299
353

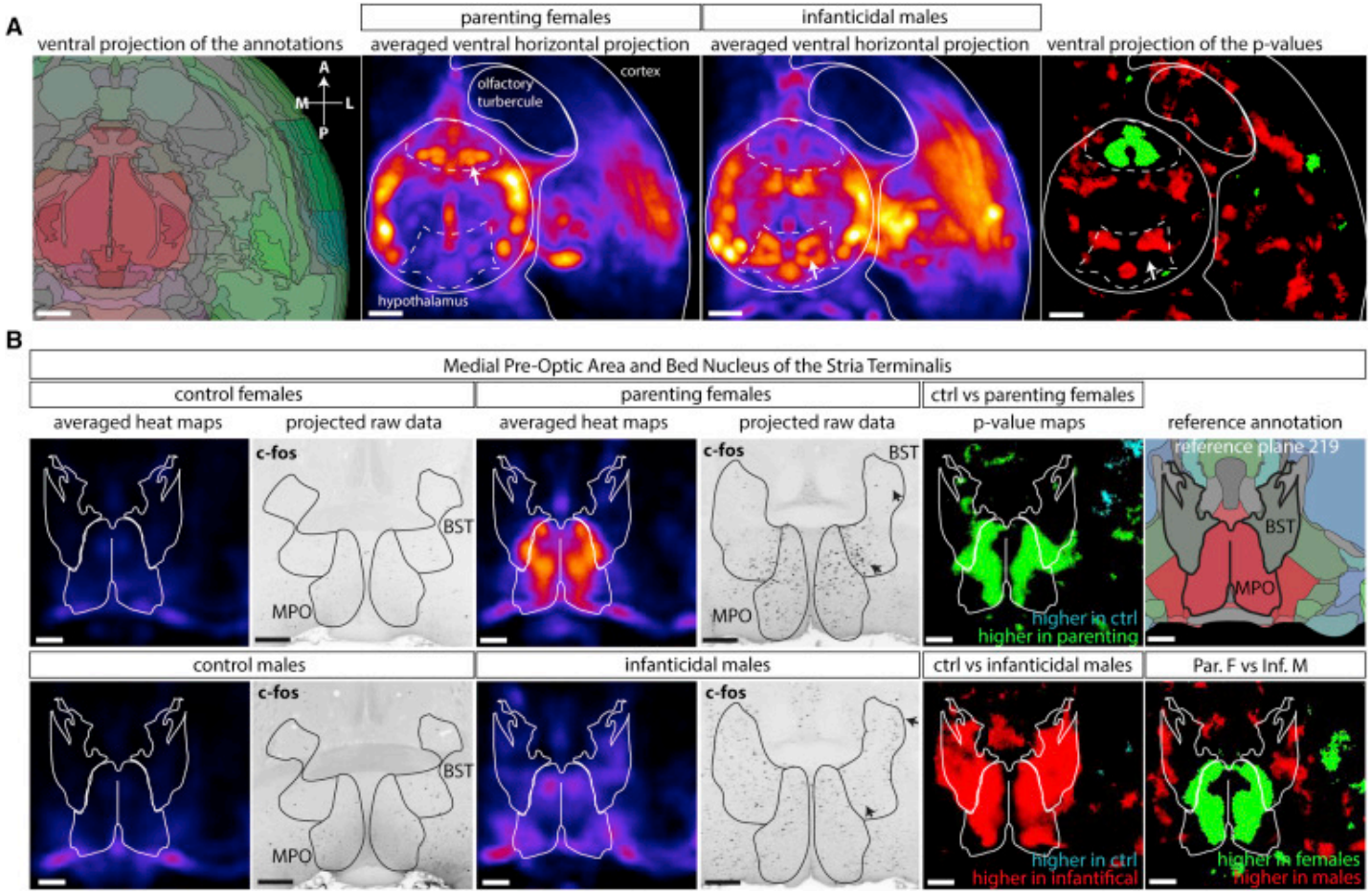

A
B
arc 2 projection, manual 3D annotation
c-Fos autofluo.
C
cortical layers, manual projections
autofluo. (L4)
autofluo. c-Fos (L4)
D
automated isolation of the somatosensory cortex S1
cortex
A row spared
B row spared
C row spared
D row spared
E row spared
E
D
C
B
A

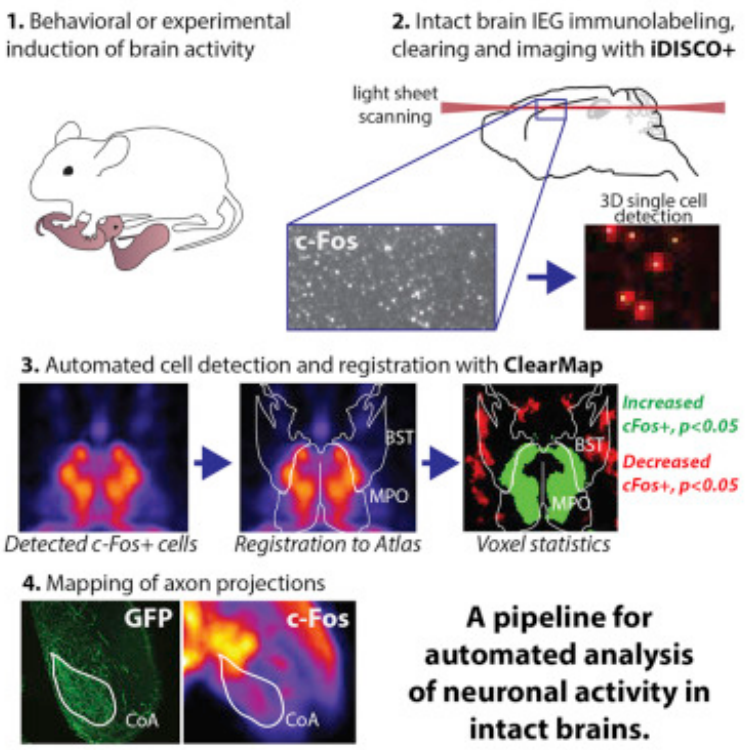
Whole-brain map of activated neuronal populations after Haloperidol treatment




10
100
1000
10000
100000
Globus pallidus, internal segment
Supplemental somatosensory area (L6)
Nucleus accumbens
Substantia innominata
Caudoputamen
Dorsal premammillary nucleus
Medial mammillary nucleus
Primary somatosensory area (L6 Mouth)
Globus pallidus, external segment
Primary somatosensory (L6 Nose)
Mediodorsal nucleus of thalamus
Central lateral nucleus of the thalamus
Zona incerta
Striatum
Lateral hypothalamic area
Olfactory tubercle
Fundus of striatum
Interfascicular nucleus raphe
Pallidum
Lateral habenula
Intermediodorsal nucleus of the thalamus
Ventral premammillary nucleus
Suprachiasmatic nucleus
***
**
*
Haloperidol
Saline
10
100
1000
Reticular nucleus of the thalamus
Red nucleus
anterior commissure
Posterior amygdalar nucleus
Anterior hypothalamic nucleus
Dorsomedial nucleus of the hypothalamus
Substantia nigra (p.r.)
Central medial nucleus of the thalamus
Anteroventral preoptic nucleus
Medial preoptic nucleus
Primary visual area
Peripeduncular nucleus
Medial preoptic area
Paraventricular hypothalamic nucleus
Superior colliculus
Midbrain reticular nucleus
Supramammillary nucleus
Ventral tegmental area
ventral tegmental decussation
Nucleus of reuniens
Primary visual area
Substantia nigra (p.c.)
...
*
trending

Volume Survey of Brain Activity Applied to Parental Behavior in the Mouse





Highlights

1. ClearMap, a pipeline for automated activity mapping in intact samples

2. iDISCO+ preserves morphology and size of cleared samples for automated registration

3. ClearMap to study brain regions involved in various behaviors

Limitations

1. IEG expression in the brain is not limited to neurons.

2. The ability to assess real-time neural activity

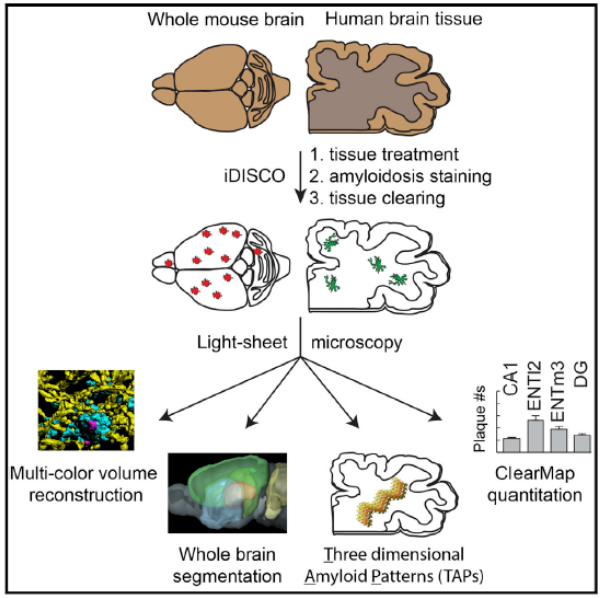
Potential applications

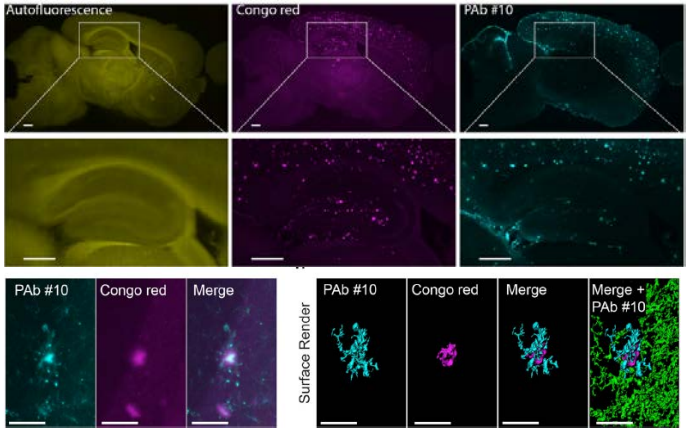
How medications that are administered acutely or chronically alter brain activity. Another use will be to study changes in brain activity as a result of such processes as exercise, sleep, aging, or neurodegeneration.

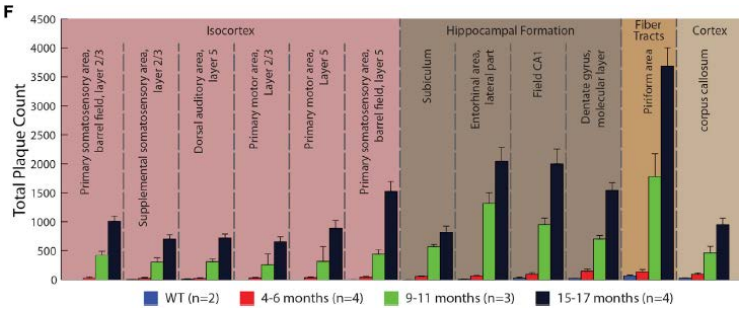
Cell Reports
Resource

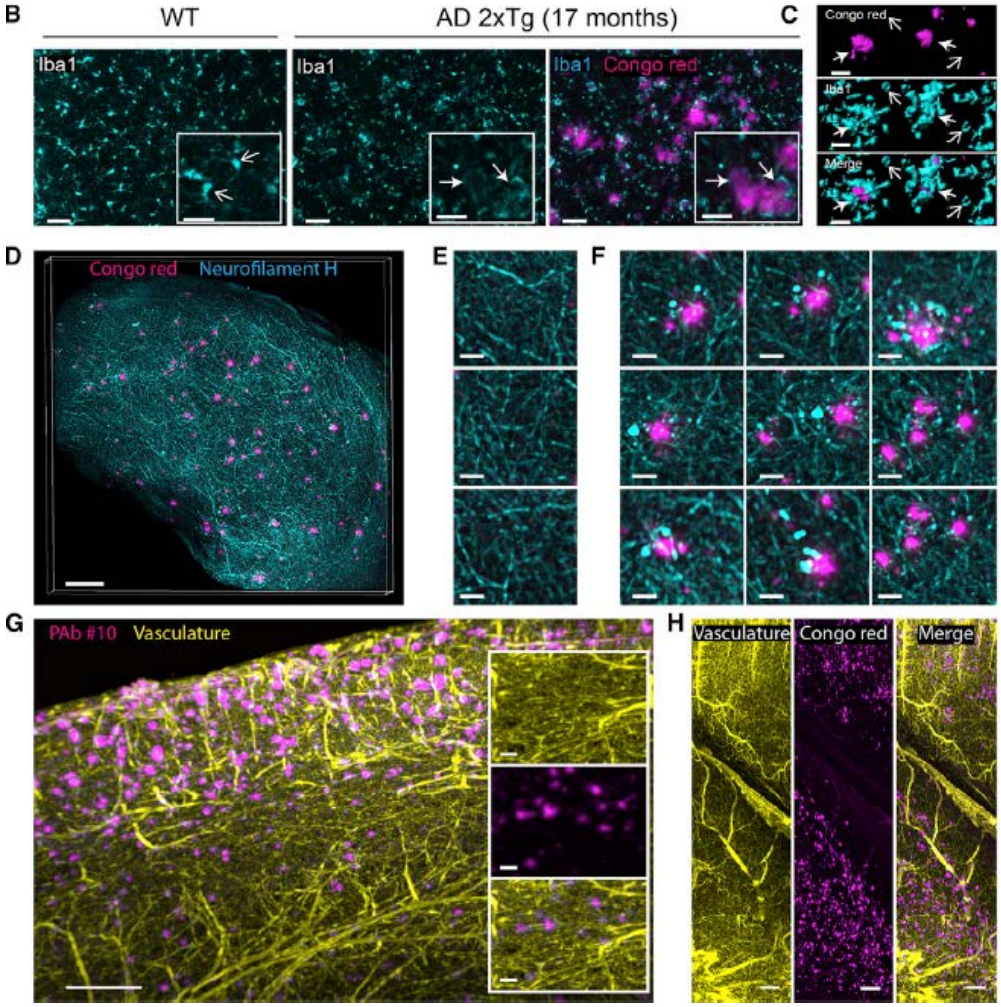
Three-Dimensional Study of Alzheimer's Disease Hallmarks Using the iDISCO Clearing Method

Thomas Liebmann,[1] Nicolas Renier,[2] Karima Bettayeb,[1] Paul Greengard,[1] Marc Tessier-Lavigne,[2] and Marc Flajolet[1,*]
[1]Laboratory of Molecular and Cellular Neuroscience
[2]Laboratory of Brain Development and Repair
The Rockefeller University, New York, NY 10065, USA
*Correspondence: marc.flajolet@rockefeller.edu
http://dx.doi.org/10.1016/j.celrep.2016.06.060

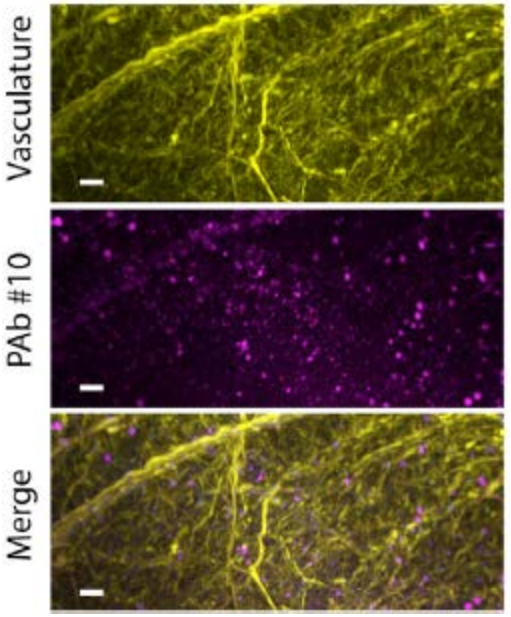




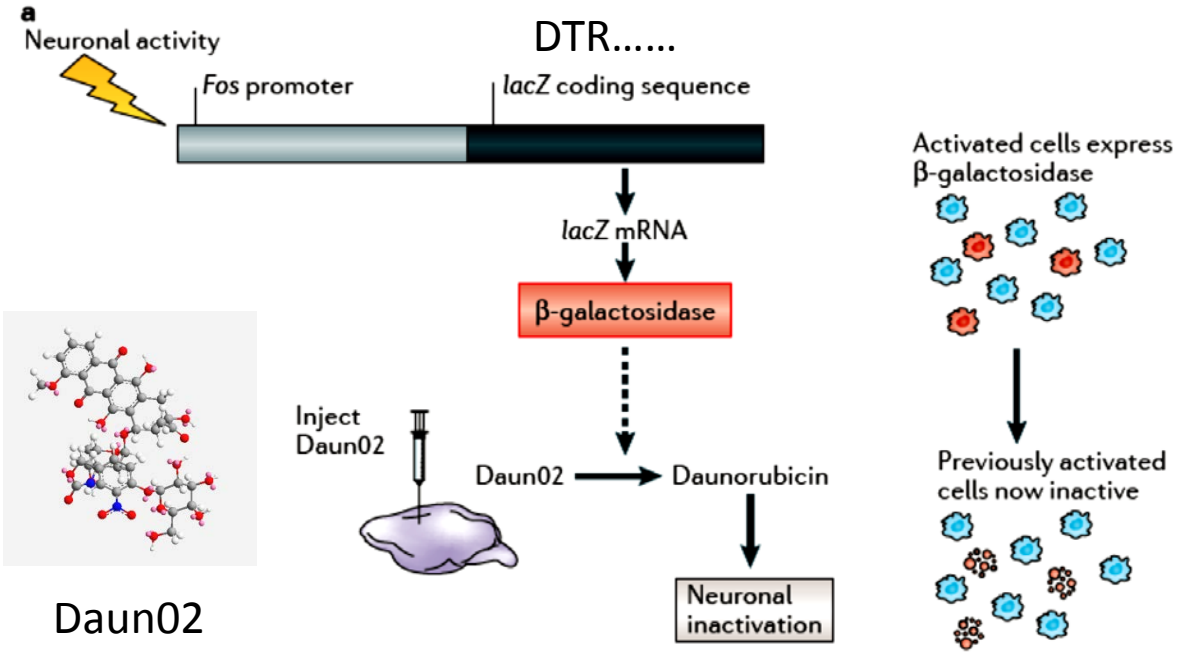



B
WT
AD 2xTg (17 months)
Iba1
Iba1
Iba1 Congo red
C
Congo red
Iba1
Merge
D
Congo red Neurofilament H
E
F
G
PAb #10 Vasculature
H
Vasculature
Congo red
Merge

Human brain


Vasculature
PAb #10
Merge

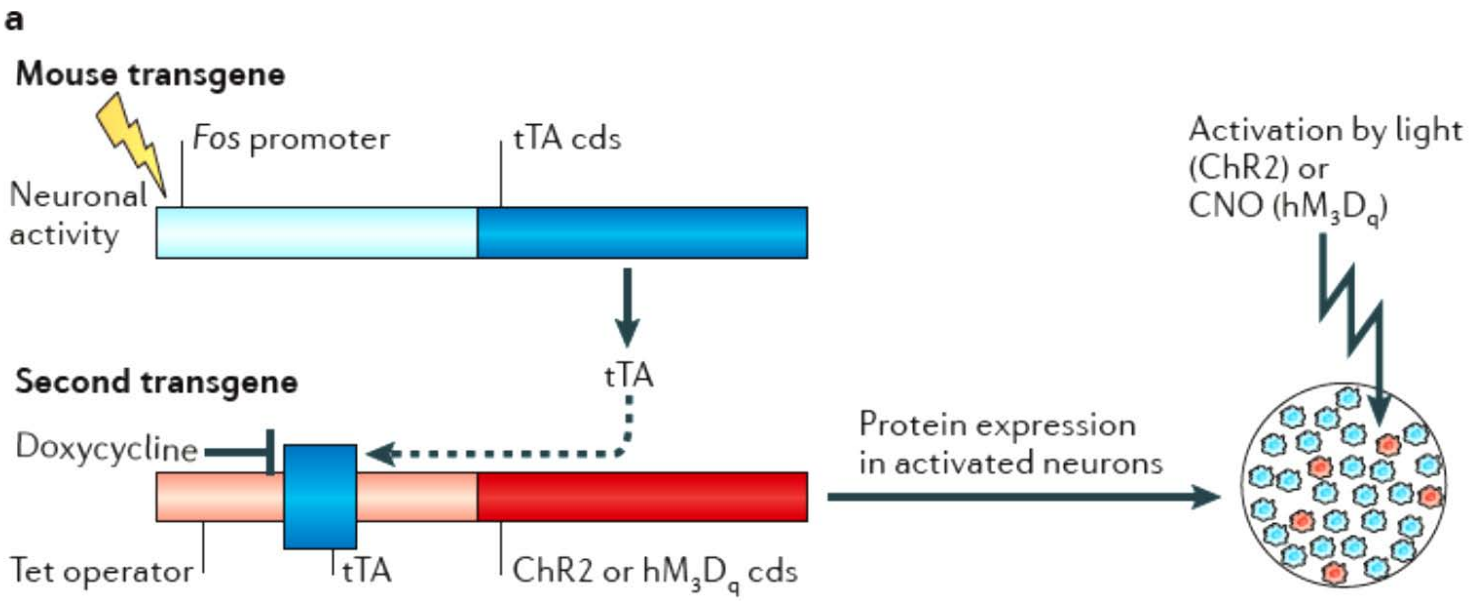
Daun02 system for the selective ablation of activated neuronal assembles in vivo



Daun02

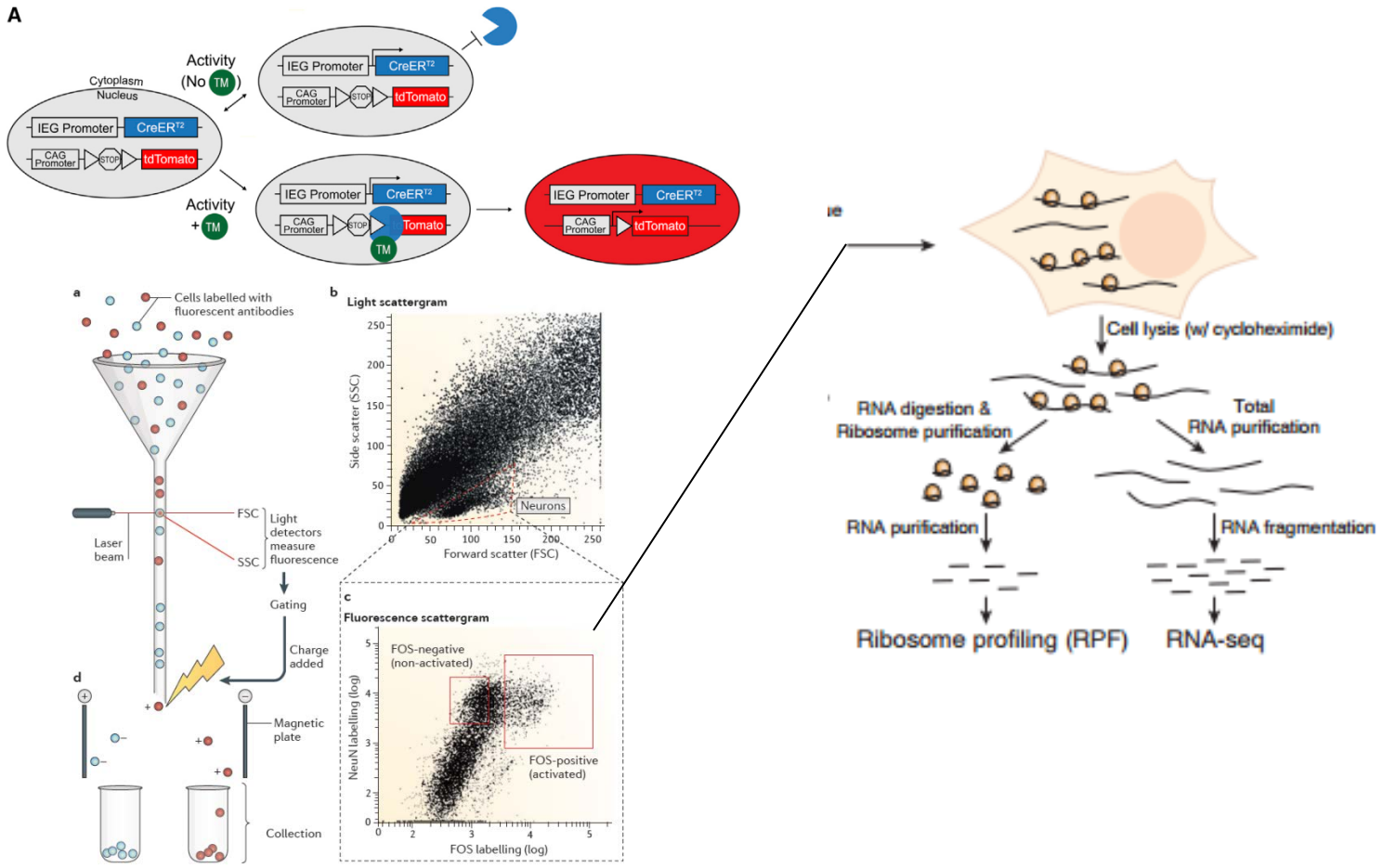
Fabio C. Cruz and Bruce T. Hope et al., 2013, Nature Reviews Neuroscience

Strategies for selective manipulation of activated neuronal assembles in vivo



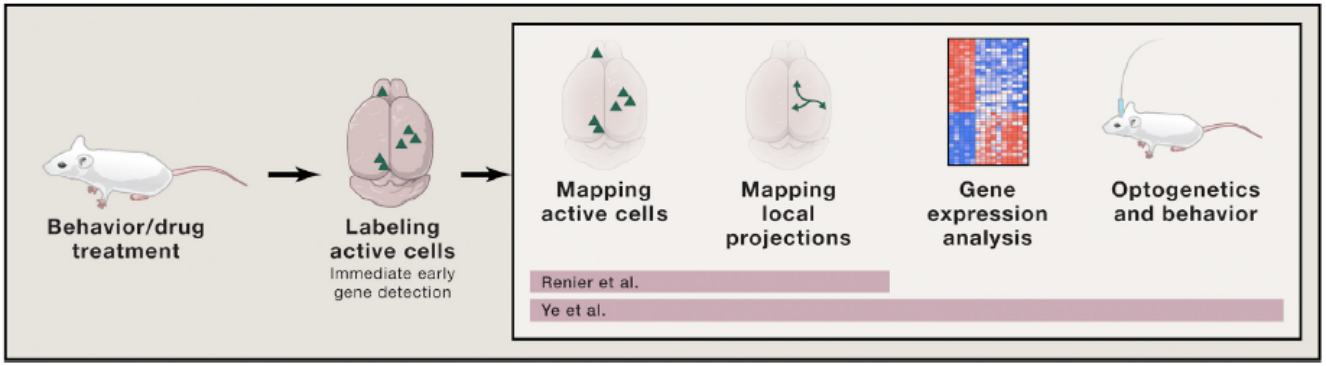
Fabio C. Cruz and Bruce T. Hope et al., 2013, Nature Reviews Neuroscience

FACS sorting of activated neurons for further analysis



Casey J. Guenthner and Liqun Luo et al., 2013, Neuron

Fabio C. Cruz and Bruce T. Hope et al., 2013, Nature Reviews Neuroscience



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for your
ATTENTION!

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