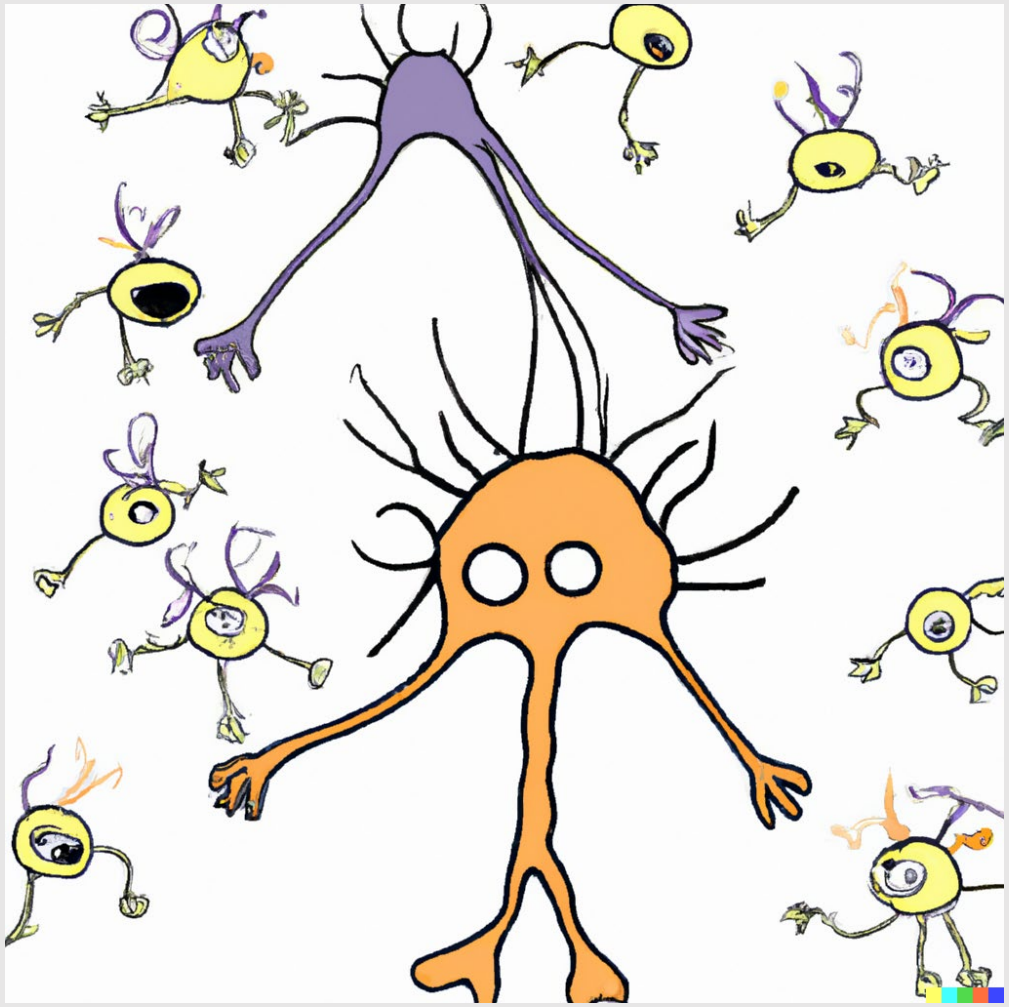
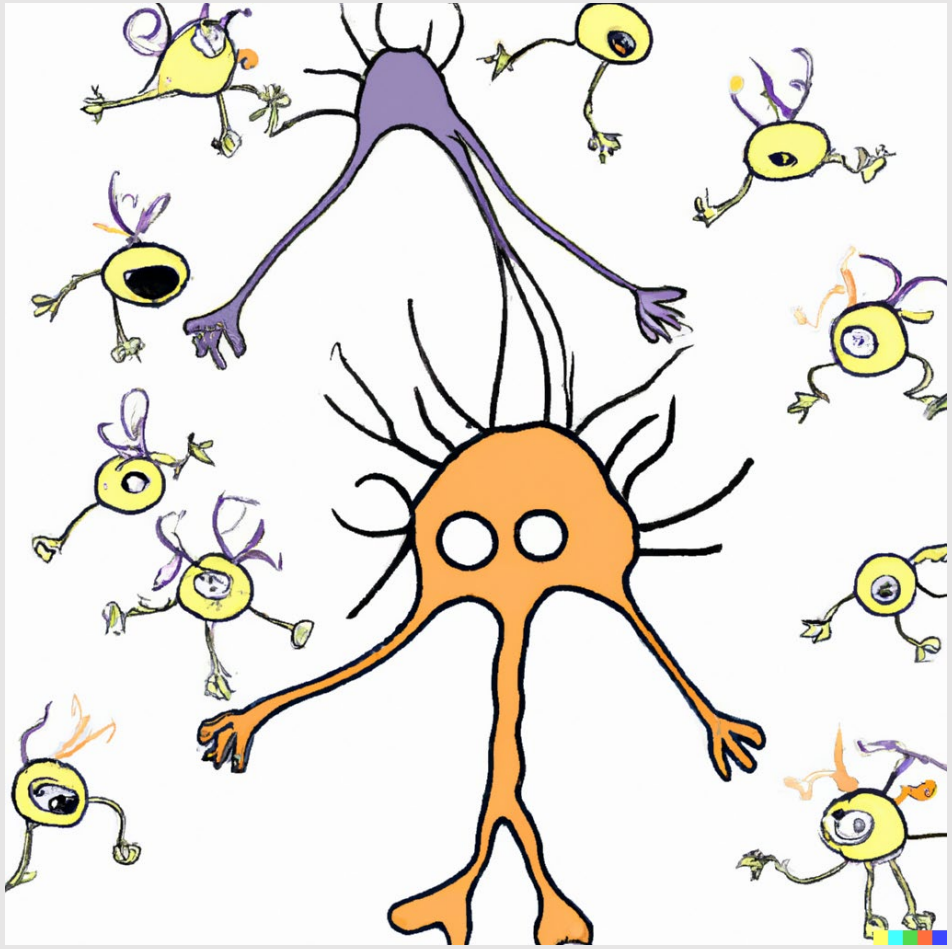
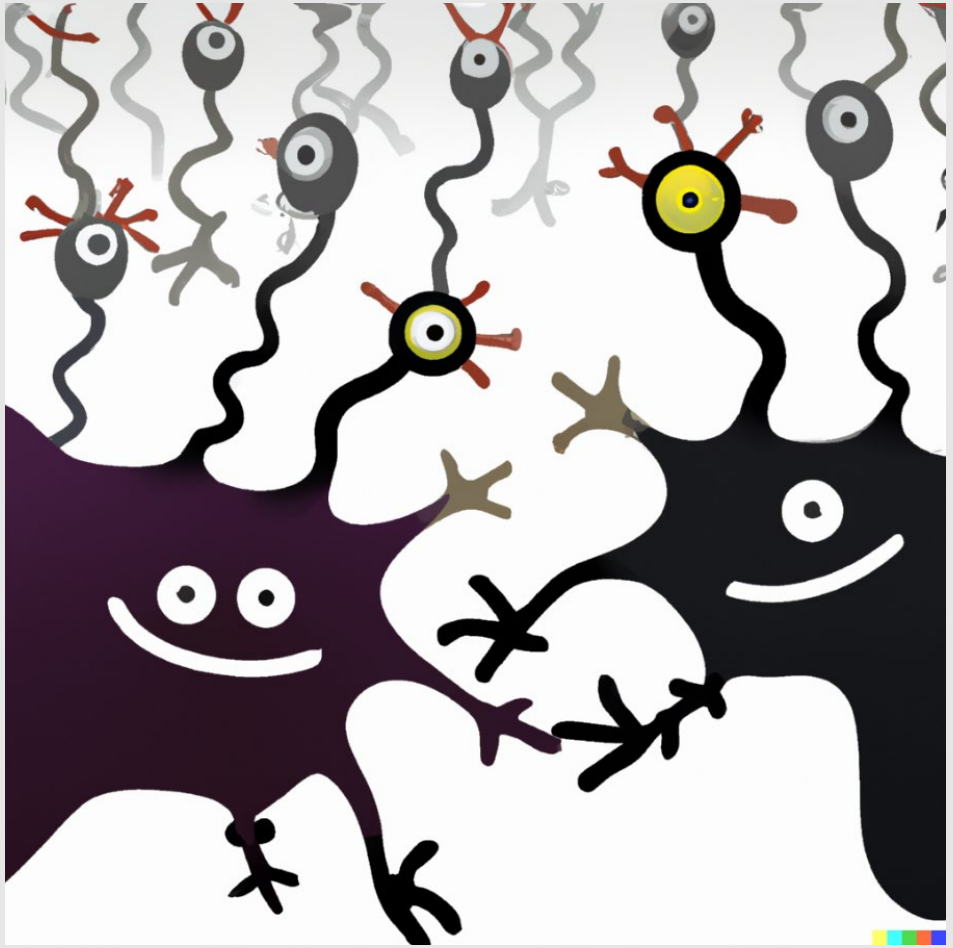
Candidate Pharmacological Therapies for Stroke Recovery



S. Thomas Carmichael, M.D., Ph.D.
Professor and Chair
Department of Neurology
David Geffen School of Medicine at UCLA

The **Take-Home Message**, from AI drawings of neurons in poor and successful stroke recovery



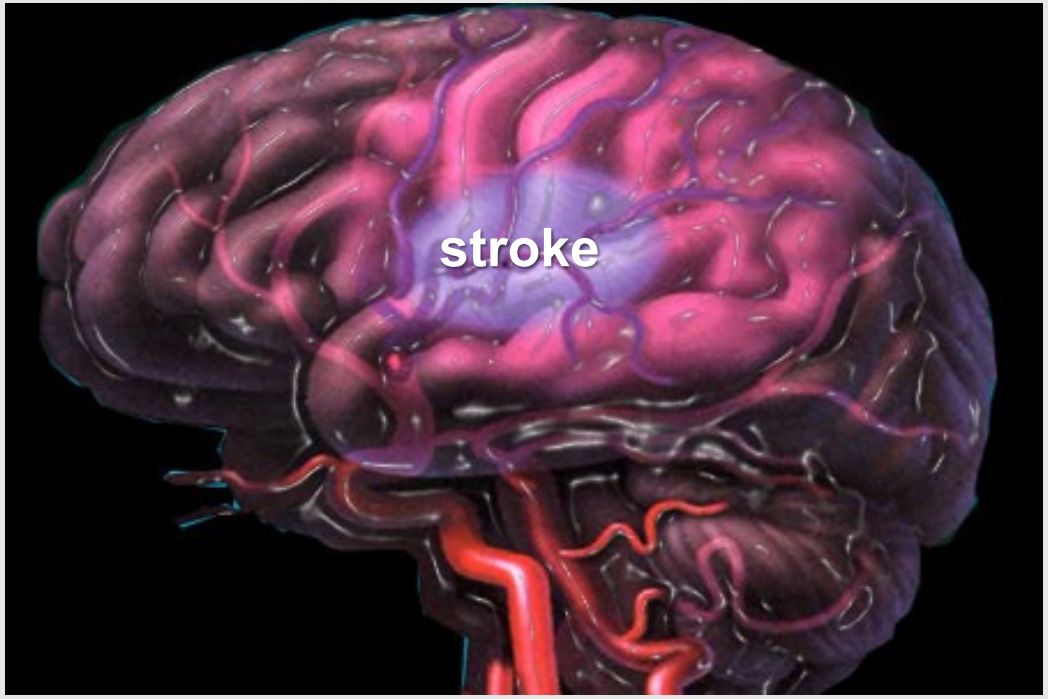


“Draw a group of neurons that do not care about each other”. Dall·E OpenAI

“Draw a group of neurons that care about each other”. Dall·E OpenAI

Mechanisms of Neural Repair and Recovery after Stroke

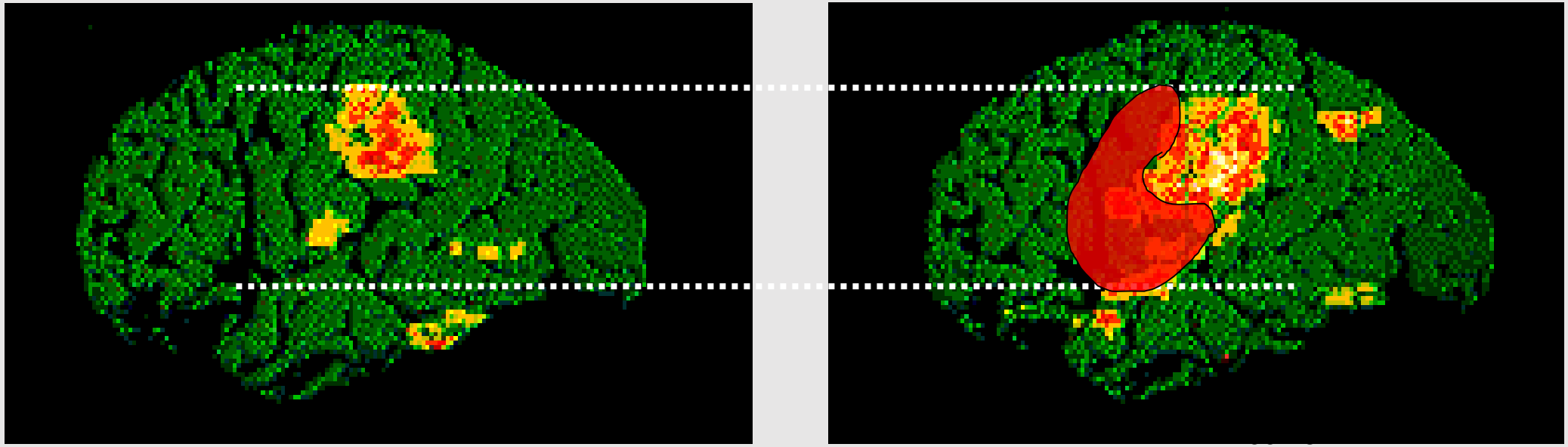
The most significant functional reorganization in the brain after stroke occurs in tissue adjacent to the stroke site.



Recovery after Stroke

Control

Recovered Stroke

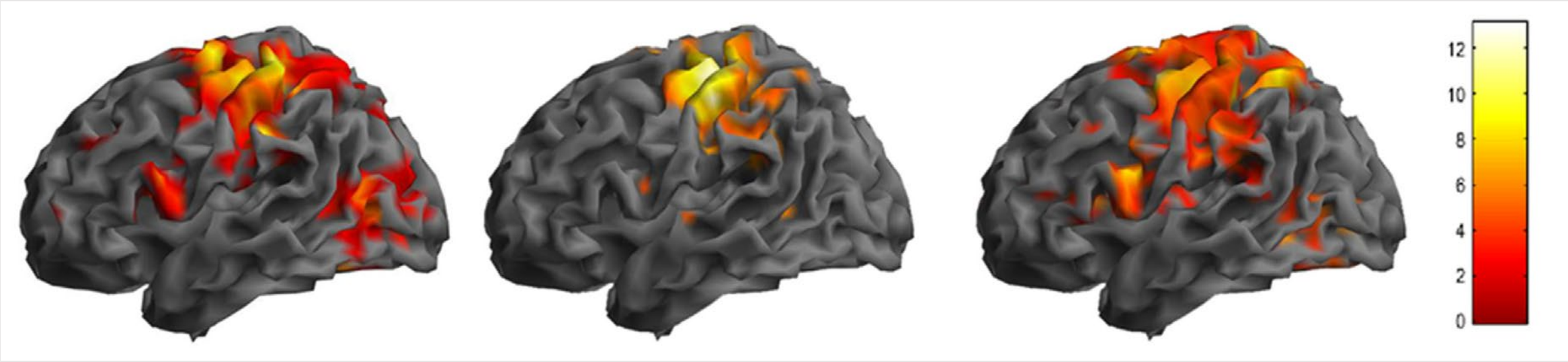


- Sensory and motor maps expand in peri-infarct and connected cortical areas.
- This process correlates most closely with good recovery.

Healthy Control

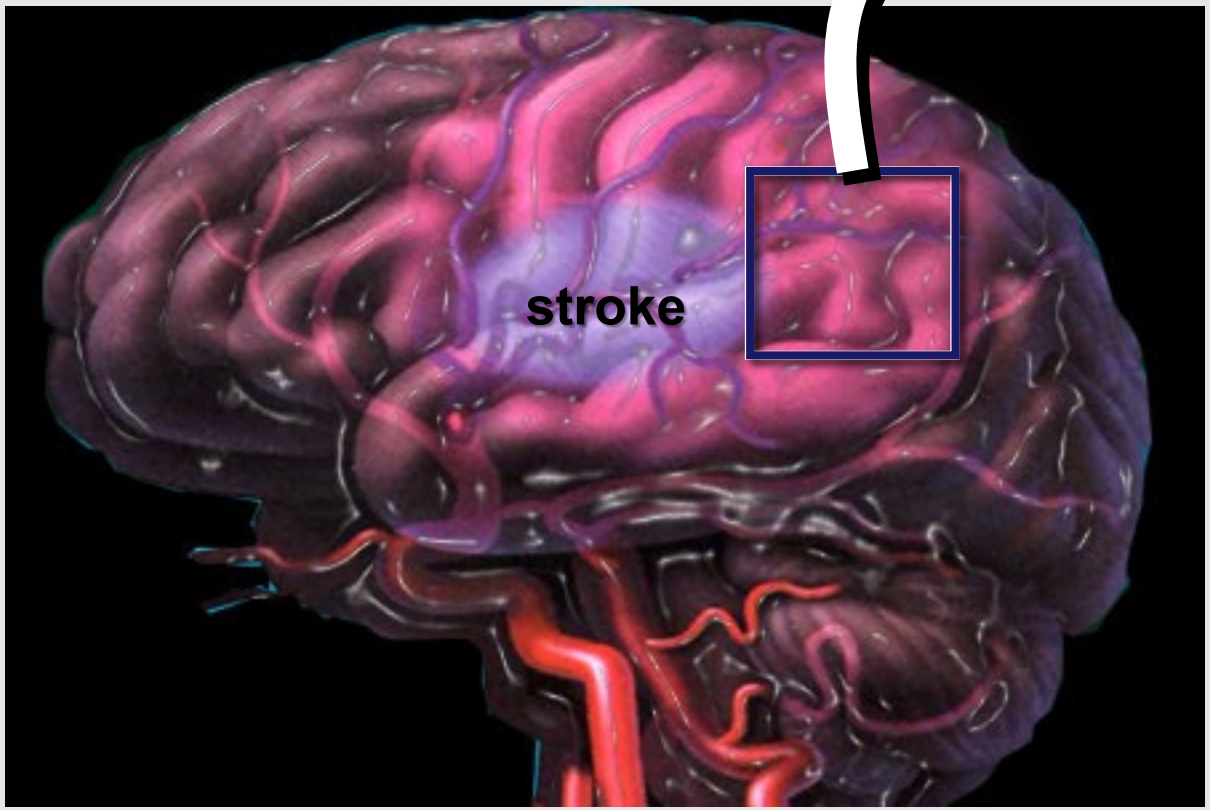
Post-Stroke, pre-rehab

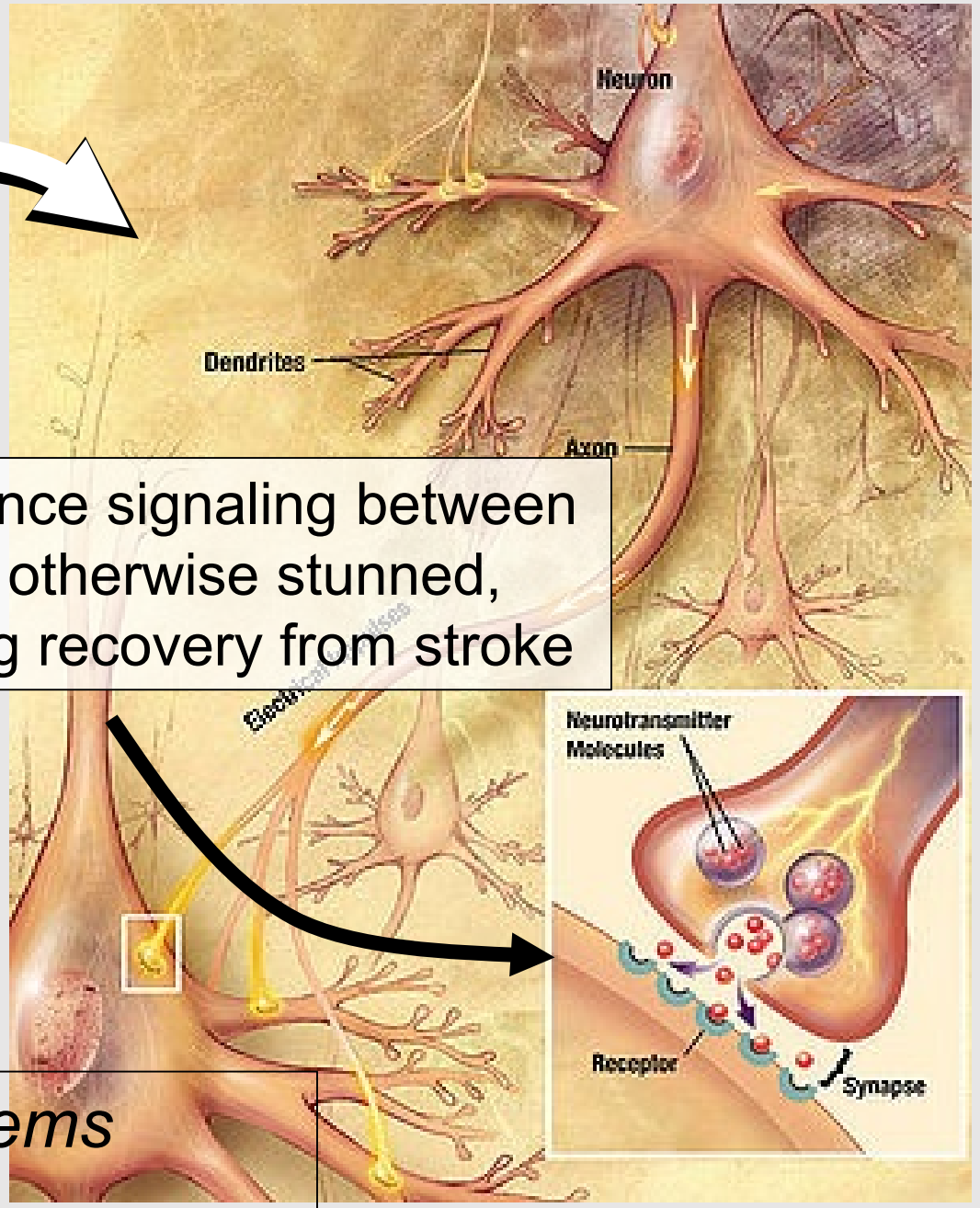
Post-Stroke, post-rehab



Horn et al. / Behavioural Brain Research 308 (2016) 152–159

Brain Connections in Recovering Tissue



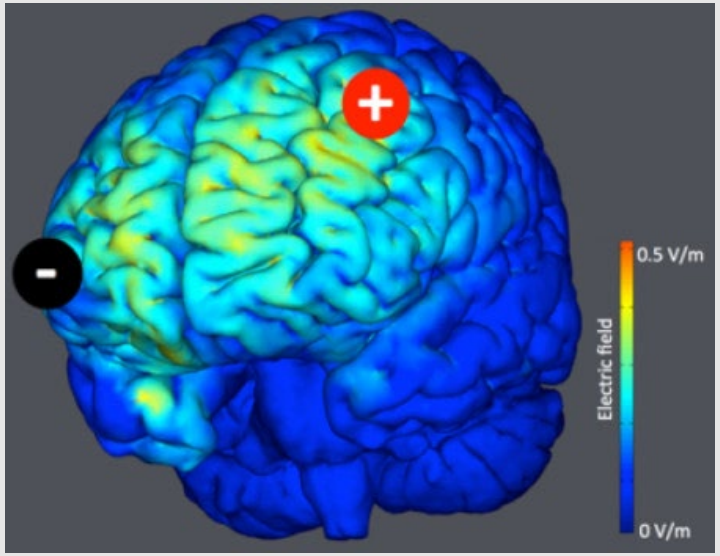


Enhance signaling between cells, otherwise stunned, during recovery from stroke

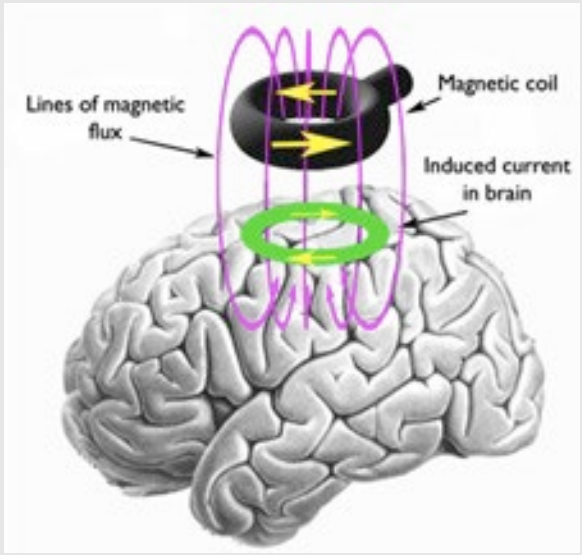
Can treatments that enhance memory systems promote behavioral recovery in stroke?

Pharmacological Therapy for Stroke Recovery

Common principle: modulating neuronal excitability after stroke



tDCS



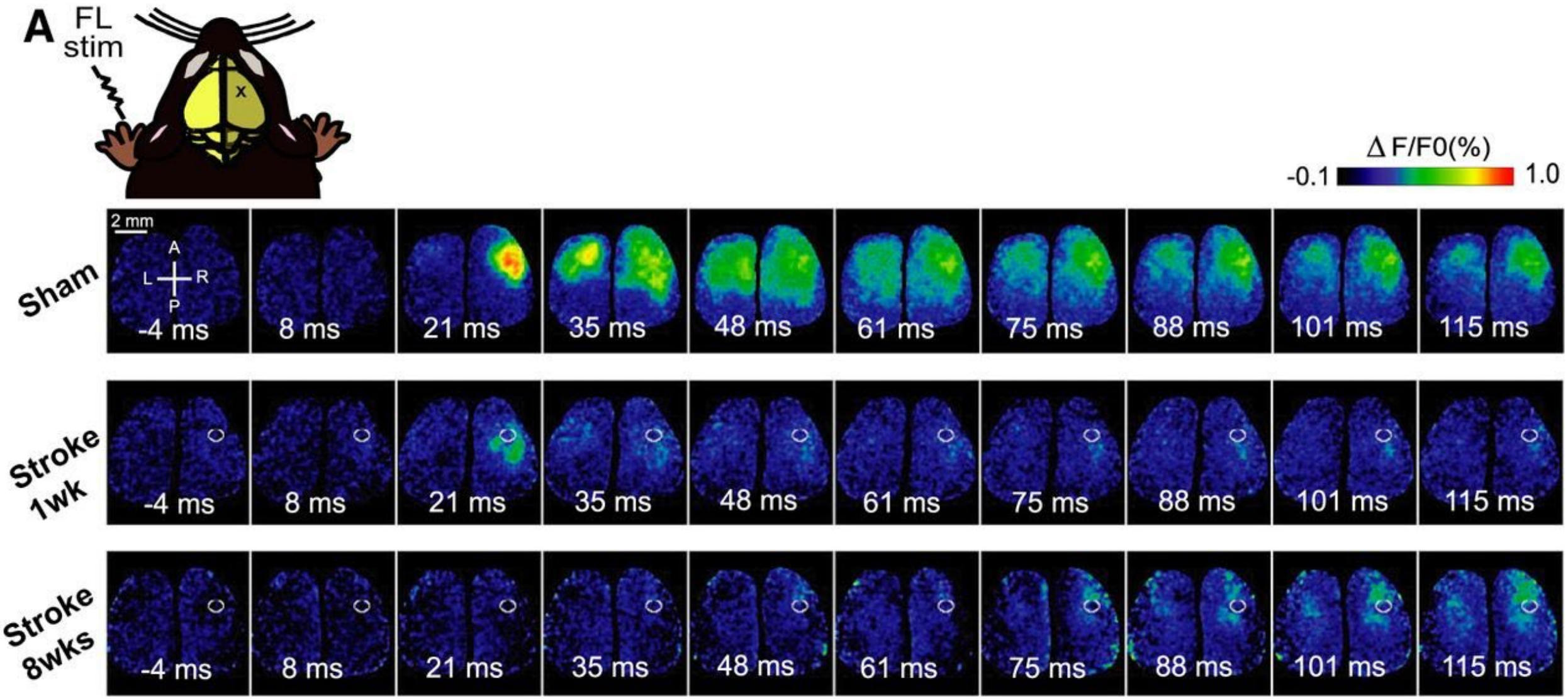
TMS

Can we do this with drugs?
Will they be selective enough?

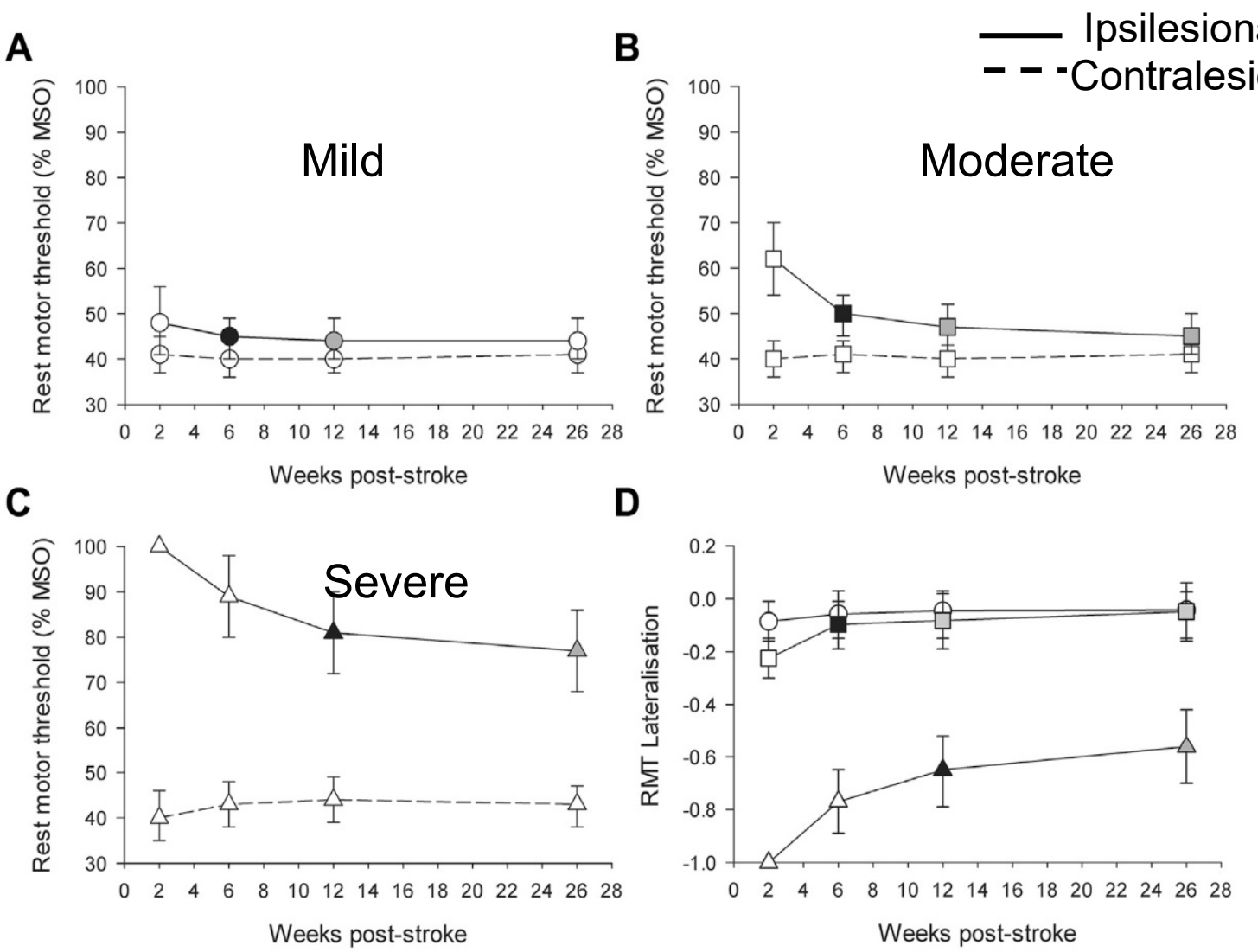
Pharmacologically Amenable Principles of Recovery after Stroke

- Recovery occurs when brain areas adjacent to or connected with the stroke site can take over some of the lost function
- Therapies that activate these brain areas may stimulate recovery
- These are first or most amenable principles for pharmacology of stroke recovery

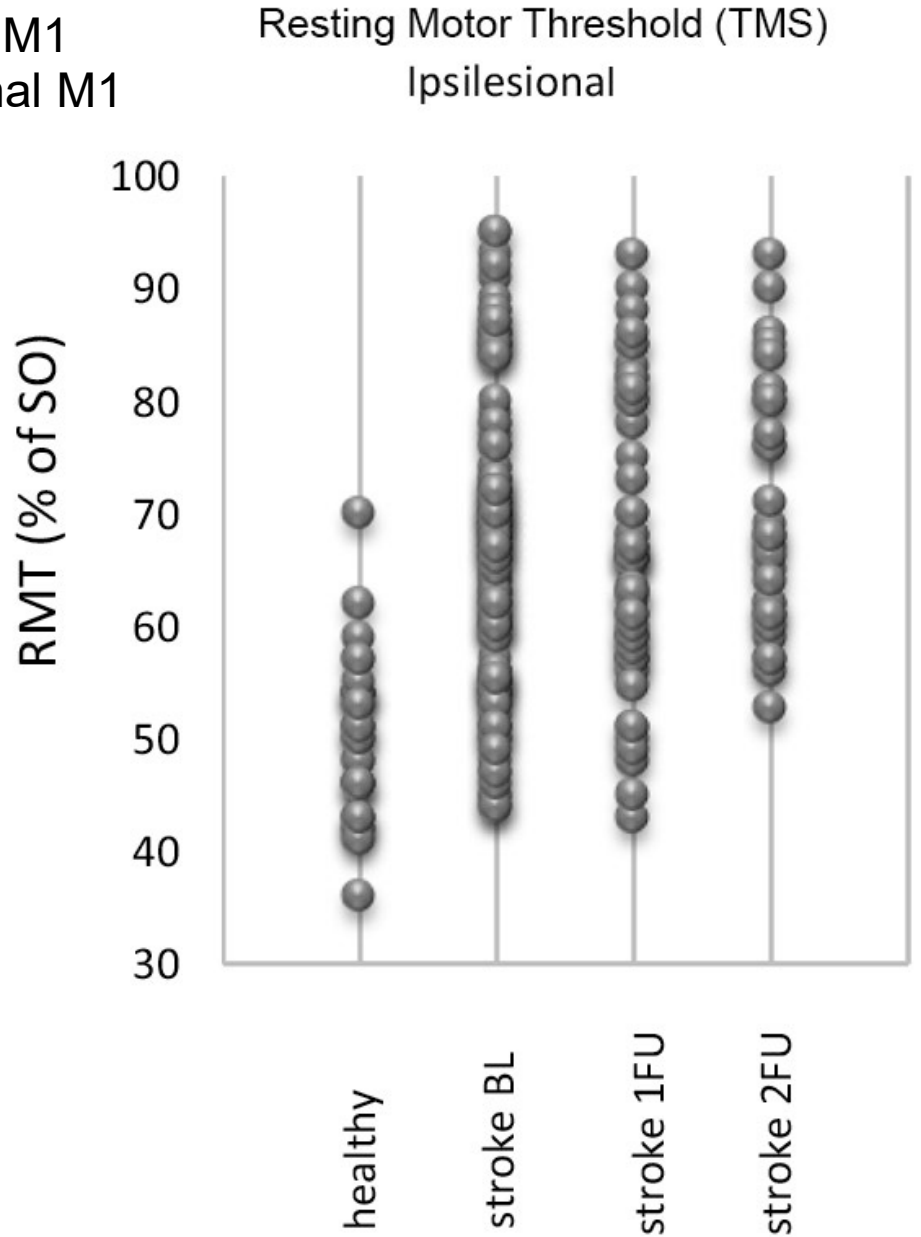
Maps of the affected limb show delayed and decreased responses in both hemispheres after stroke



Diana H. Lim et al. J. Neurosci. 2014;34:16455-16466



Stinear et al. / Brain Stimulation 8 (2015) 1183e1190



Veldema *et al. J NeuroEngineering Rehabil (2021) 18:158*
92 human studies after stroke

Rest Motor Threshold

- Tonic GABA blockade
- Enhanced AMPA receptor signaling
- Phosphodiesterase inhibition
- CCR5 blockade

All are candidate therapies for stroke recovery

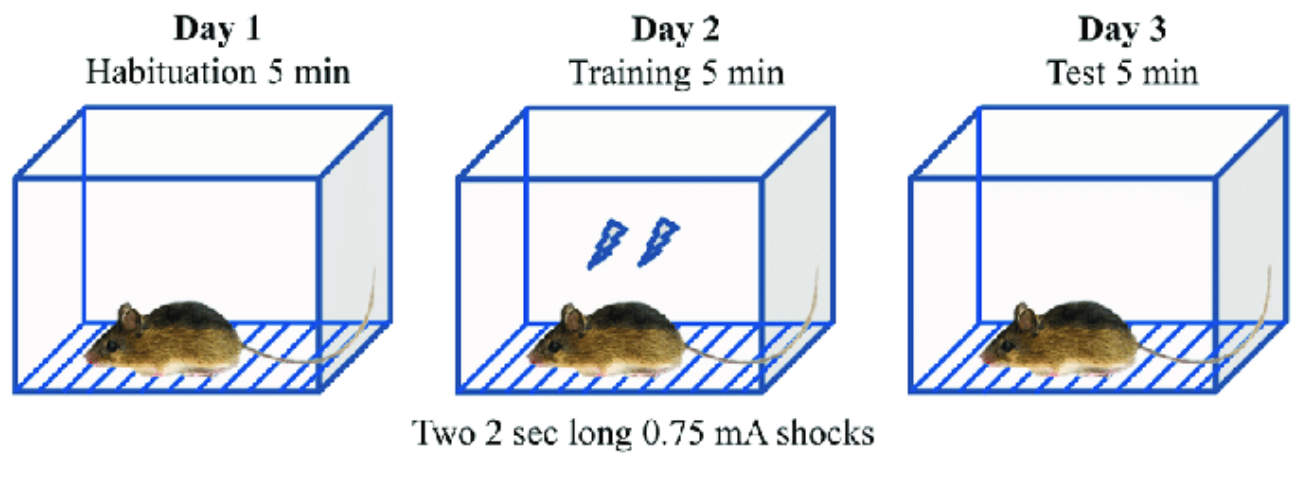
Three clinical trials in this group

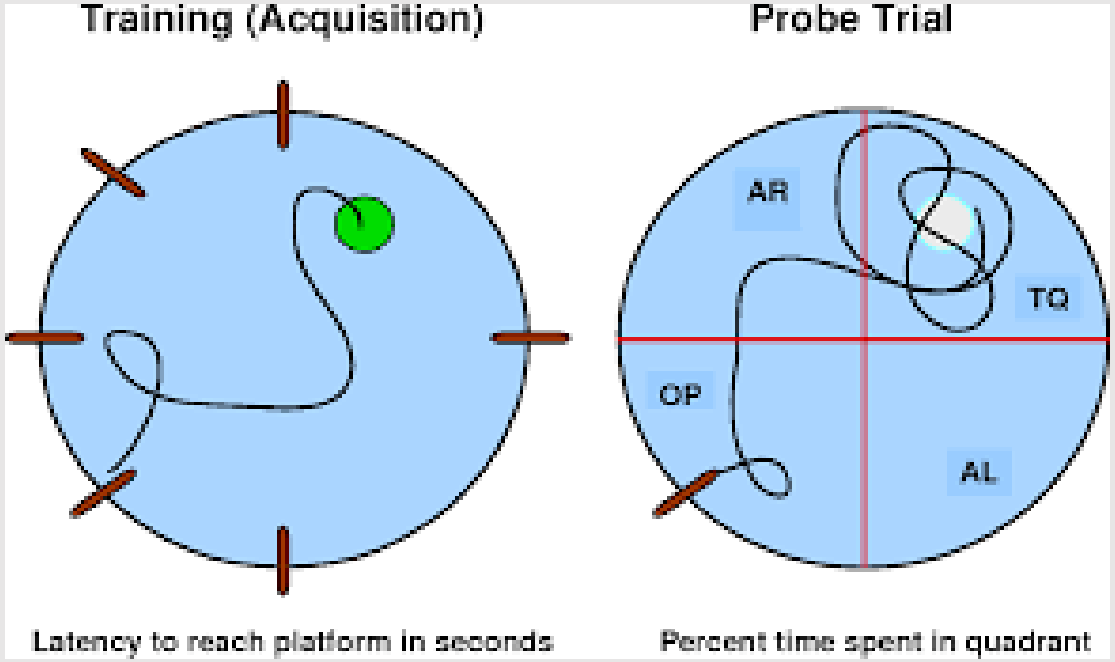
What is a common mechanism?

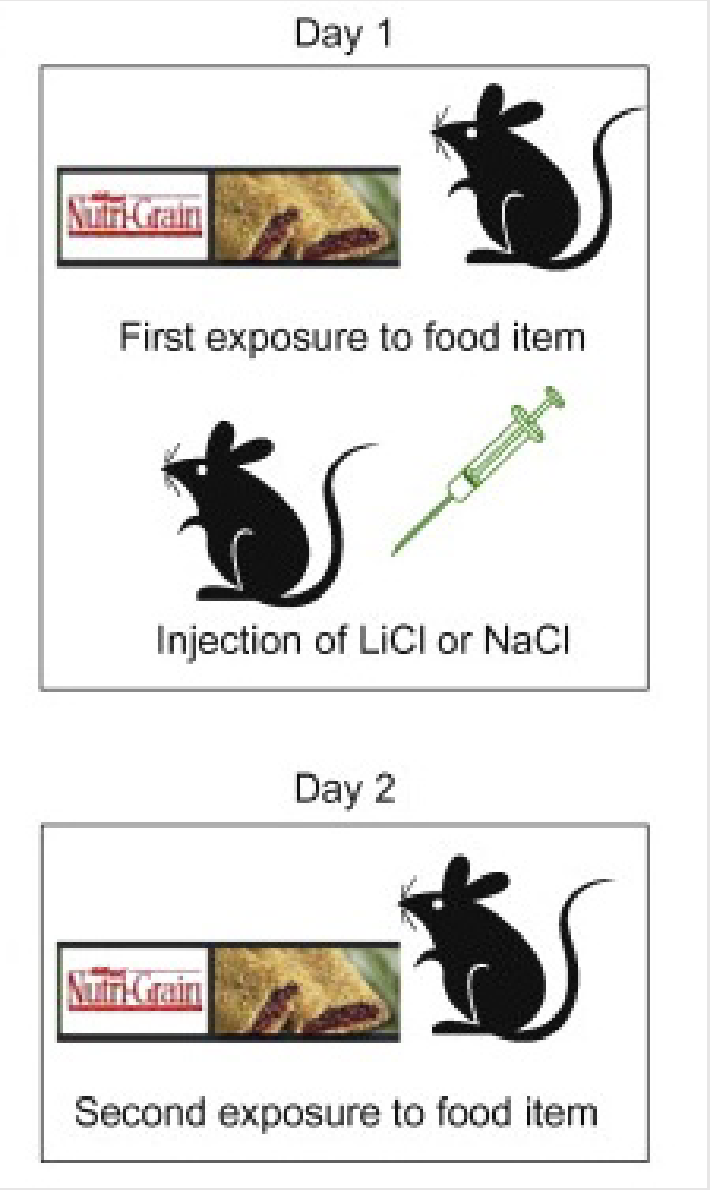
What can we learn about the biology of recovery from stroke?

Neuronal Allocation in Stroke Recovery

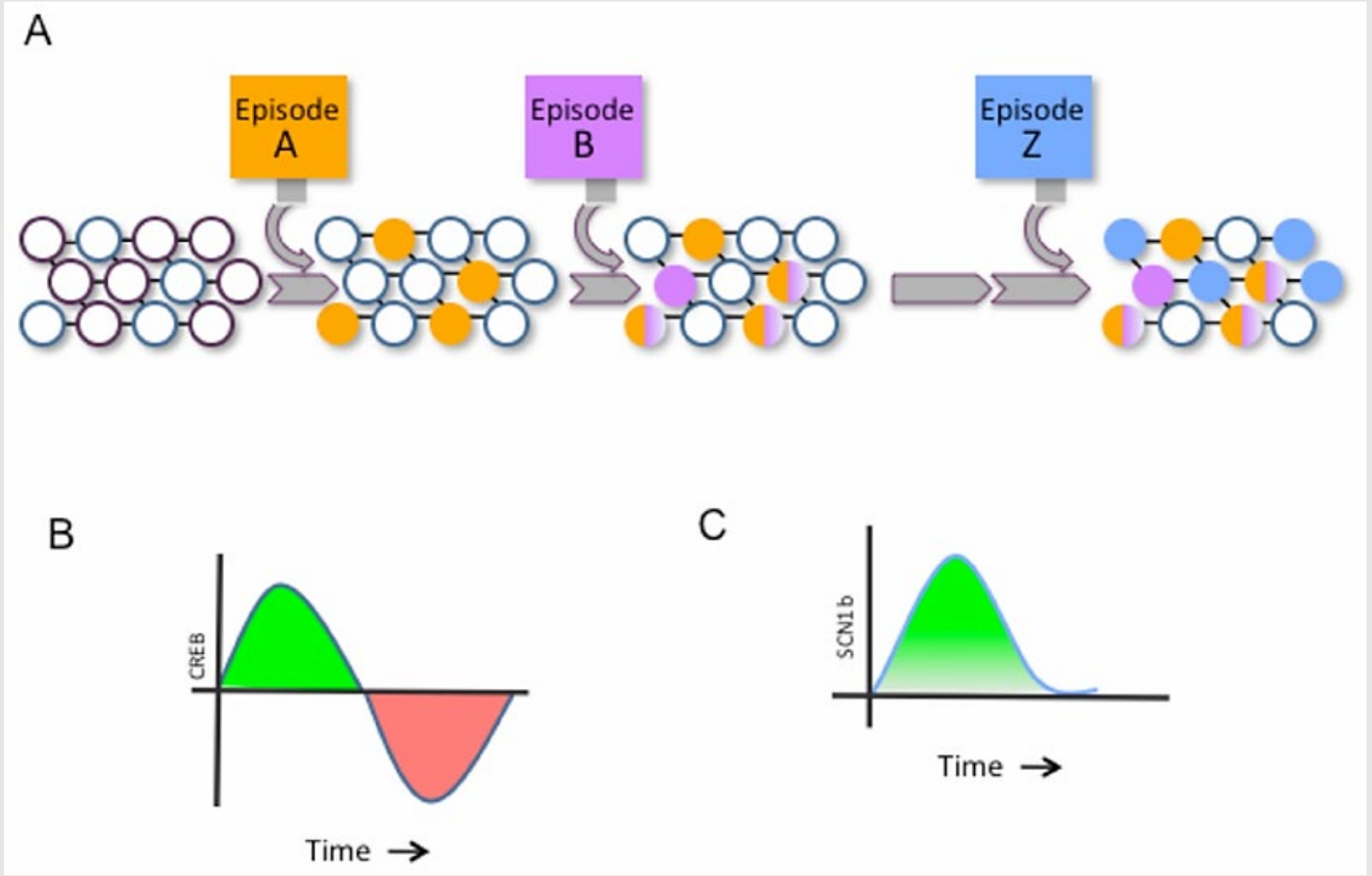
The Neuronal Engram





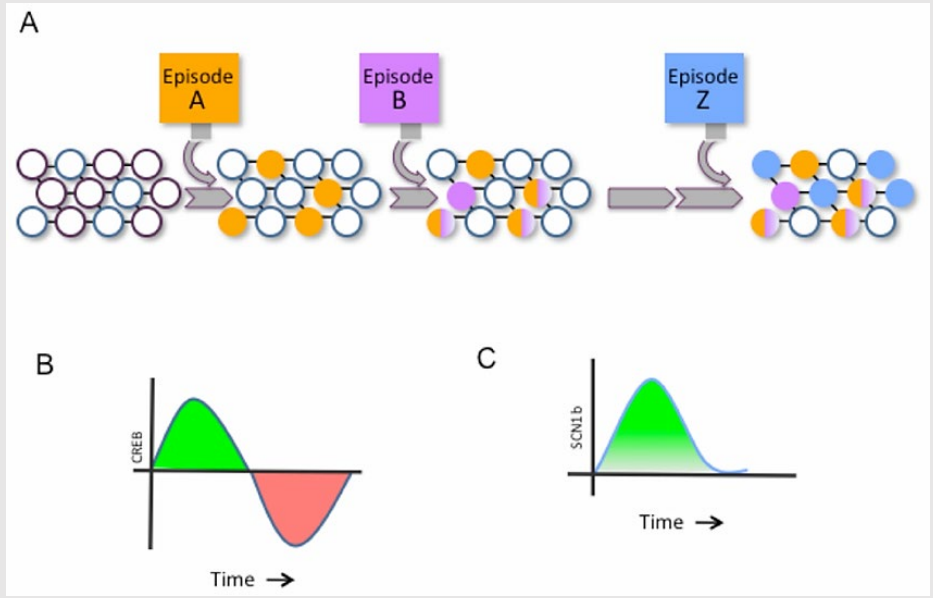


The Neuronal Engram

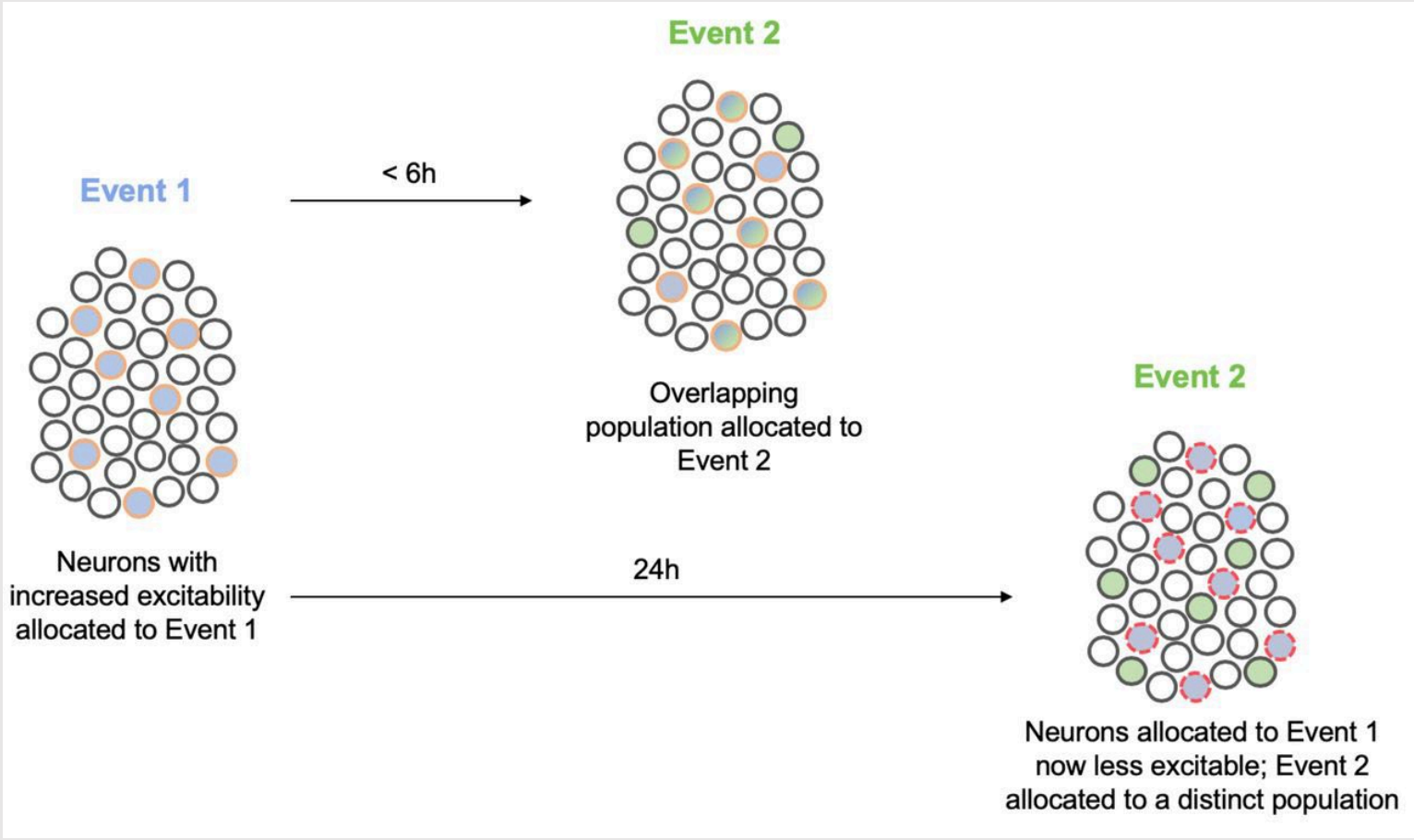


Silva et al. *Science* Oct, 2009

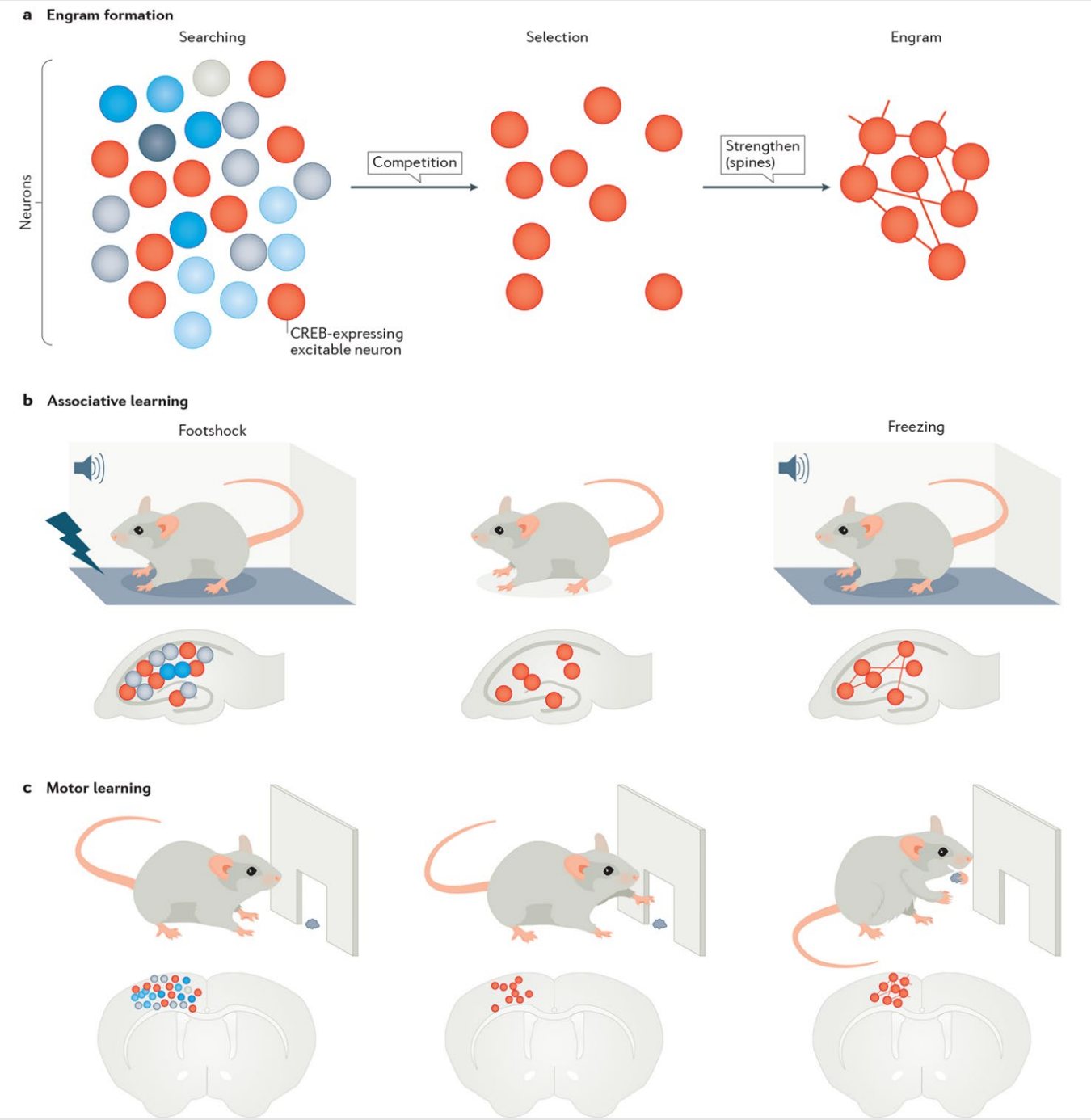
The Neuronal Engram



Silva et al. *Science* Oct, 2009

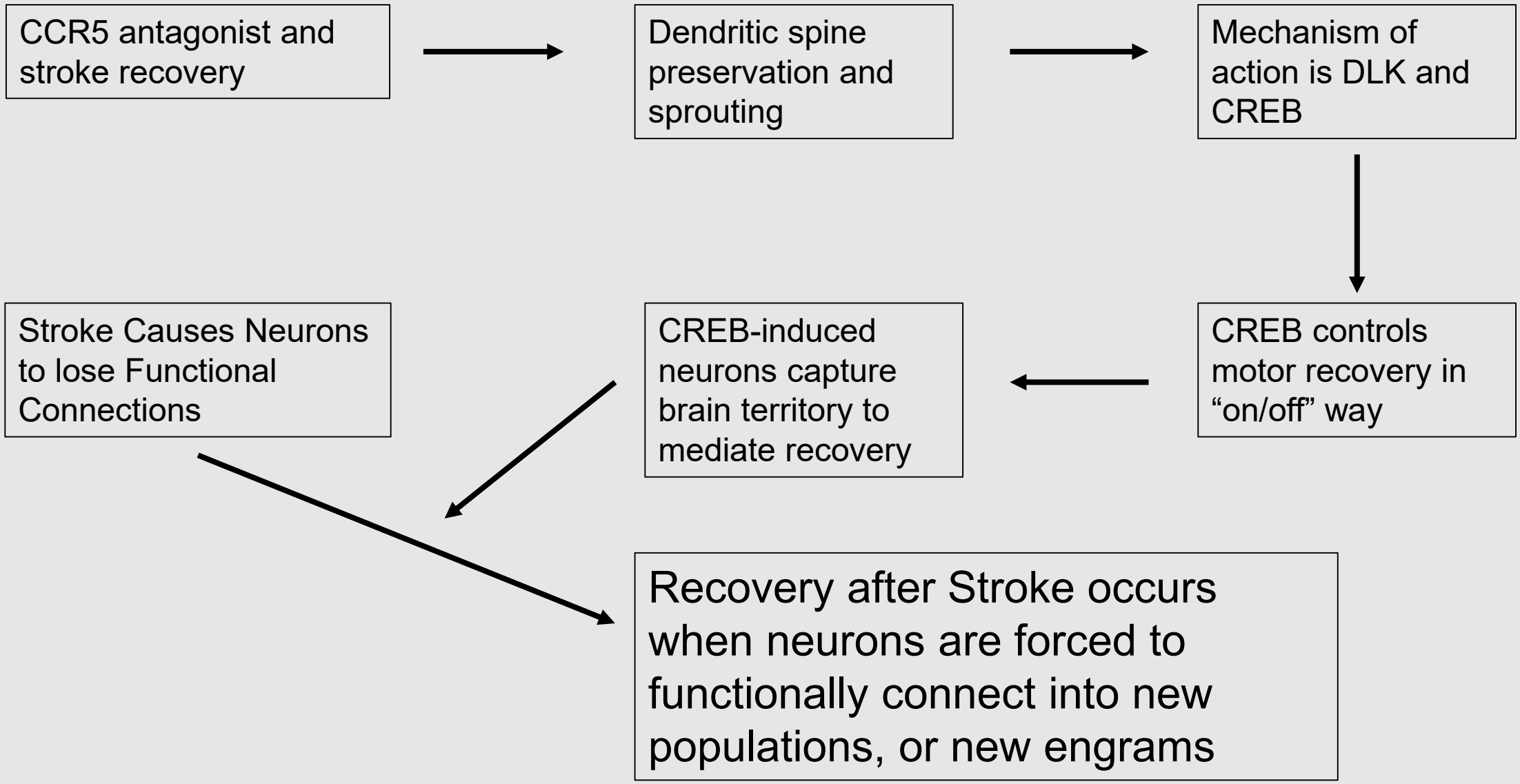


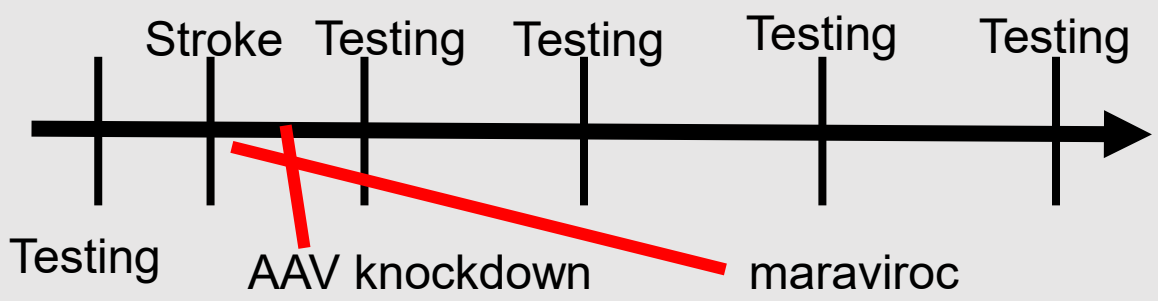
Josselyn, Tonegawa *Science Jan, 2020*



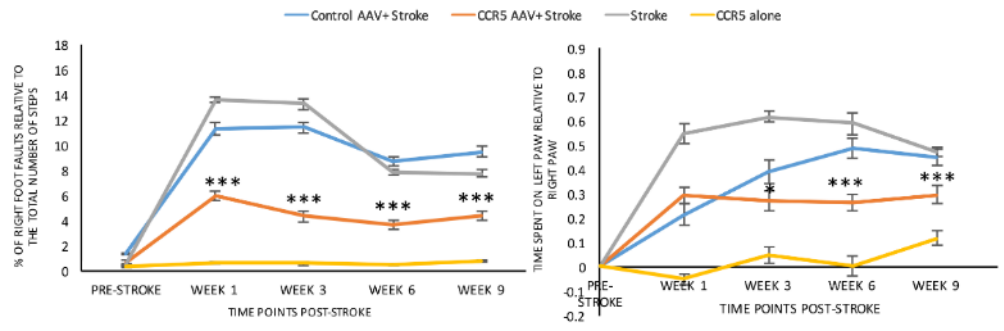
Joy and Carmichael, *Nat Rev Neurosci*. 2020

The Recovery Neuronal Engram



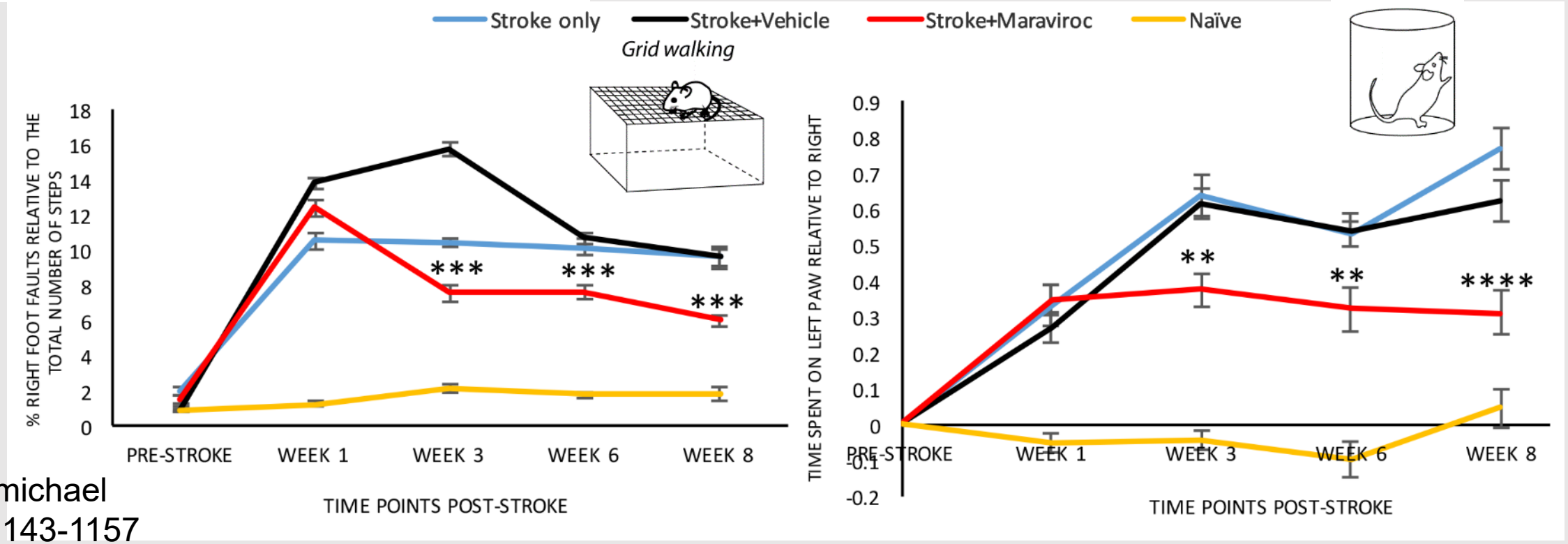


Behavioral Testing for Motor Recovery with CCR5 blockade



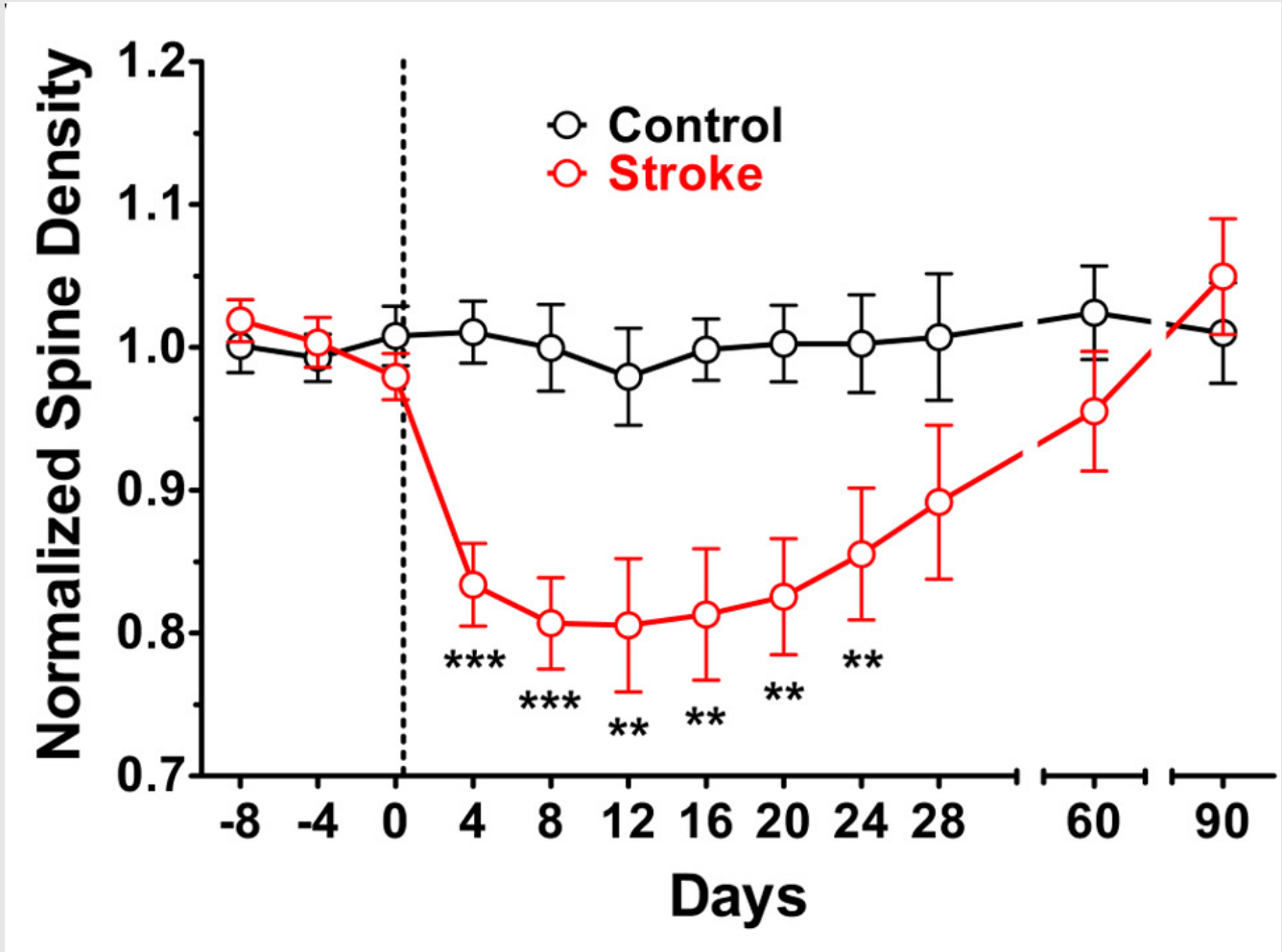
Drug or gene blockade or CCR5 promotes significant and long lasting motor recovery after stroke

Cylinder task



Joy…Carmichael
Cell.176:1143-1157

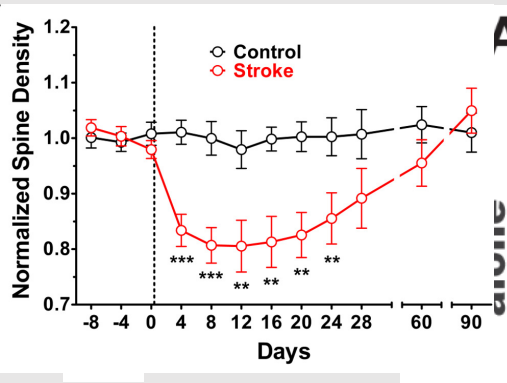
Mechanism of Action: dendritic spine sparing

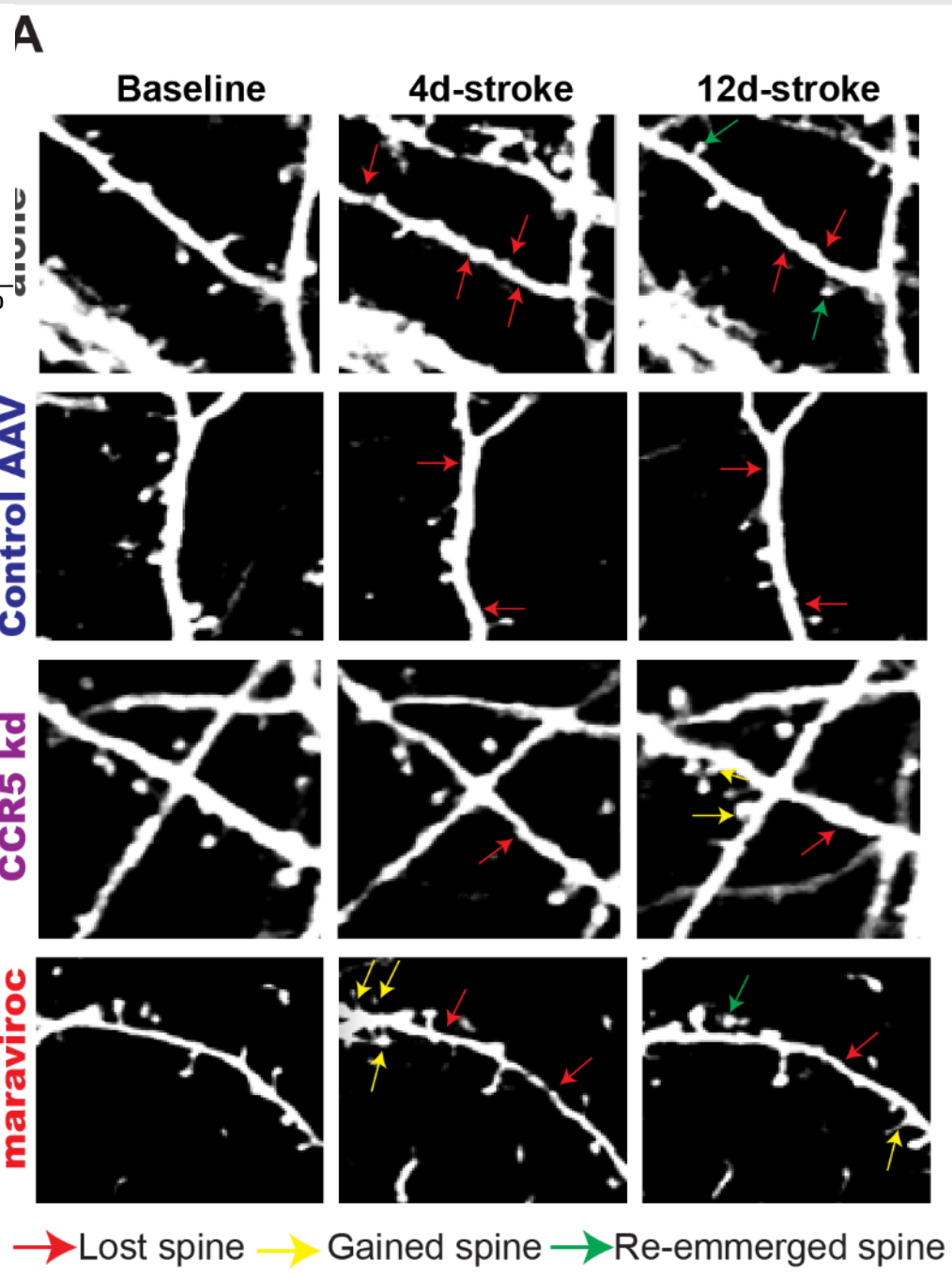


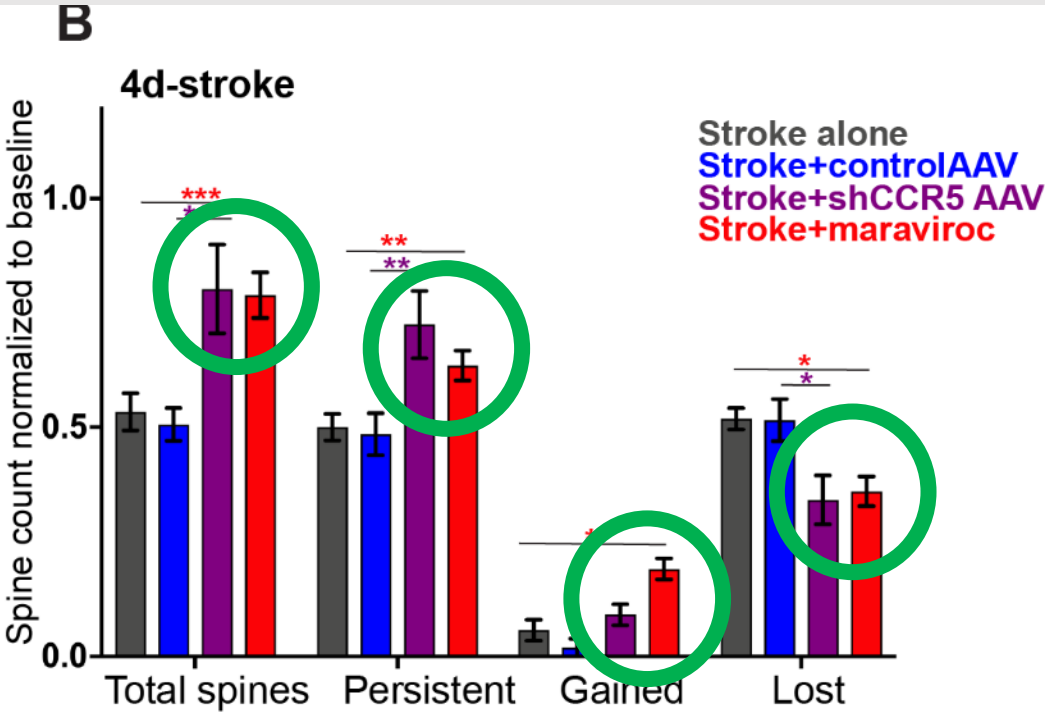
Stroke causes loss of dendritic spines in cortex adjacent to the stroke site

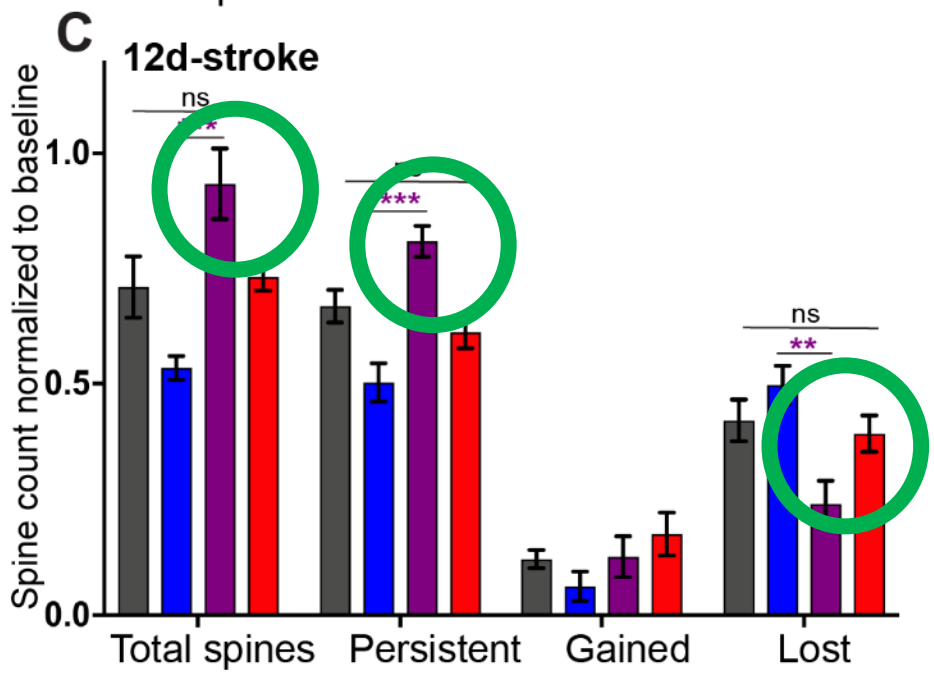
Mostany et al. J Neurosci 30:14116 (Portera-Cailliau)

Mechanism of Action: dendritic spine sparing







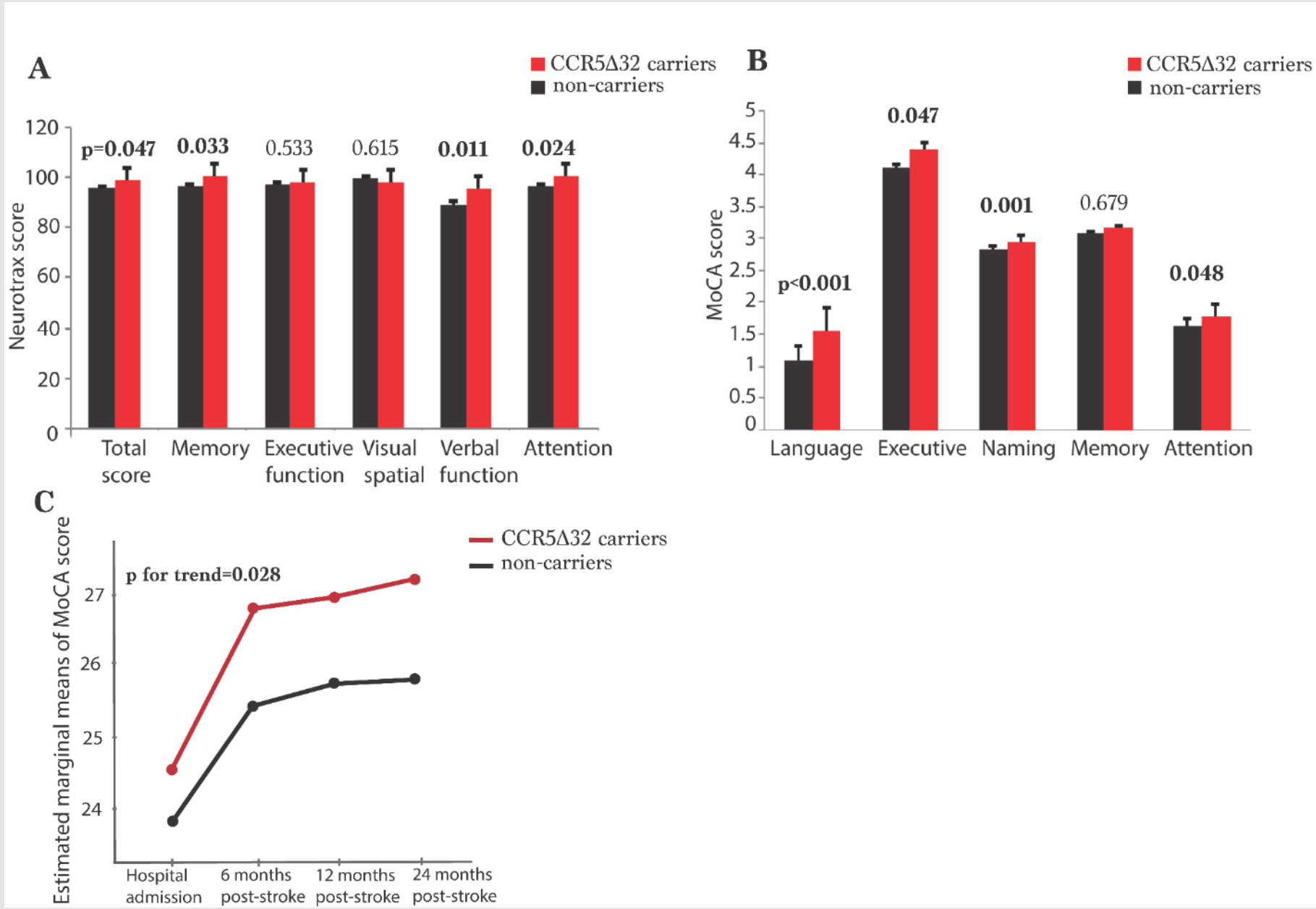


TABASCO (Tel-Aviv Brain Acute Stroke Cohort) Study

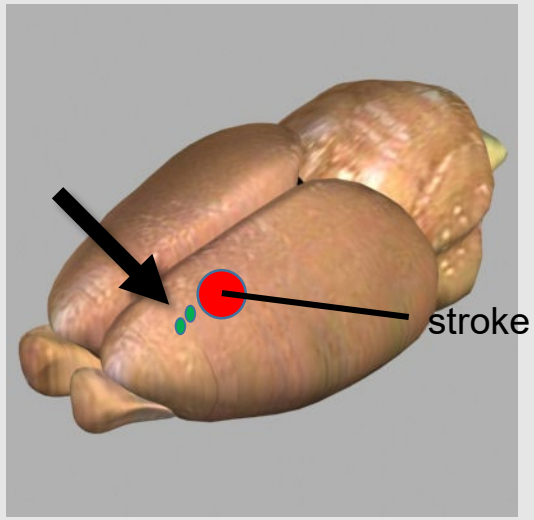
- Recent (within 72 h) first-ever acute ischemic stroke or TIA
- Neurological assessment: NIH Stroke Scale (NIHSS), Cognitive assessment Montreal
- Cognitive Assessment (MoCA), a computerized battery of neuropsychological tests for memory, attention and executive functions (“Neurotrax”)
- Admission, 3, 6, 12 months

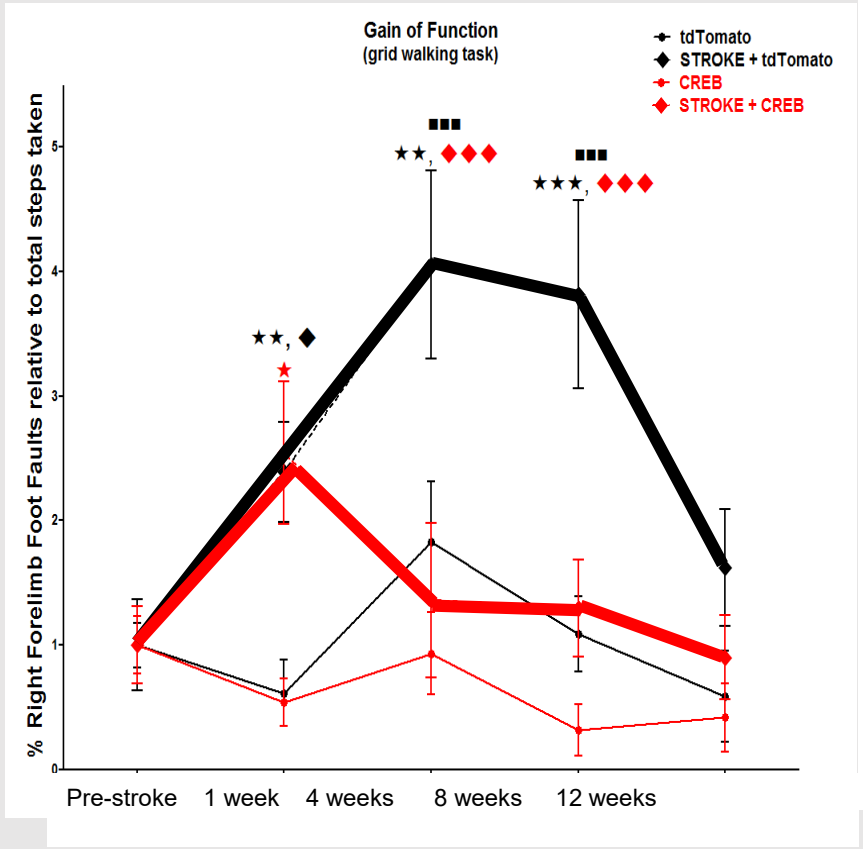
Einor Ben Assayag

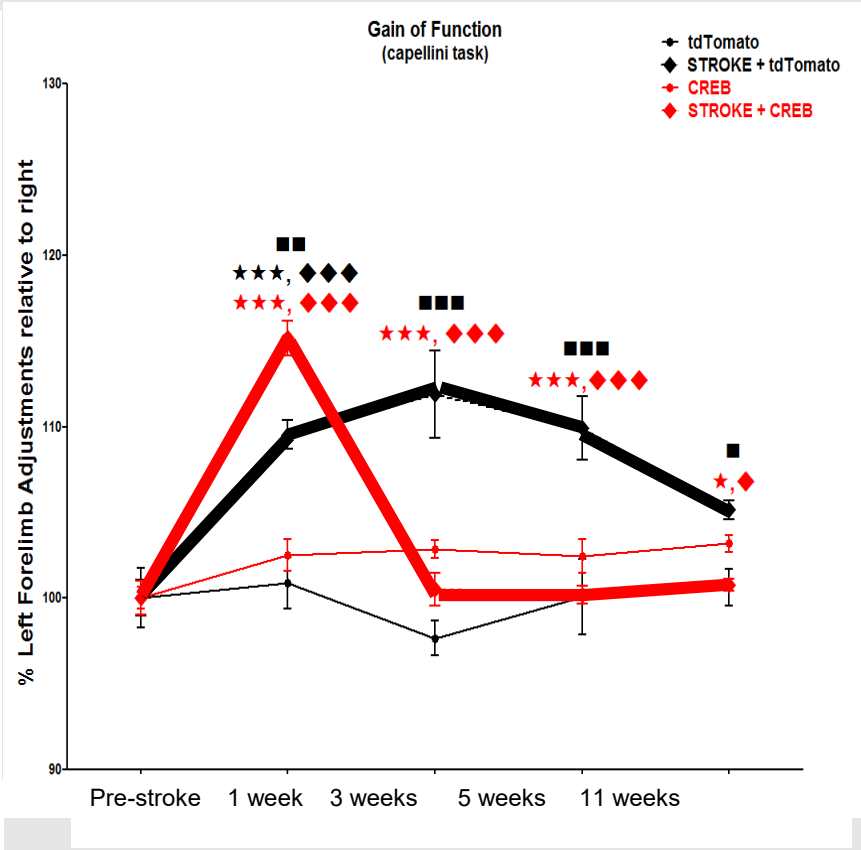
	CCR5-Δ32 non-carriers	CCR5-Δ32 carriers	p
N	328	68	
Age, years (SD)	66.7 (9.5)	66.8 (9)	0.093
Male Gender, n (%)	215 (65.5)	37 (54.5)	0.025
Education, years (SD)	13.1 (3.5)	14.9 (4.3)	<0.001
Body-mass index, kg/m^2 (SD)	26.9 (4.2)	27.9 (3.8)	0.064
Ethnicity, Ashkenazi, n (%)	189 (57.6)	61 (89.7)	<0.001
Admission Systolic blood pressure, mmHg (SD)	146.4 (22.8)	151.7 (25.4)	0.087
Current smokers, n (%)	125 (38.1)	30 (44.1)	0.339
Diabetes mellitus, n (%)	83 (25.3)	21 (30.9)	0.329
Dyslipidemia, n (%)	172 (52.4)	37 (54.4)	0.772
Hypertension, n (%)	189 (57.6)	41 (60.3)	0.666
APOE ε4 allele, n (%)	62 (18.9)	8 (11.8)	0.166
Admission NIHSS, median (IQR)	2 (1-4)	1 (0-3)	<0.001
Delta NIHSS from admission to 1 year	2 (0-4)	1 (0-3)	0.016
Cognitive scores 1 year post-stroke			
Computerized Total cognitive score (SD)	95.5 (13.4)	98.5 (10.2)	0.047
Memory score (SD)	96.4 (16.5)	100.6 (13.4)	0.033
Executive function score (SD)	96.9 (12.9)	98 (12.2)	0.533
Visuospatial score (SD)	99.6 (17.8)	98.3 (16.9)	0.615
Verbal functioning score (SD)	89.1 (23.6)	95.9 (17.5)	0.011
Attention score (SD)	96.6 (14.7)	100.4 (11.3)	0.024

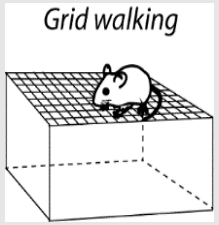

A
CCR5Δ32 carriers
non-carriers
Neurotrax score
120
100
80
60
40
20
0
p=0.047
0.033
0.533
0.615
0.011
0.024
Total score
Memory
Executive function
Visual spatial
Verbal function
Attention
B
CCR5Δ32 carriers
non-carriers
MoCA score
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
p<0.001
0.047
0.001
0.679
0.048
Language
Executive
Naming
Memory
Attention
C
CCR5Δ32 carriers
non-carriers
Estimated marginal means of MoCA score
27
26
25
24
p for trend=0.028
Hospital admission
6 months post-stroke
12 months post-stroke
24 months post-stroke

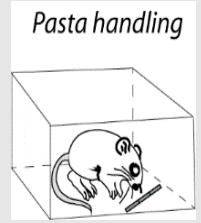
Molecular Control of CREB in a Specific Motor Circuit Improves Stroke Recovery





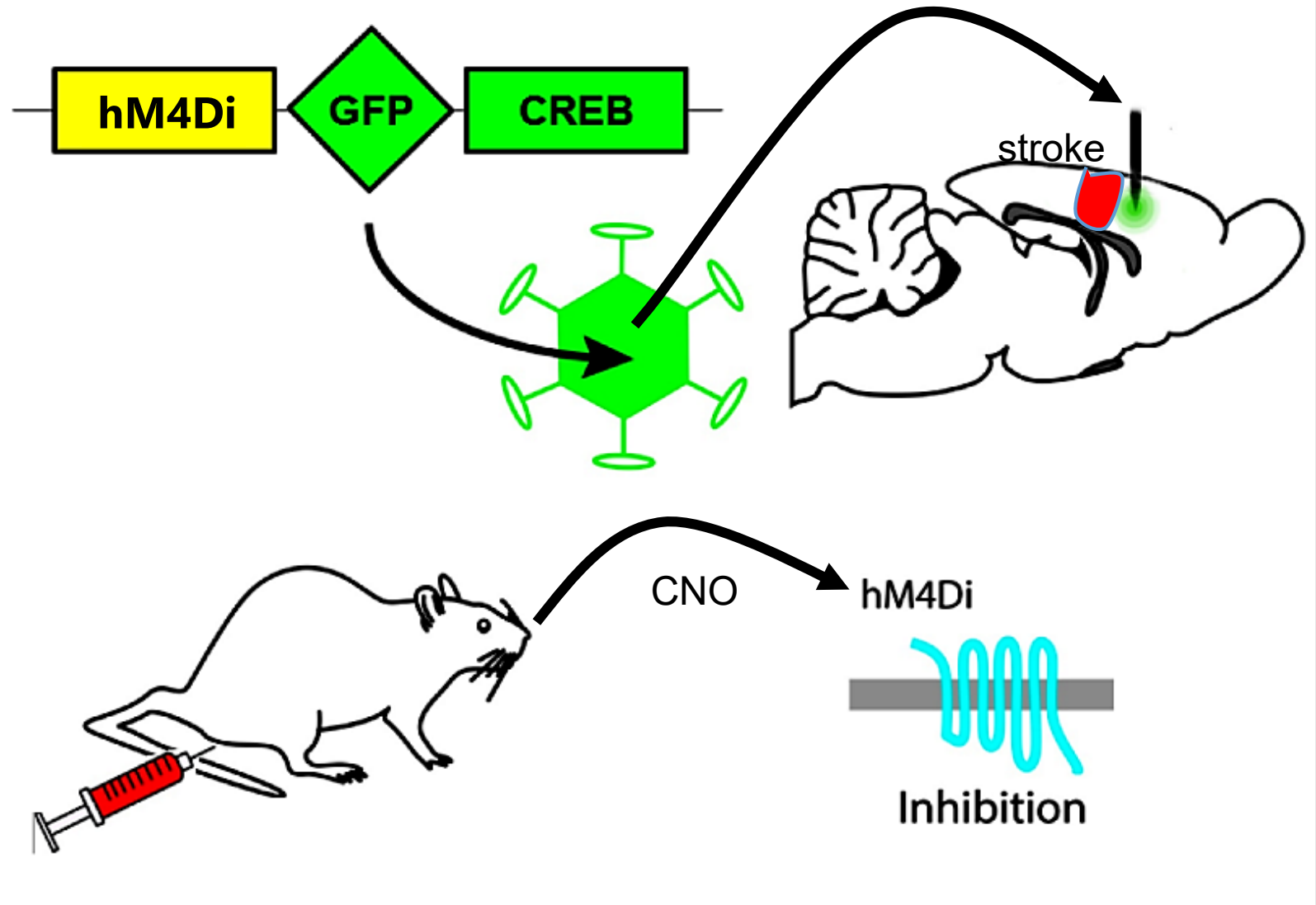






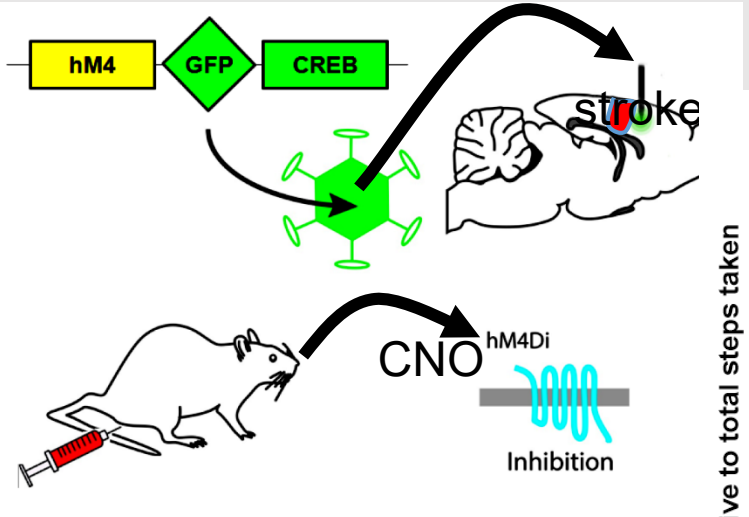
Caracciolo…Carmichael. Nature Communications, 2018, 9:2250

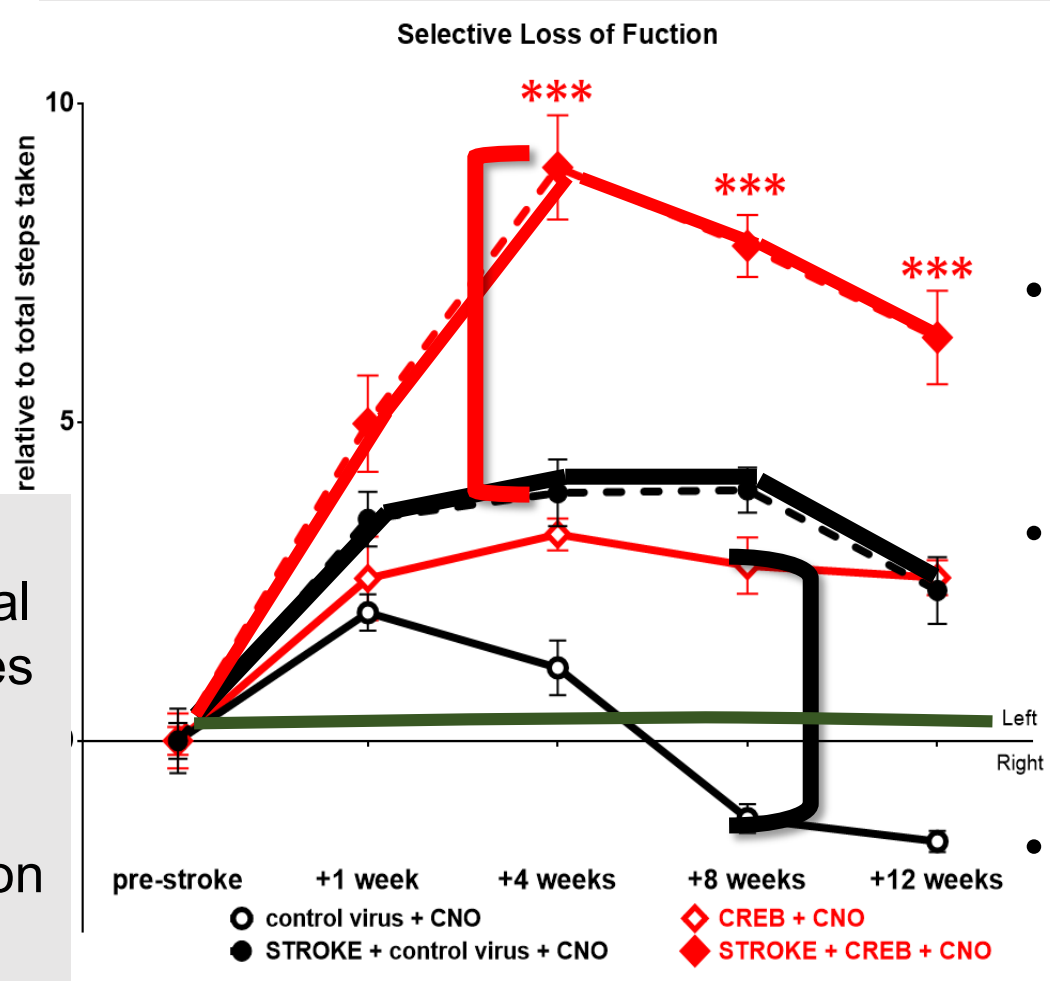
Turning Off CREB-Induced Motor Neurons During Recovery Process

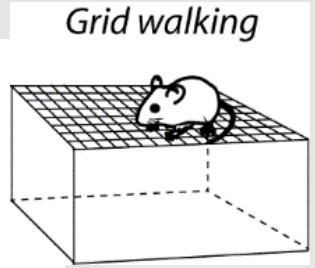


Designer Receptors Exclusively Activated by Designer Drugs (DREADD)

Turning Off CREB-Induced Motor Neurons During Recovery Process







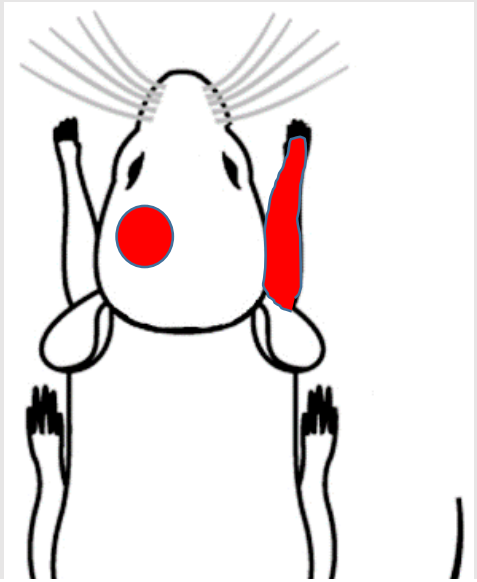
CREB effect:

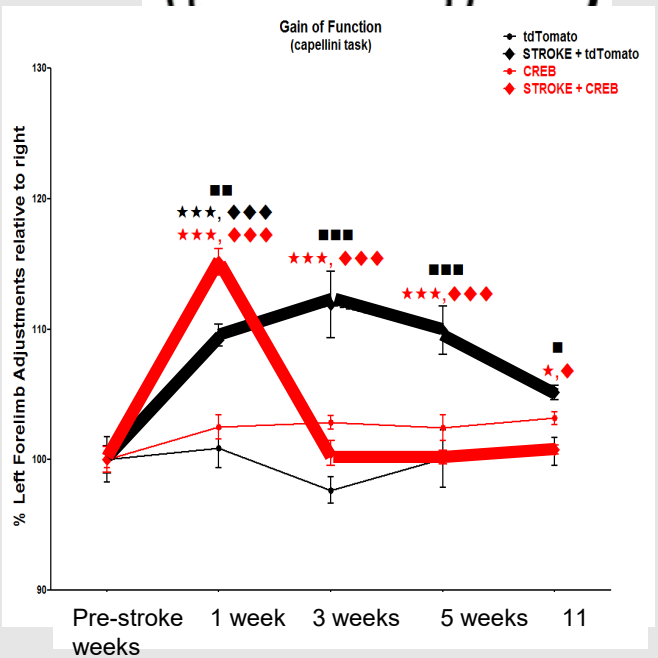
- Simply turning a motor cortical neuron off with DREADD does not impair motor control
- But inducing CREB first, and then turning off a motor neuron profoundly impairs motor control

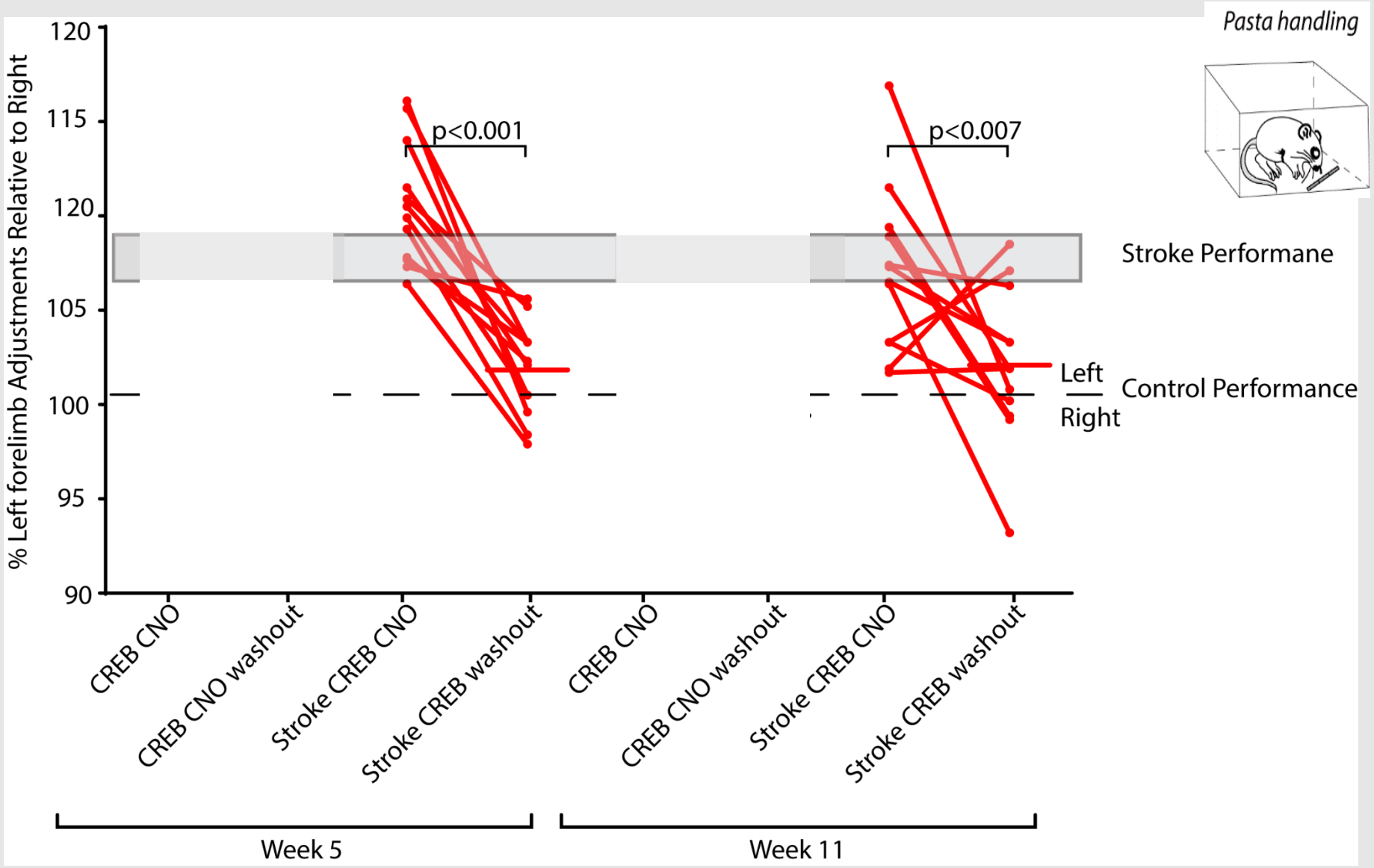
- Blocking Creb-induced motor cortical neurons blocks recovery
- In fact makes mice much worse in their stroke deficit than in stroke alone
- Blocking Creb-induced neurons in mice without a stroke causes a “stroke-like” deficit

Caracciolo…Carmichael. Nature Communications, 2018, 9:2250

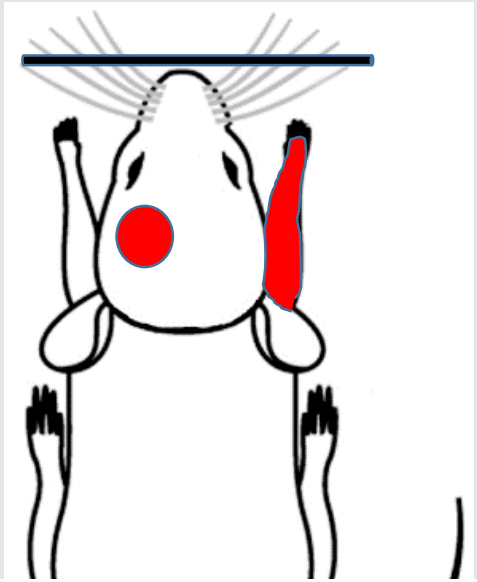
CREB Induction and Neuronal Inactivation in Motor Recovery:
Turning on and off motor recovery in stroke

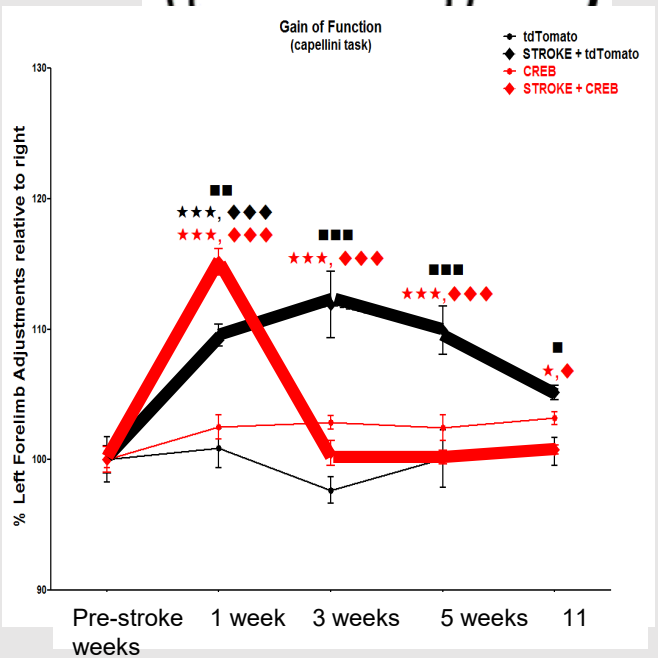


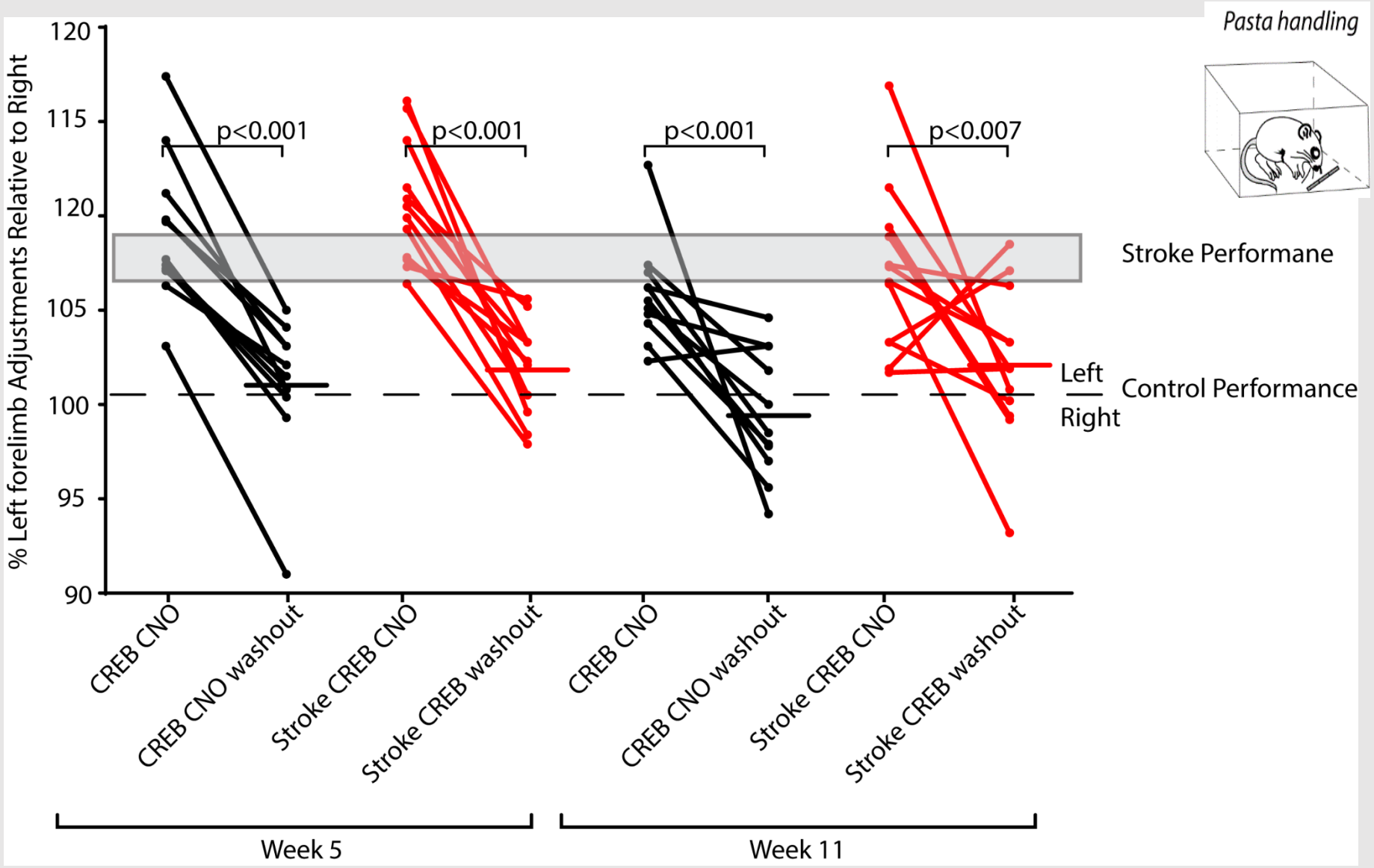




CREB Induction and Neuronal Inactivation in Motor Recovery:
Turning on and off motor recovery in stroke

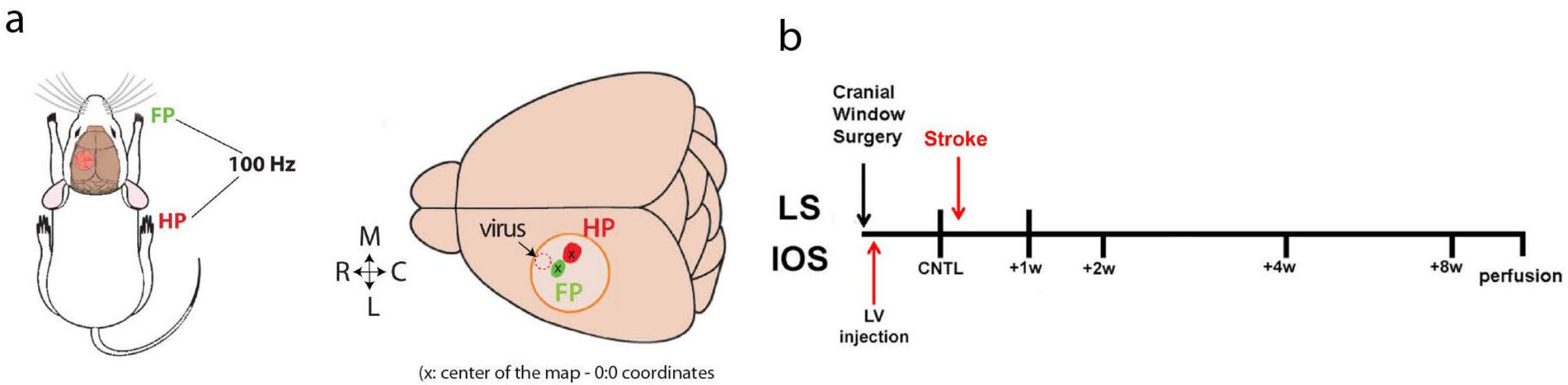






Neuronal CREB Induction and Circuit Effects in Stroke

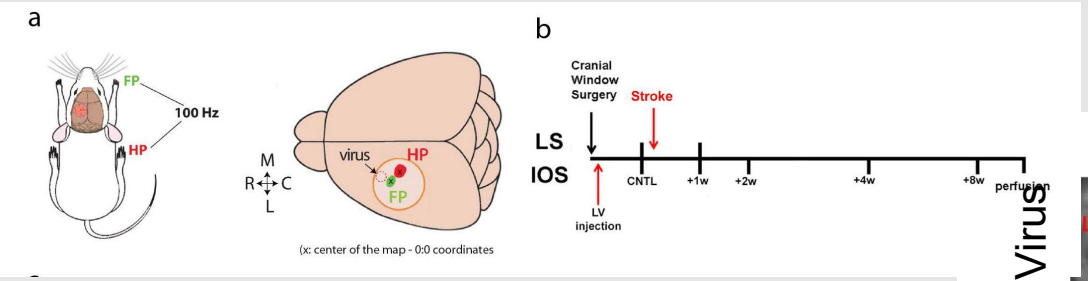
Intrinsic Optical Signal Mapping

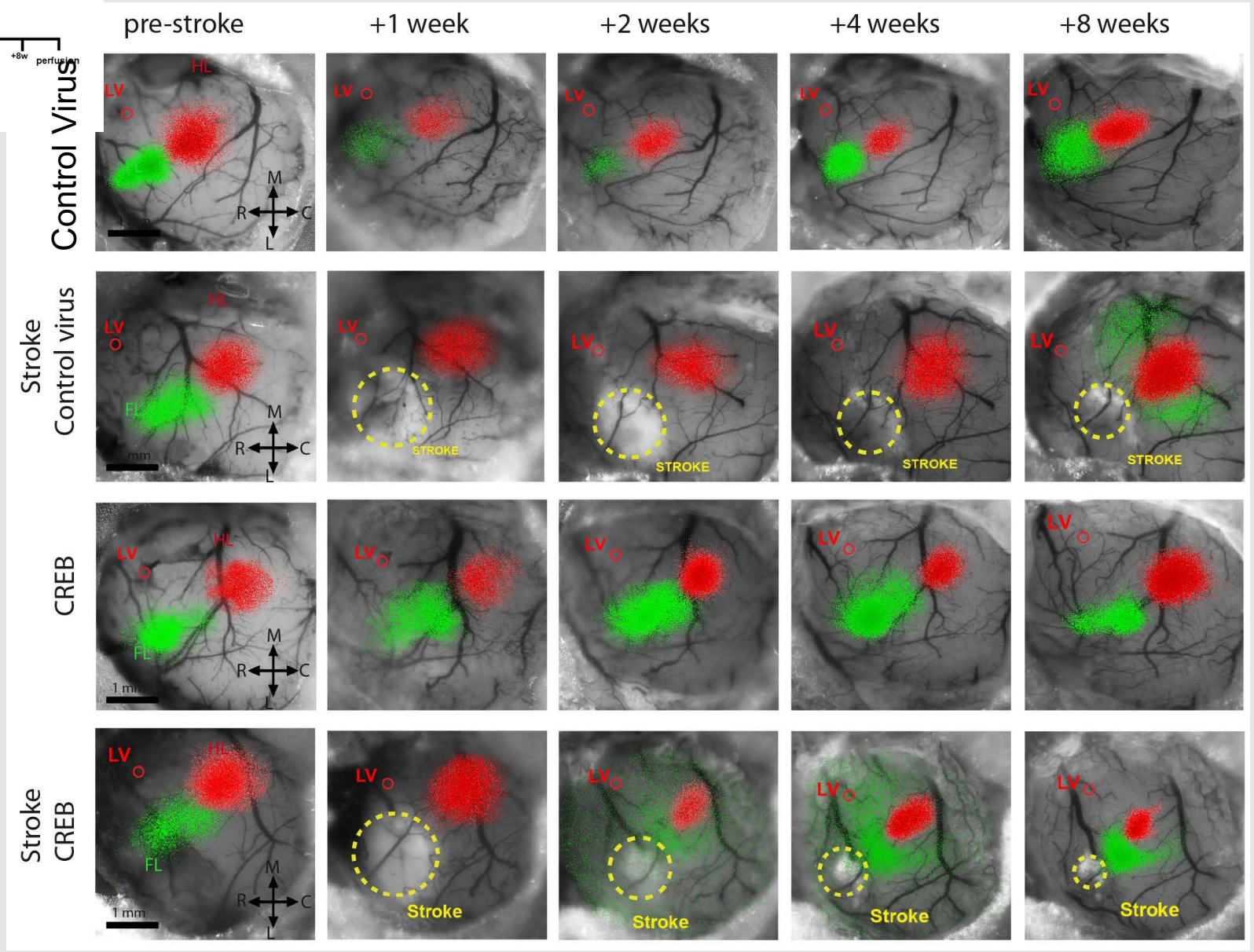


Mate Marosi, Carlos Portera-Cailliau

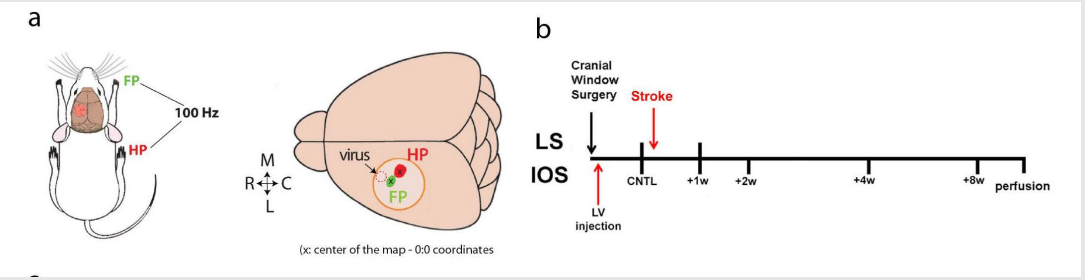
Caracciolo…Carmichael. Nature Communications, 2018, 9:2250

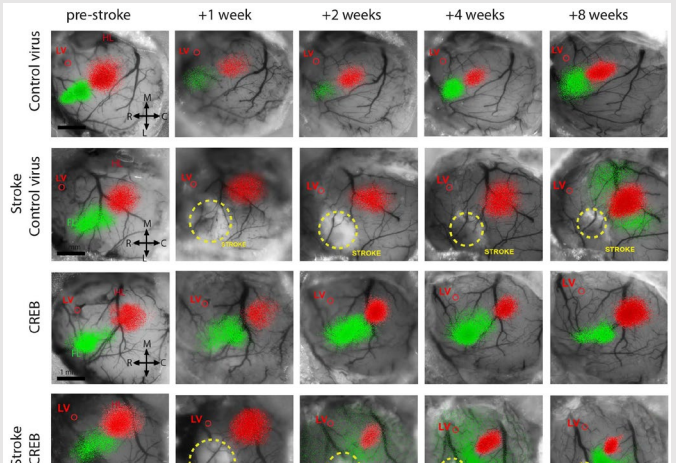
Neuronal CREB Induction and Circuit Effects in Stroke

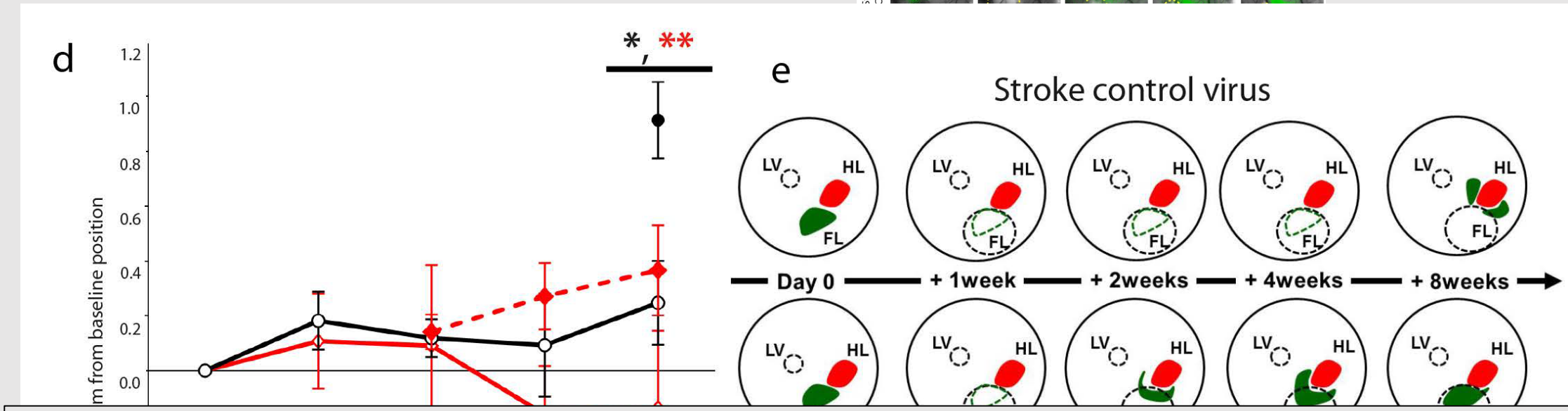




Neuronal CREB Induction and Circuit Effects in Stroke



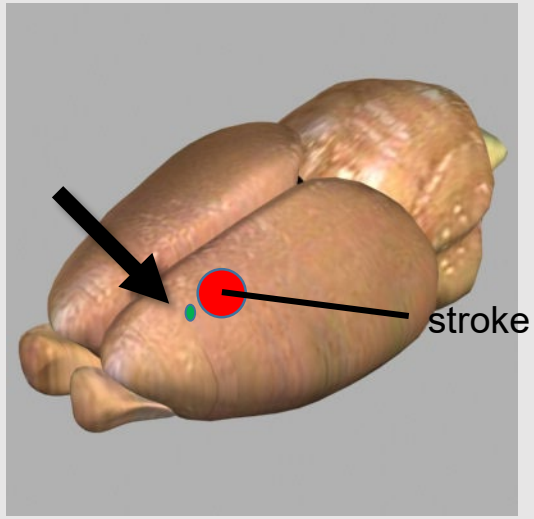




CREB induction in a subset of motor cortex circuits remaps somatosensory body representations faster and in a more normal pattern

control virus CREB Stroke control virus Stroke CREB

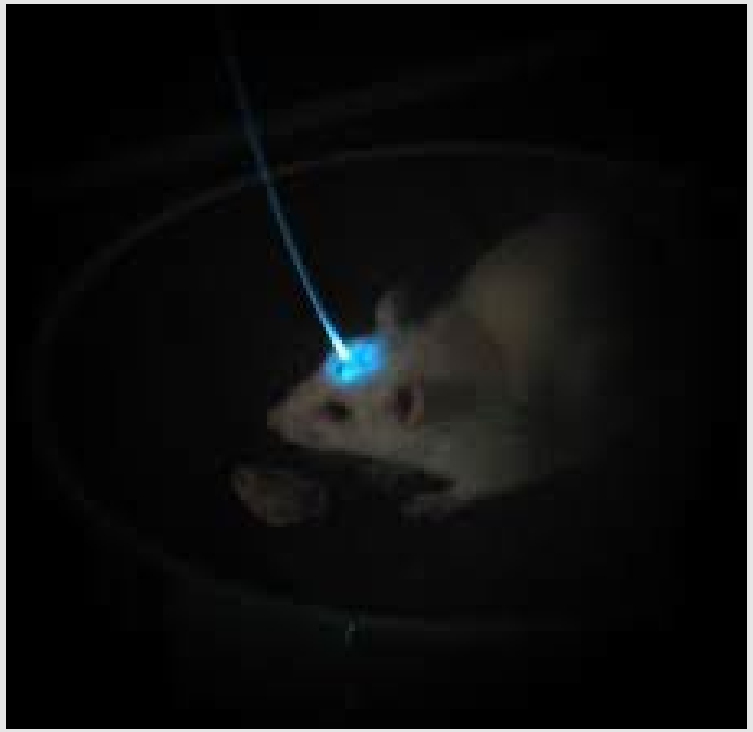
Neuronal CREB Induction and Circuit Effects in Stroke



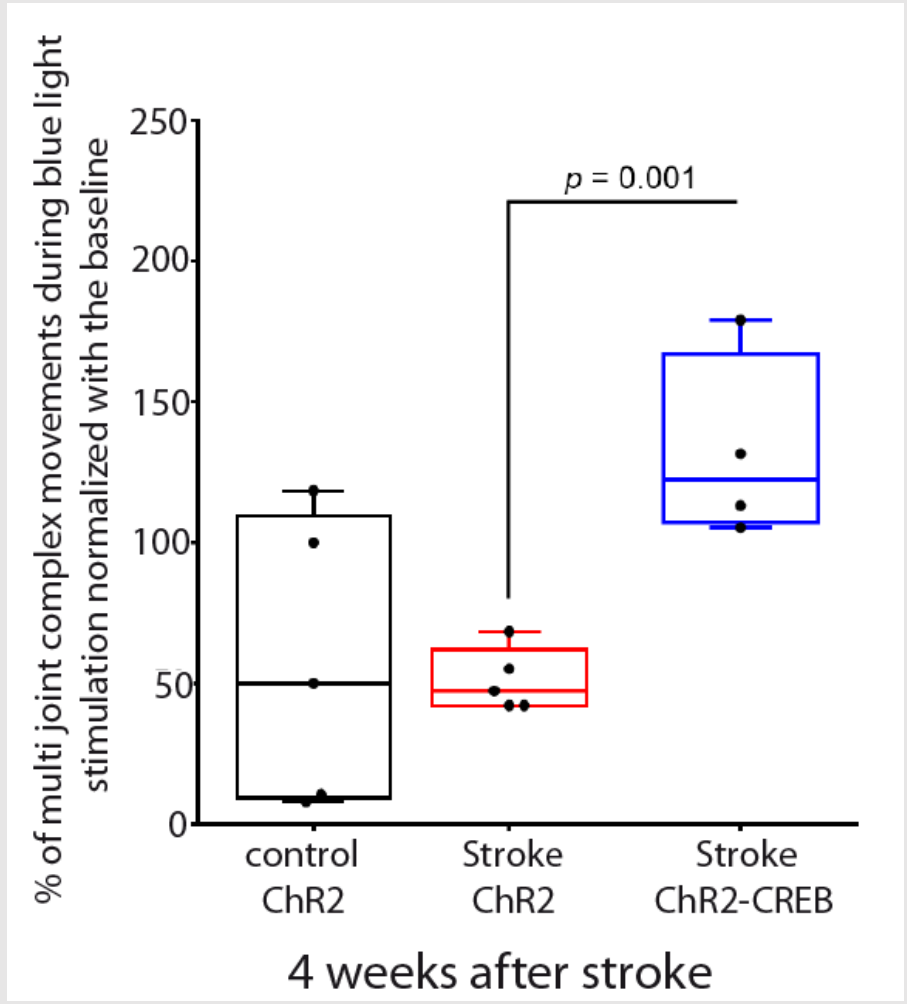
Motor Mapping

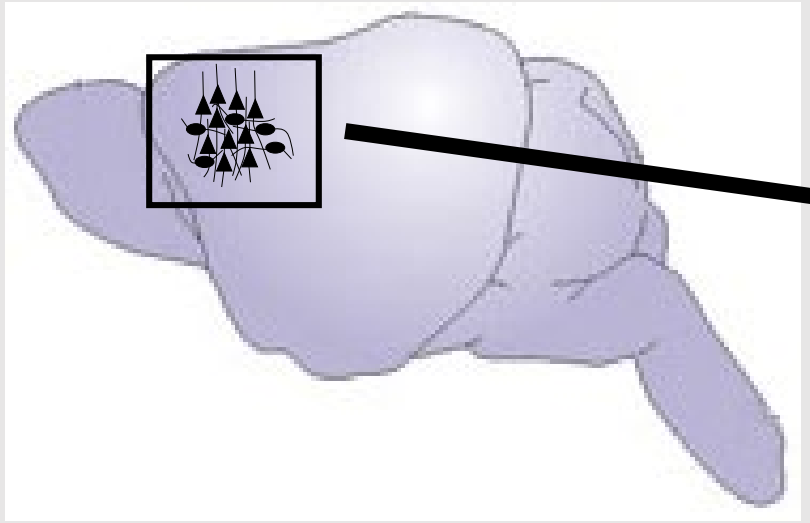
Lenti-pCAMKII-ChR2-CREB

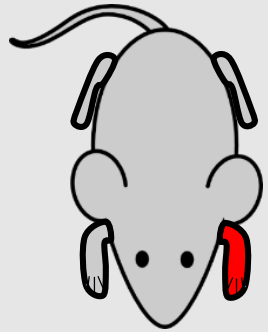
Lenti-pCAMKII-ChR2

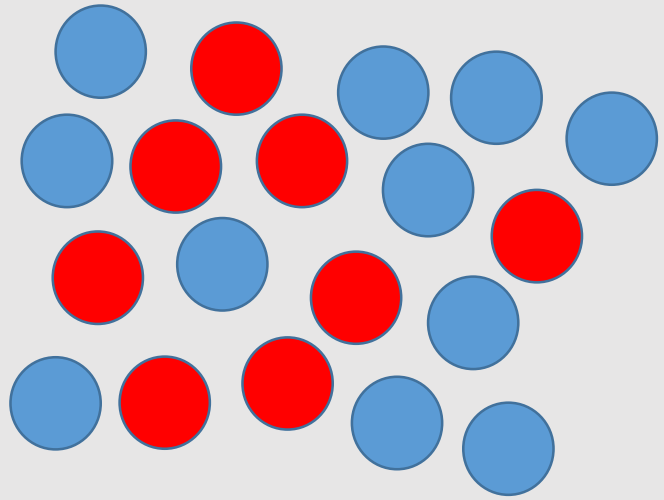


- Activating CREB in recovering motor neurons in forelimb motor cortex allows these neurons to take over control of more than just the forelimb
- These CREB-neurons control trunk, face and other body parts

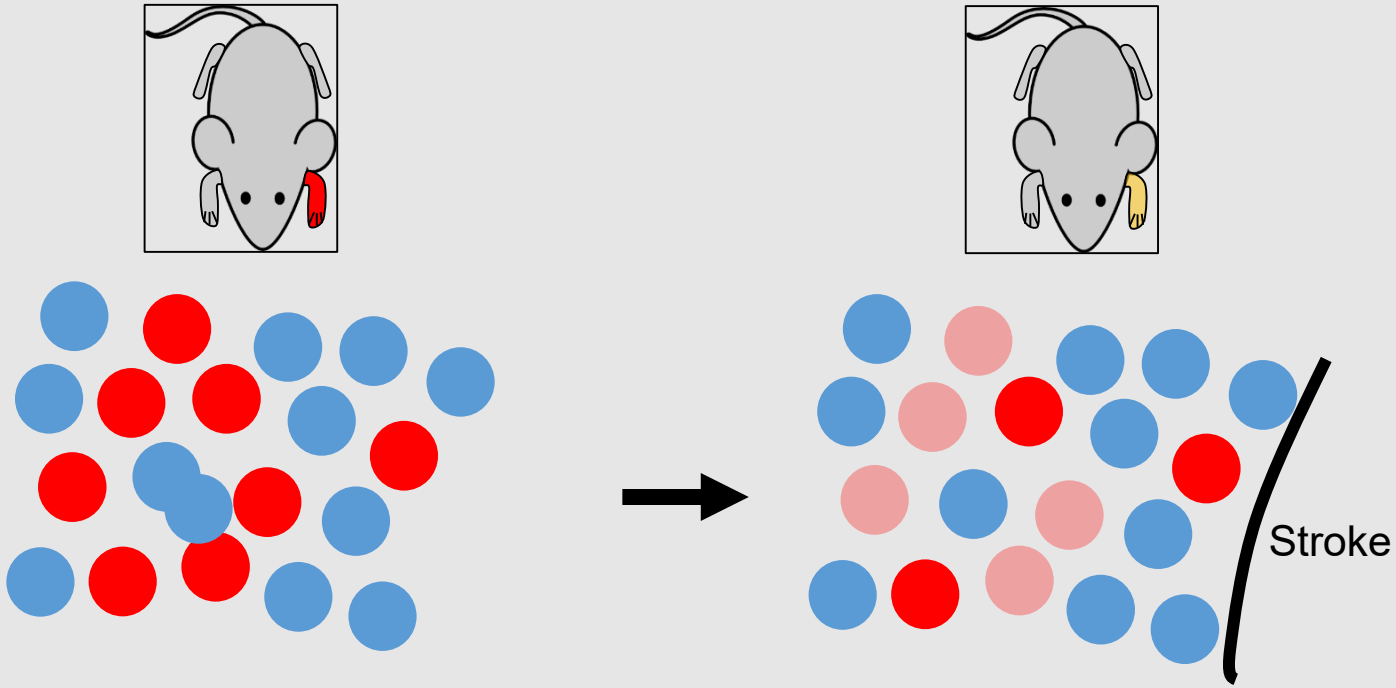






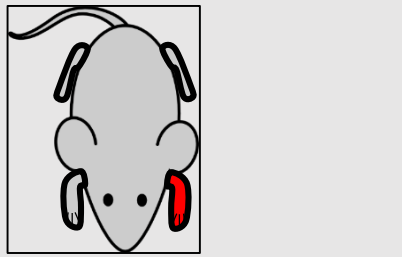


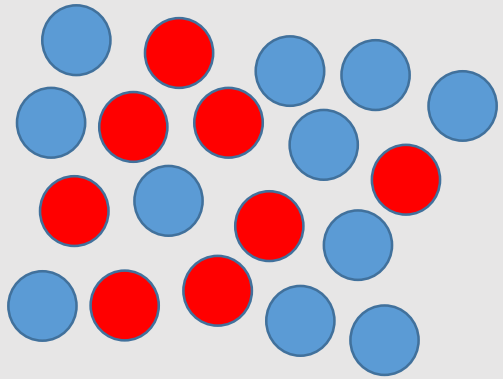
The Motor “Engram”:
Circuit of co-activated neurons that move the forelimb



The Motor “Engram”:
Circuit of co-activated neurons that move the forelimb

The Degraded “Engram”:
Stroke reduces the network of functionally activated neurons that move the forelimb





The Motor “Engram”:
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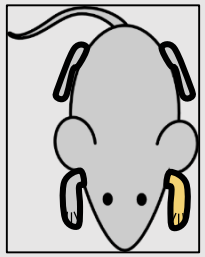

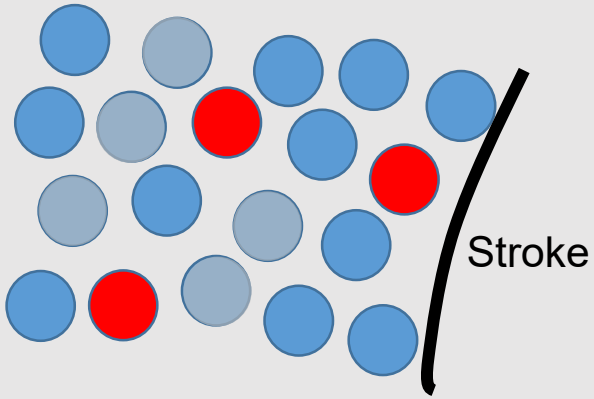

The Degraded “Engram”:
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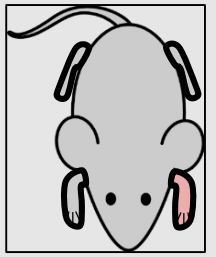
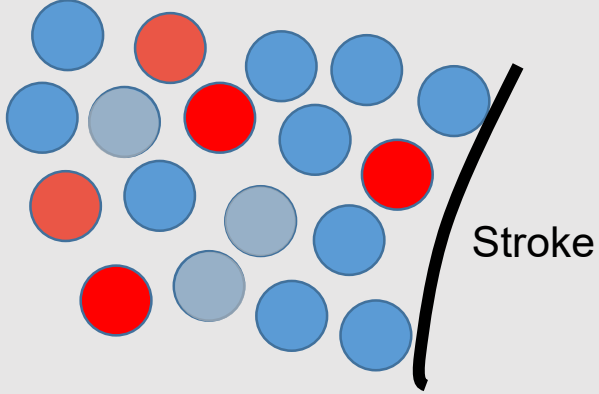
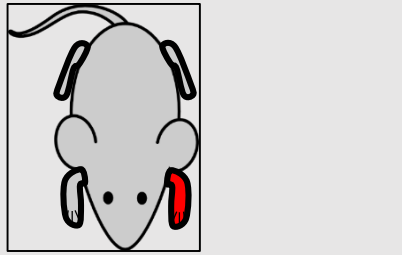
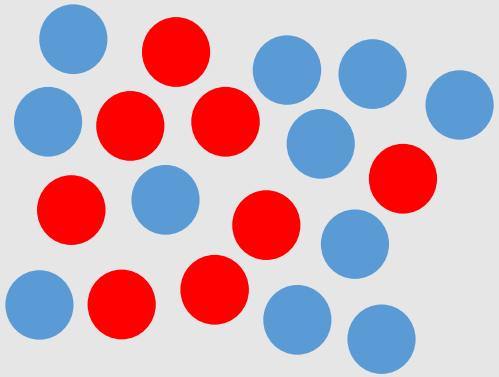




Normal, incomplete recovery:
Partial re-allocation of neurons into the motor engram





The Motor "Engram":
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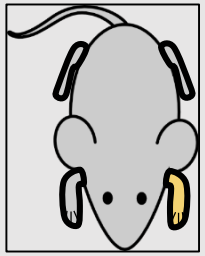
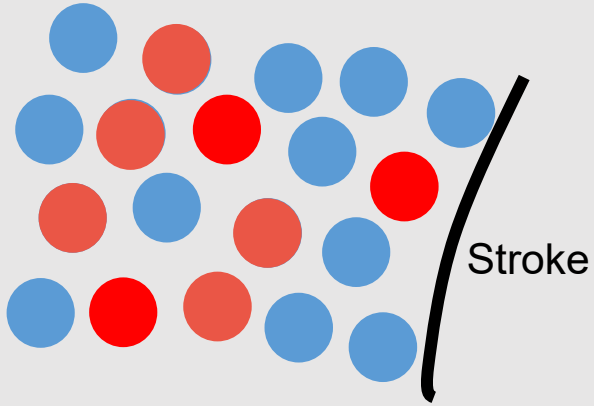
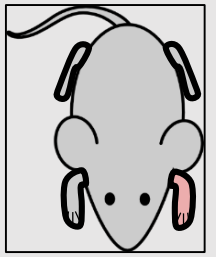
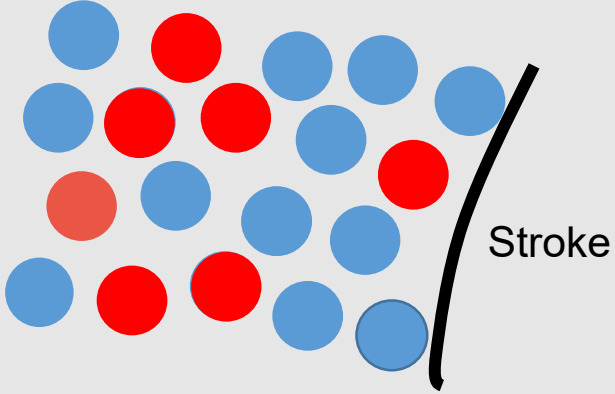




The Degraded "Engram":
Stroke reduces the network of functionally activated neurons that move the forelimb





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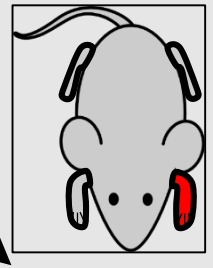
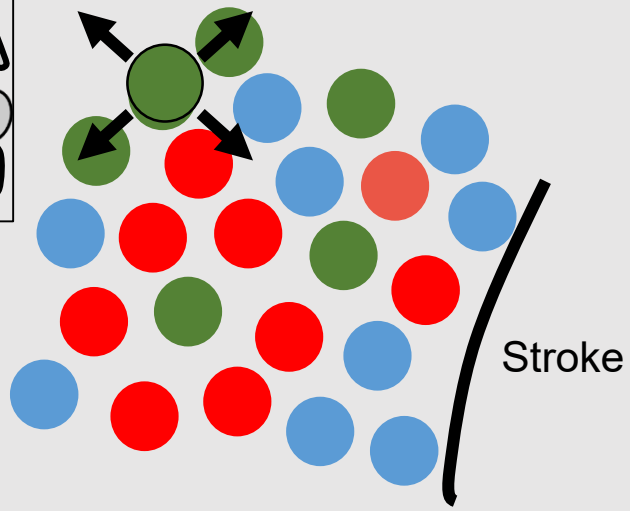




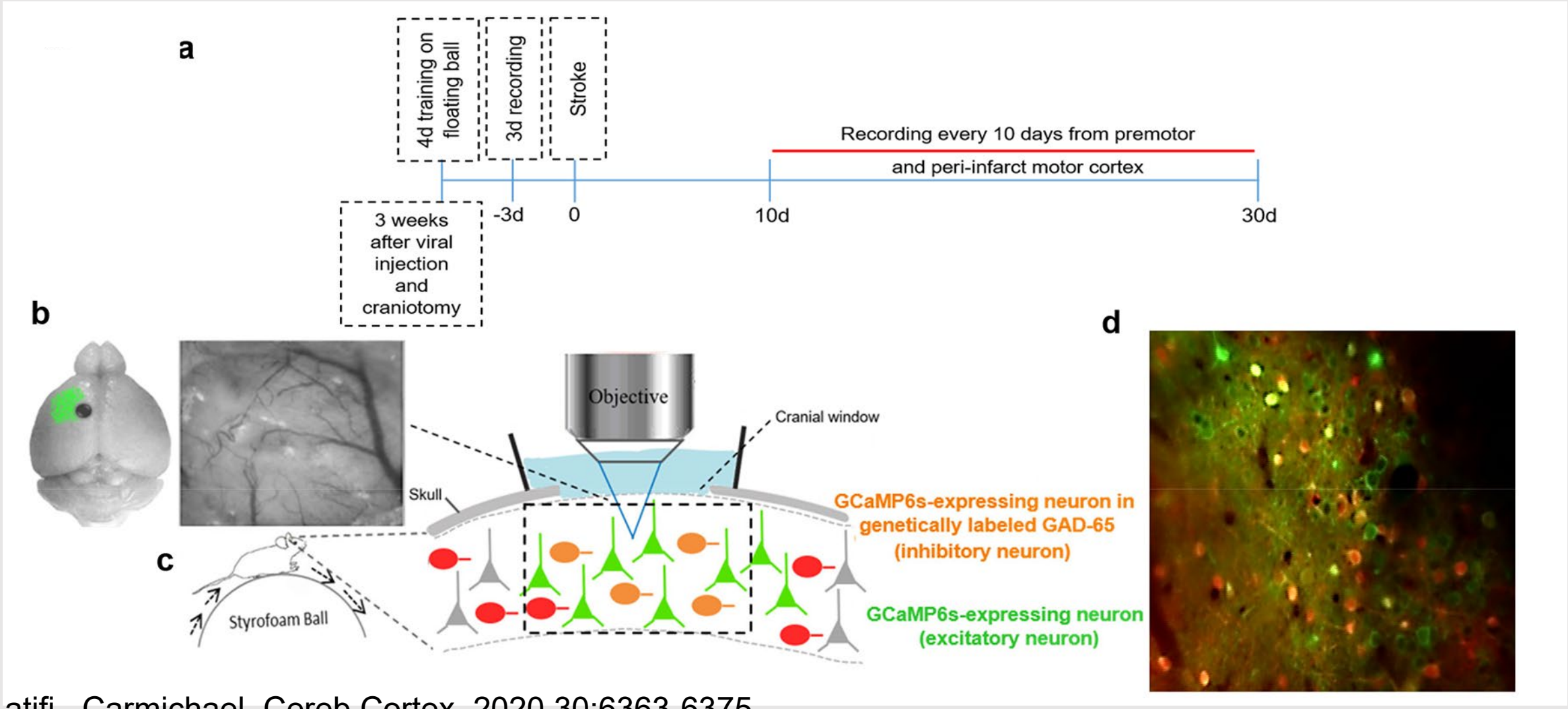
Enhanced recovery with CCR5/Creb/DLK:
New neuronal allocation into an expanded motor engram

- Tonic GABA antagonists
- AMPA receptor enhancers
- PDE isoform inhibitors
- CCR5 Antagonists

- Pharmacological Targets for Stroke Recovery Drugs
- Three clinical trials in this group of drugs

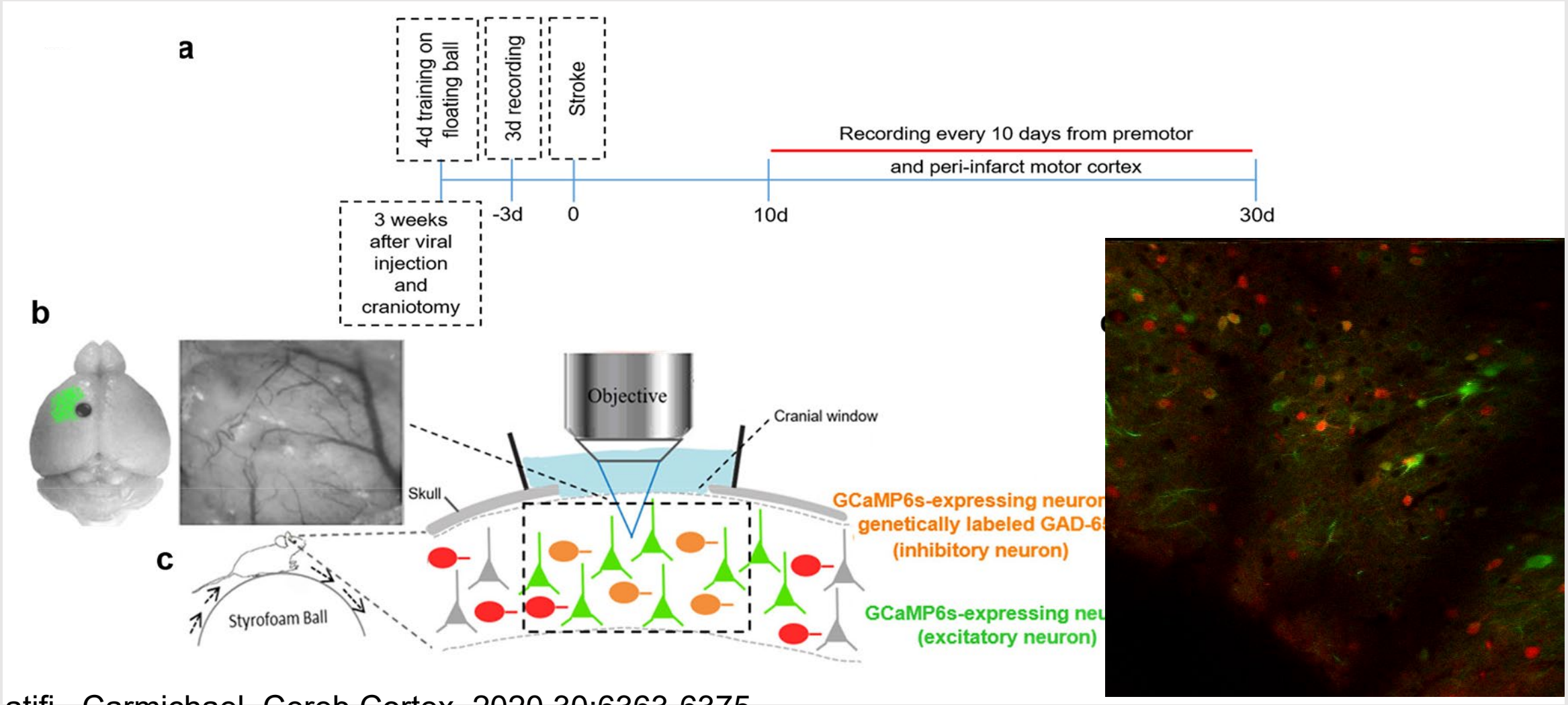
If a common mechanism is neuronal allocation, what does this look like?

Strategy for Visualizing Neuronal Circuits over Time before and after Stroke



Latifi...Carmichael. Cereb Cortex. 2020 30:6363-6375

Strategy for Visualizing Neuronal Circuits over Time before and after Stroke



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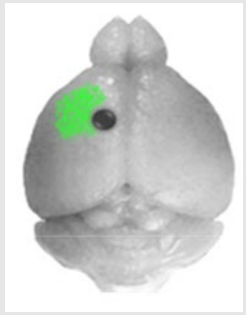
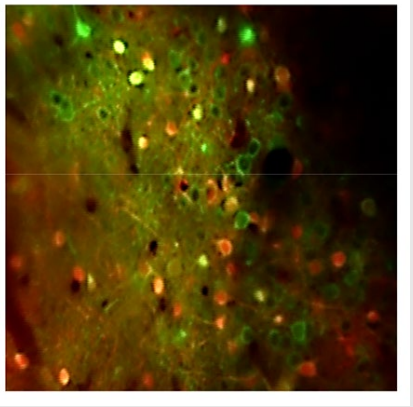
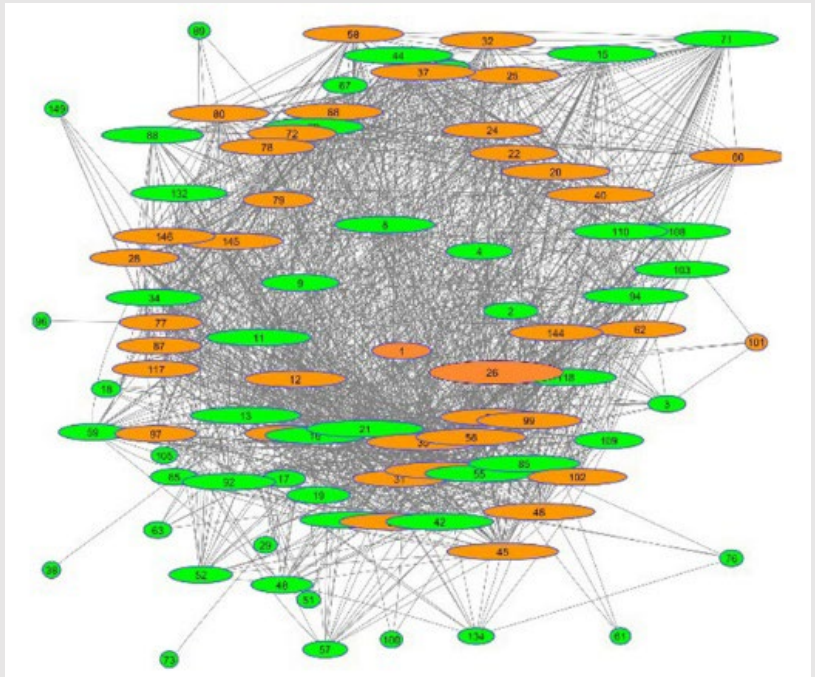


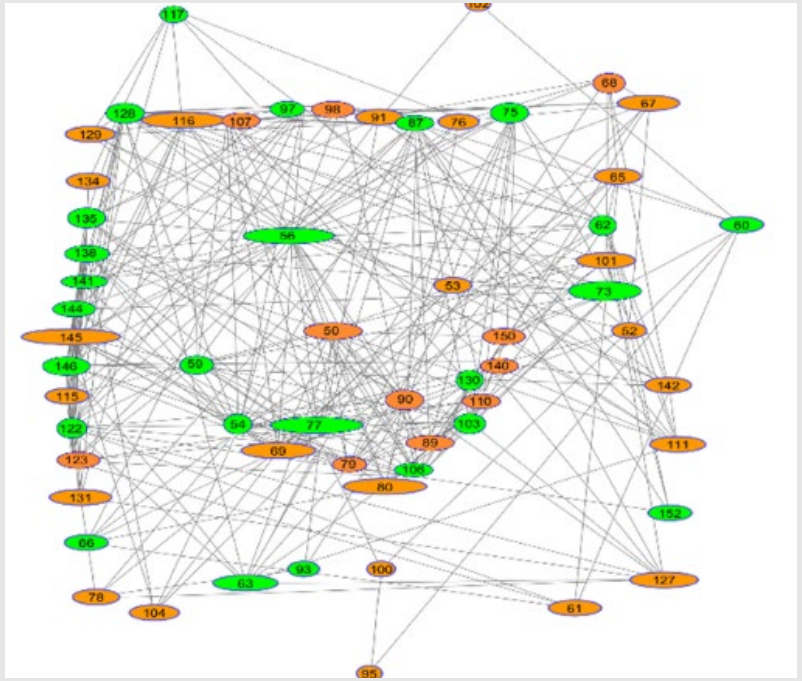


Motor Cortex Neuronal Activity During Movement

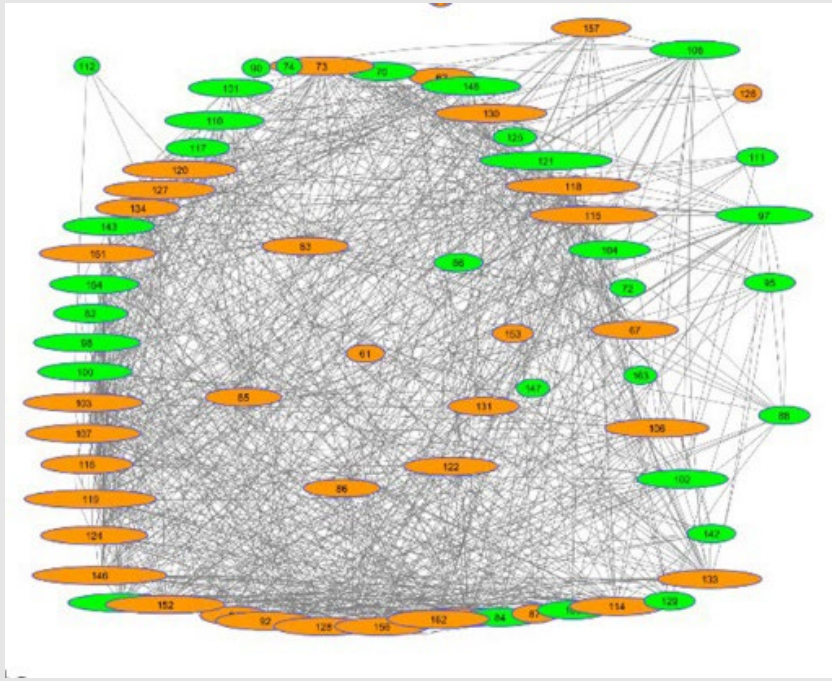
Before Stroke



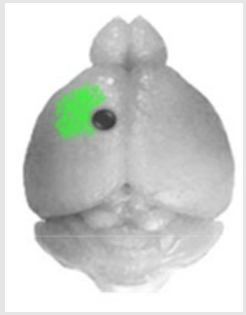
After Stroke

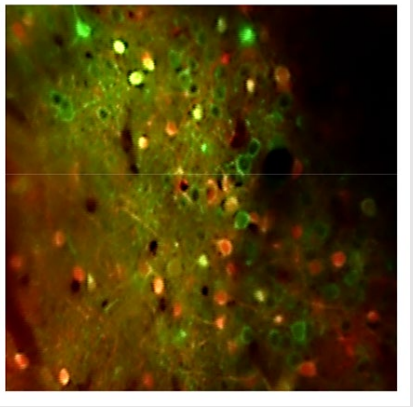


After Stroke with Recovery



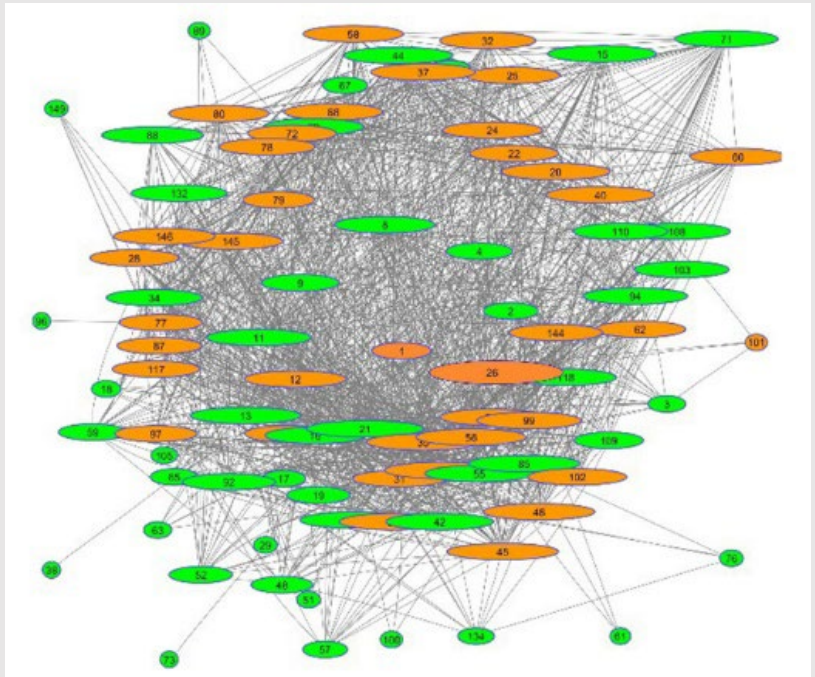
Loss of functional Interactions among a motor circuit, then re-allocation of neurons into this circuit



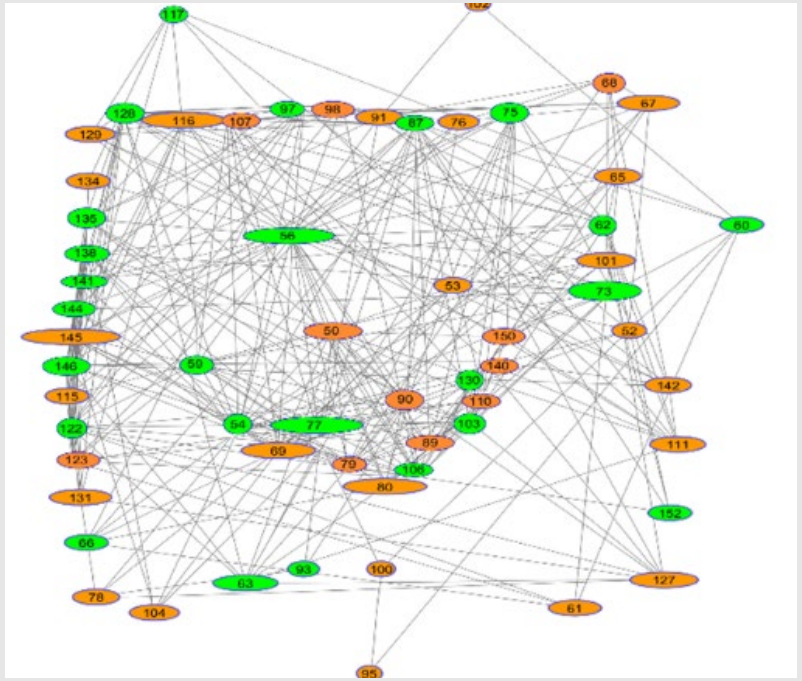


Motor Cortex Neuronal Activity During Movement

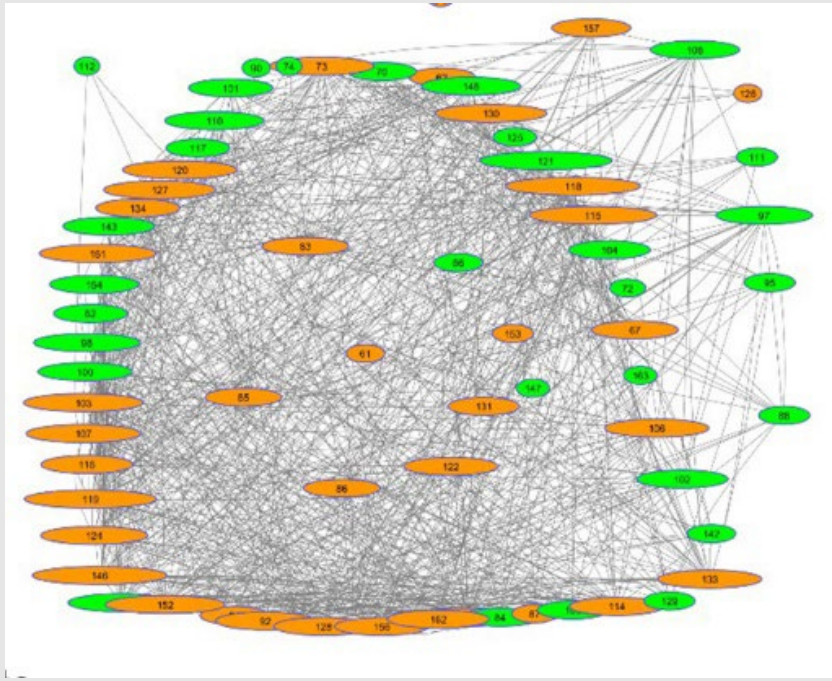
Before Stroke



After Stroke

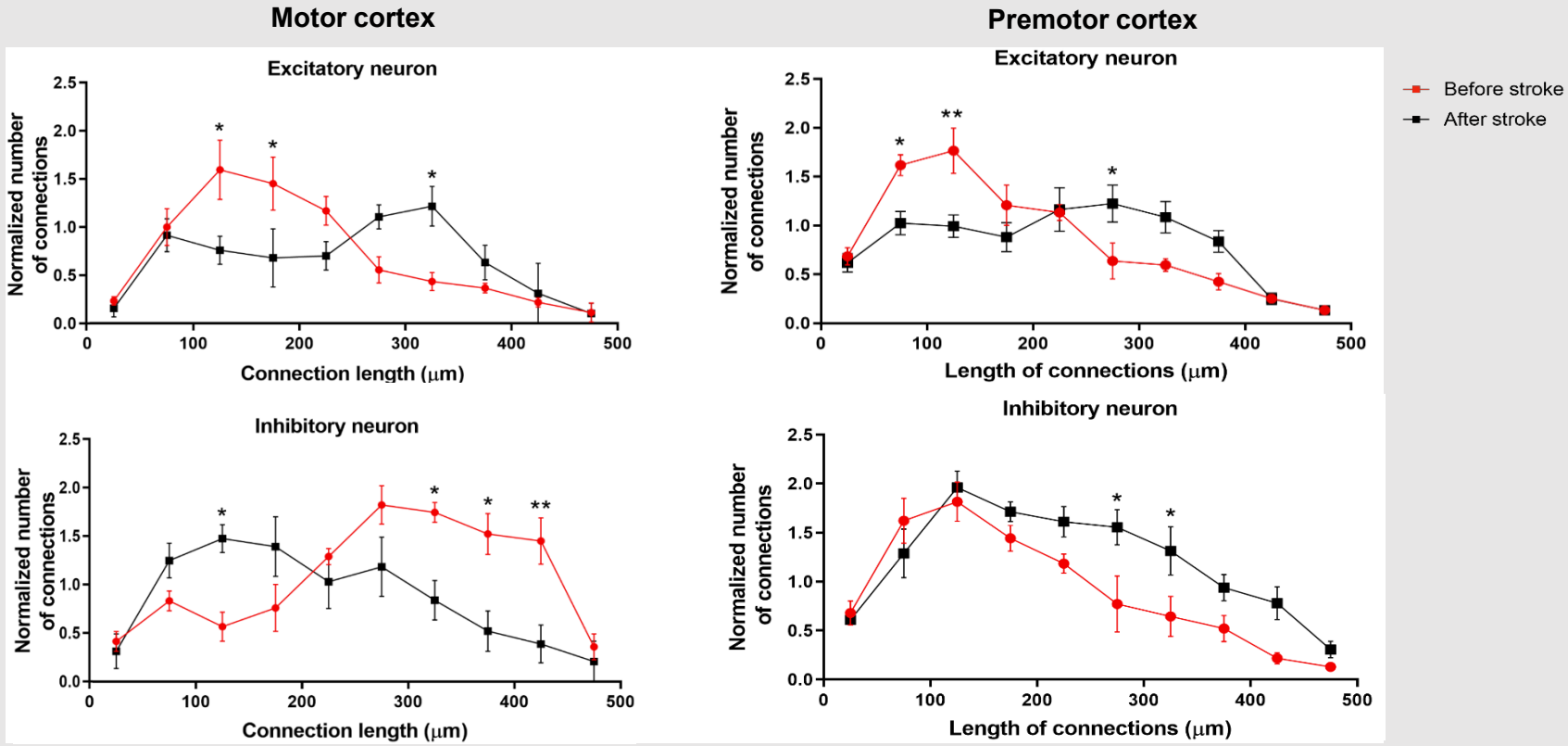


After Stroke with Recovery



Loss of functional Interactions among a motor circuit, then re-allocation of neurons into this circuit

Network Topology after Stroke—cell-specific changes



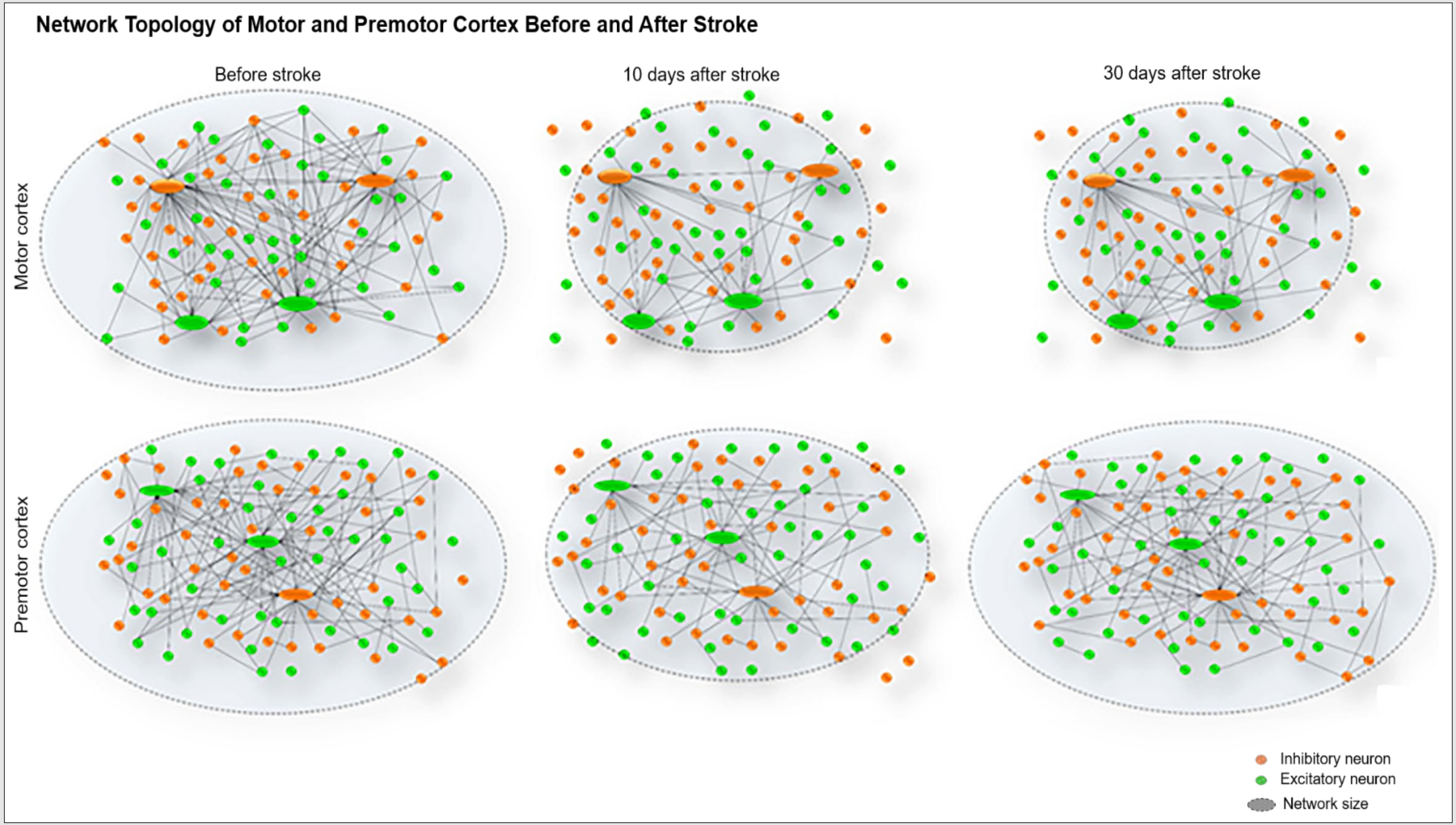
--Excitatory neurons: the strength of functional connectivity decays as the inverse of physical distance; the majority of edges in the functional network have a relatively short distance (between 50 to 250 μm)

--Inhibitory neurons: in motor cortex have predom long distance connections, in premotor cortex have short distance connections

--With stroke, the inverse action occurs:

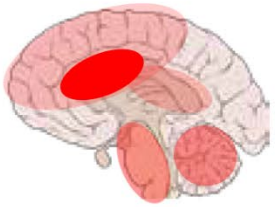
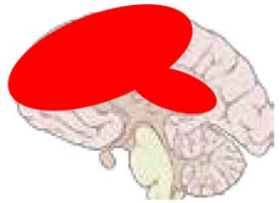
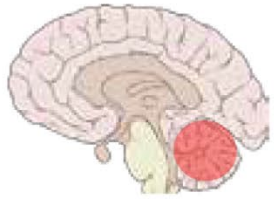
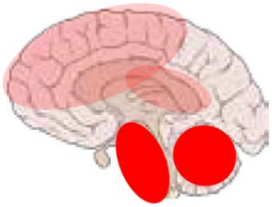
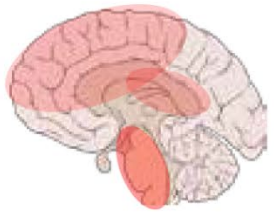
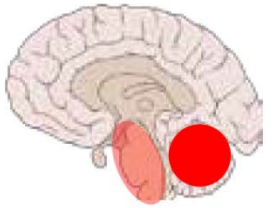
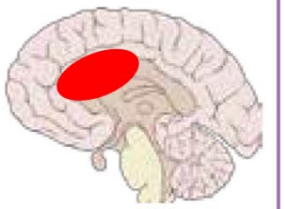
excitatory neurons lose short distance connectivity and gain long distance connectivity

inhibitory neurons in motor cortex lose long distance connections and gain short distance connections, in premotor cortex inhibitory neurons gain long distance connections


Network Topology of Motor and Premotor Cortex Before and After Stroke
Before stroke
10 days after stroke
30 days after stroke
Motor cortex
Premotor cortex
Inhibitory neuron
Excitatory neuron
Network size

PDE Inhibitors for Stroke Recovery

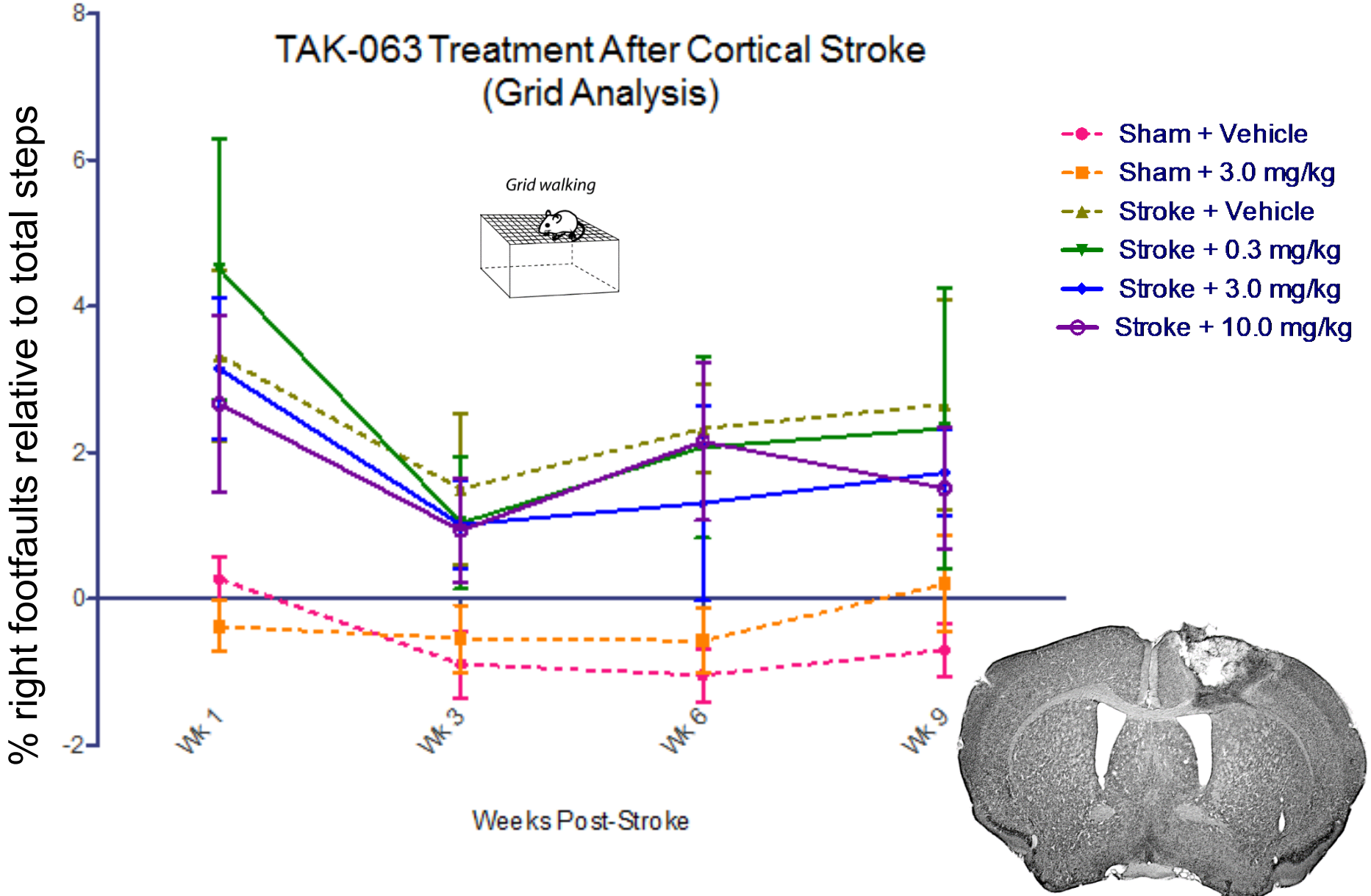
- 11 PDE gene families, comprising 21 genes that generate 100 (or more) proteins via alternative splicing of mRNA or multiple promoters and transcription start sites.
- PDE4 inhibitor (Rolipram) has been shown to promote stroke

PDE1A-C	PDE2A	PDE3A-B	PDE4A-D	PDE5A	PDE8A-B	PDE9A	PDE10A
cAMP/cGMP (mainly cAMP)	cAMP/cGMP (mainly cGMP)	cAMP	cAMP	cGMP	cAMP	cGMP	cAMP/cGMP
							
Periphery Heart	Periphery Spleen #2	Periphery Heart	Periphery multiple tissues	Periphery multiple tissues	Periphery Thyroid gland	Periphery multiple tissues	Periphery

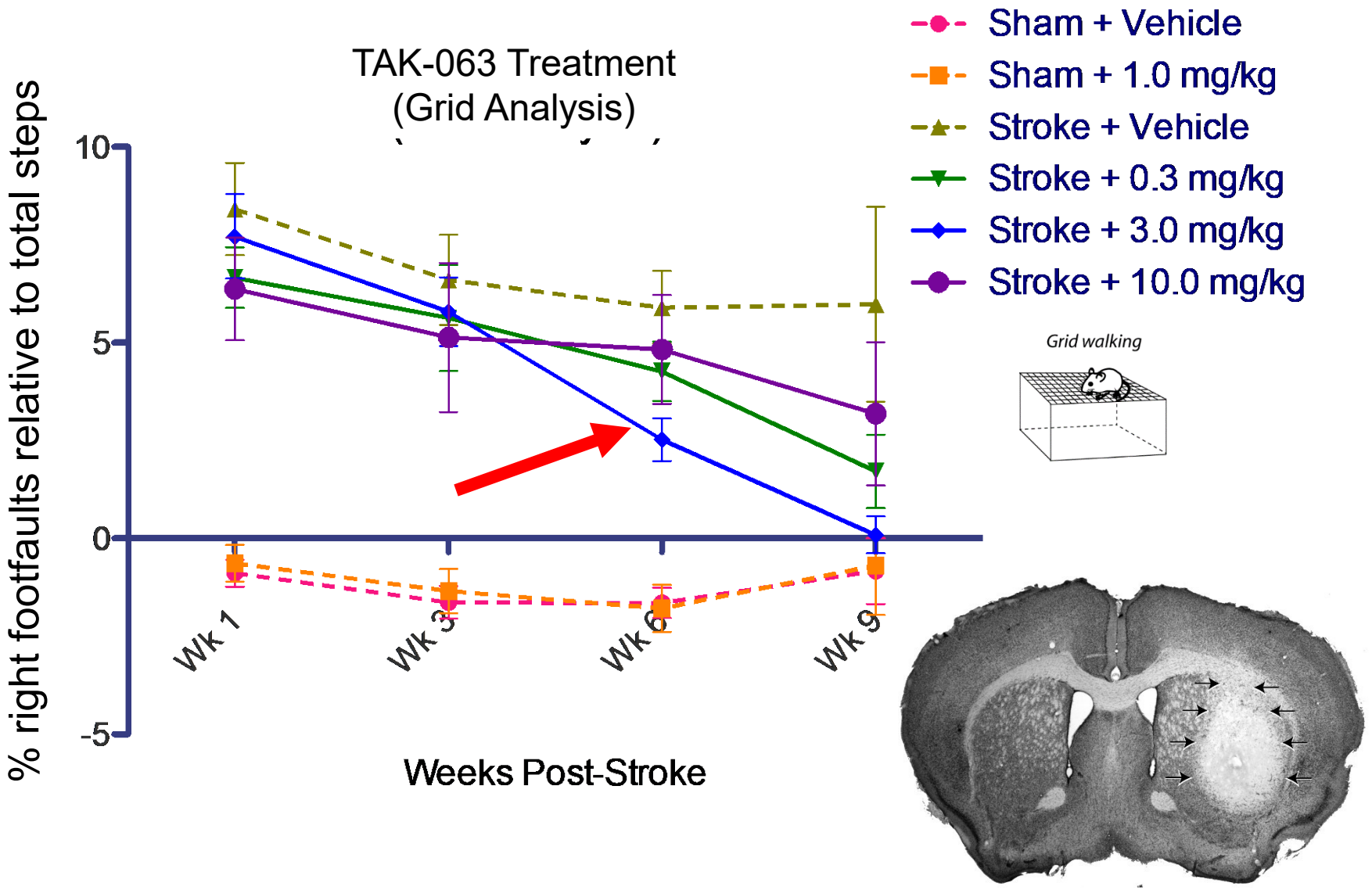
Modified from Neuropharmacology 59: 367-374 (2010)

- PDE10a inhibitor may exhibit brain region selectivity in stroke recovery.

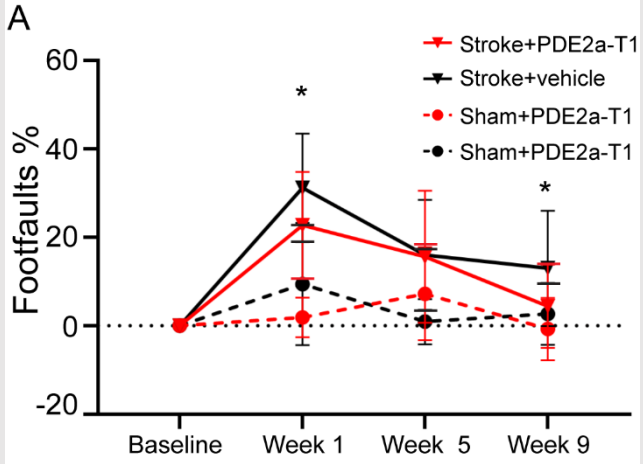
TAK-063 Does Not Promote Motor Recovery in Cortical Stroke Mouse Models

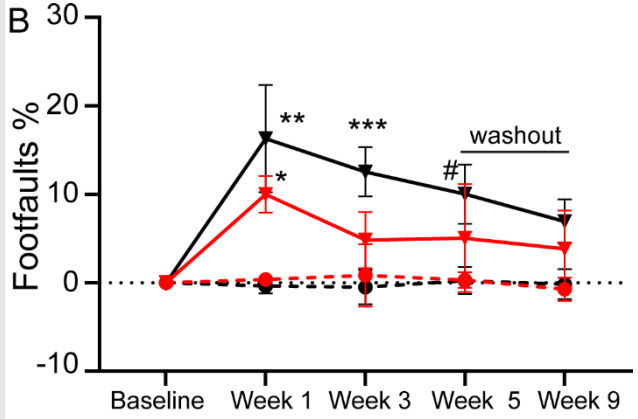


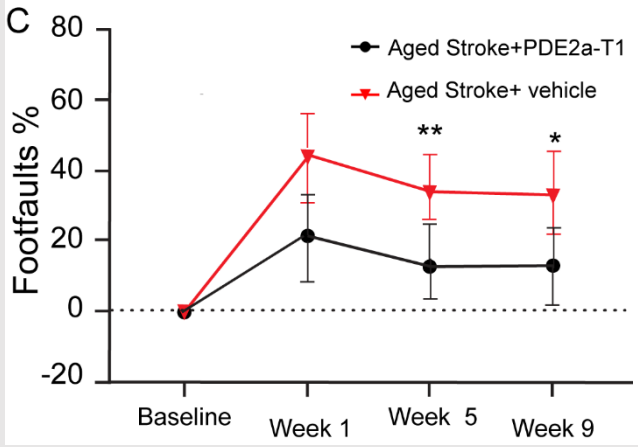
TAK-063 Promotes Motor Recovery in Striatal Stroke

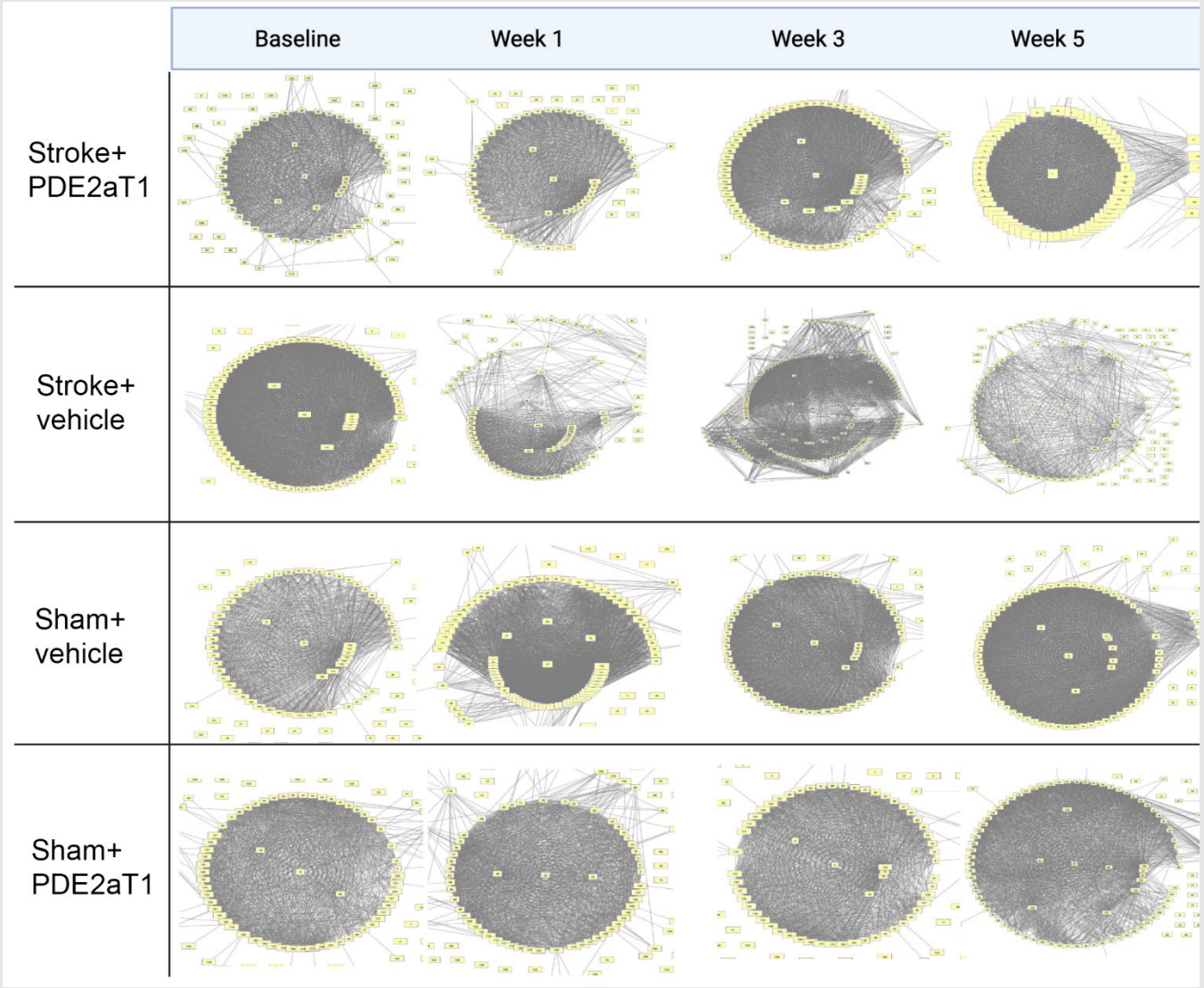


PDE2a inhibition in stroke

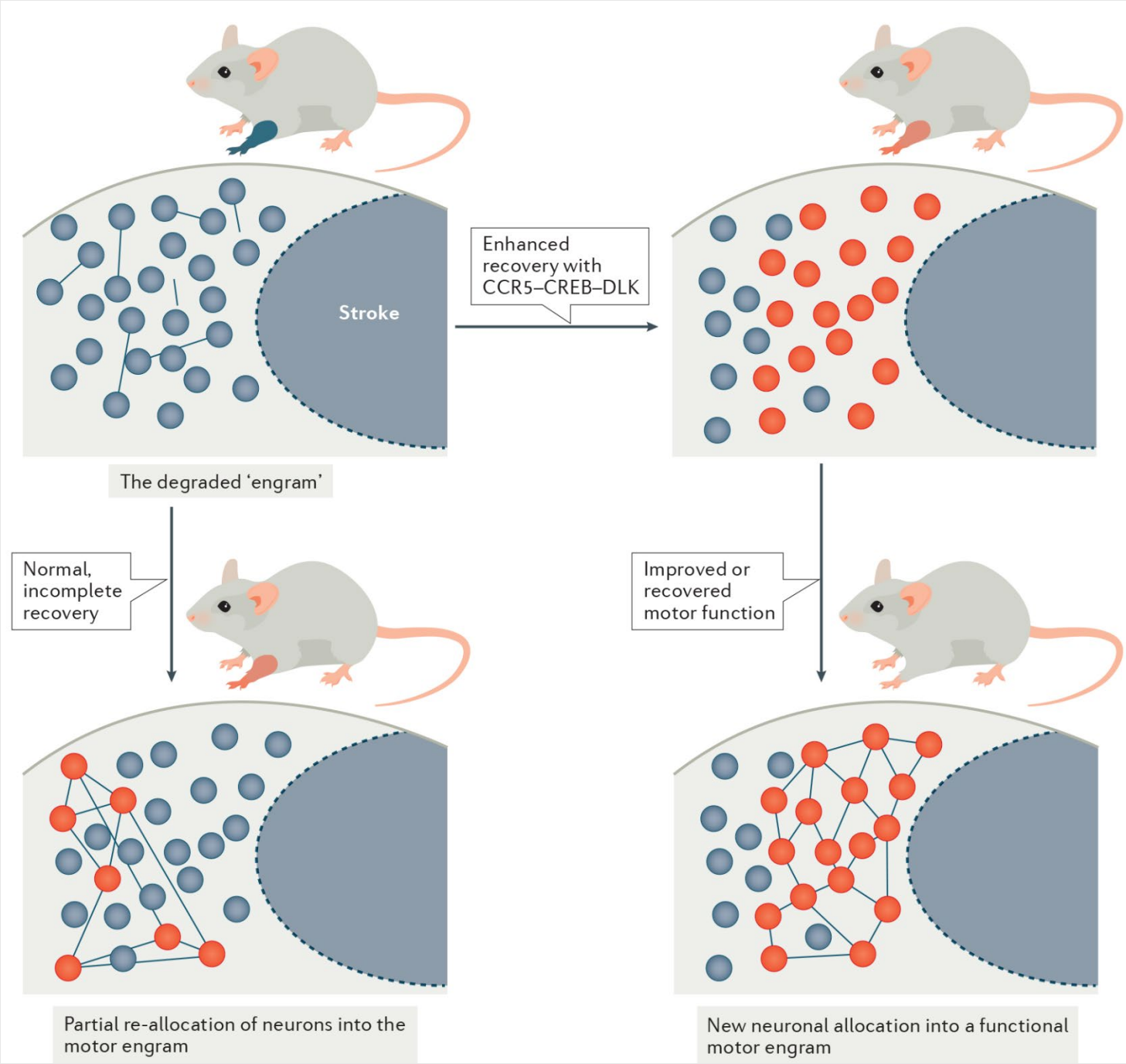




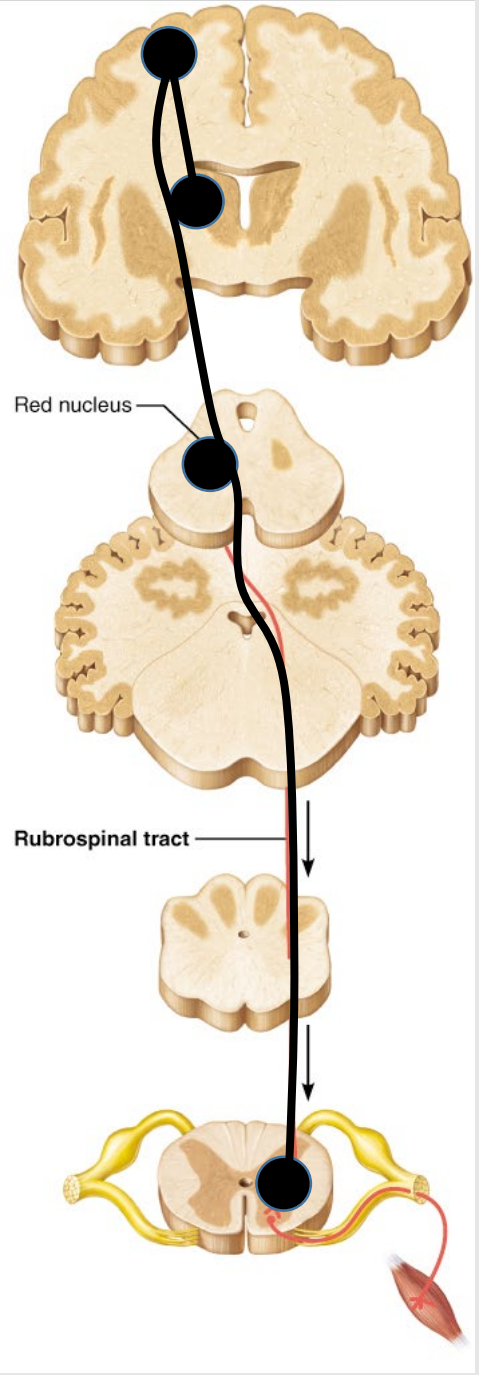




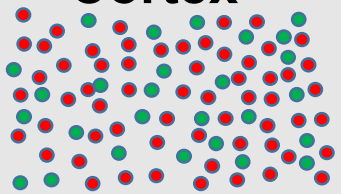
The Recovery Engram in Motor Function after Stroke



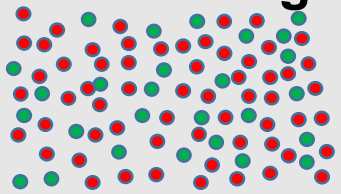
Joy and Carmichael,
Nat Rev Neurosci. 2020



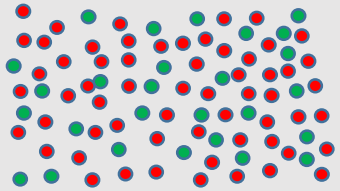
Cortex



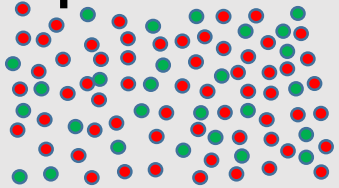
Basal Ganglia



Red Nucleus



Spinal Cord

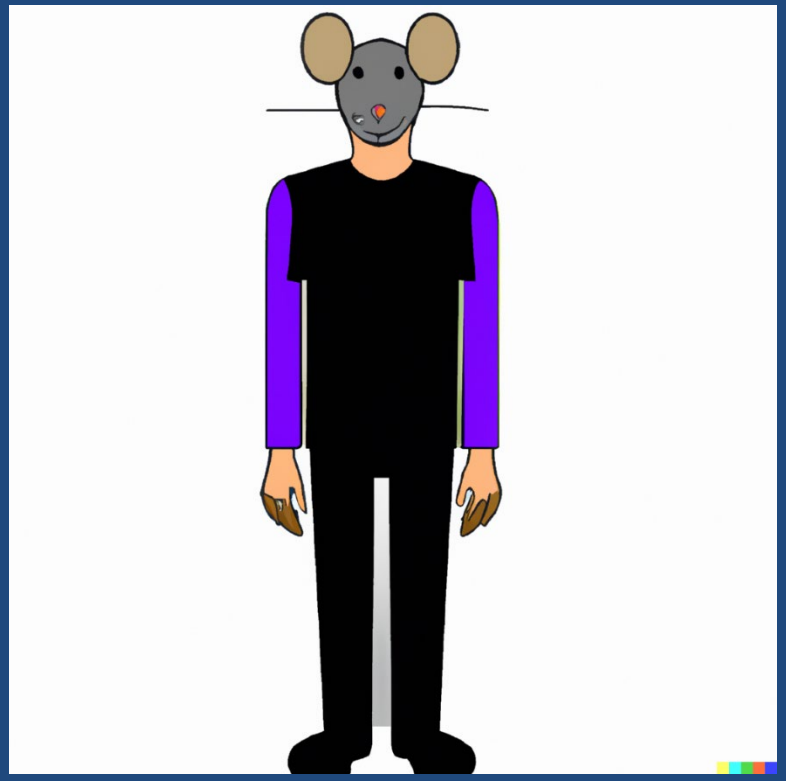


Recovery Engram in Motor Function:
Activity-dependent allocation of neurons into injured motor circuits across the motor system

What should the trial design of stroke recovery (brain repair) trials look like?

Should the Design of Clinical Trials Promoting Recovery Post Stroke be Informed by Animal Models?

Yes, and we can integrate the studies and approach from the pre-clinical to the human



No, the pre-clinical and clinical phases are distinct and trying to integrate them gives us a mashup

Should the Design of Clinical Trials Promoting Recovery Post Stroke be Informed by Animal Models?

What is the design of the most widely recognized human stroke neurorehabilitation clinical trials?

- Mostly chronic phase
- Outcome measures of motor impairment and disability
- Usually background training and activity in patients but no control or measurement for this
- ICARE, LEAPS, EXCITE, MIT Robot, ARMin robot

Should the Design of Clinical Trials Promoting Recovery Post Stroke be Informed by Animal Models?

What is the design of the most widely recognized human stroke neurorehabilitation clinical trials?

- Mostly chronic phase
- Measures of motor impairment, disability
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- ICARE, LEAPS, EXCITE, MIT Robot, ARMin robot

Recent Human Stroke Recovery Trials:

1. Fluoxetine: FOCUS Trial, AFFINITY Trial
2. Tonic GABA antag: RESTORE BRAIN

Modified Rankin Scale or its analysis through shift

Score	Description
0	No symptoms
1	No significant disability. Able to carry out all usual activities, despite some symptoms
2	Slight disability. Able to look after own affairs without assistance, but unable to carry out all previous activities
3	Moderate disability. Requires some help, but able to walk unassisted
4	Moderately severe disability. Unable to attend to own bodily needs without assistance, or unable to walk unassisted
5	Severe disability. Requires constant nursing care and attention, bedridden, incontinent
6	Dead

From [58]

Stroke Recovery Trials in Humans		Stroke Recovery Trials in Rodents
• Mostly very early or chronic phase	≠	• Mostly acute and subacute phases and short term outcomes
• Measures of disability	≠	• Mostly motor impairments
• Usually background training and activity but no control or measurement for this	≠	• Mostly no training or background activity levels

Collaborators and Funding

<u>Carmichael Lab</u>

Nora Abduljawad
Kiro Bechay
Sam Bridges
Amy Gleichman
Teena Joy
Shahrzad Latifi
Jose Mazzitelli
Irene Llorente
Alec Marin
Natalie Shih
Min Tian
Inwoo Wang
Junyu Luo
Mary Hovanesyan
Weiye Dai
Jennifer Garcia

Dan Geschwind (UCLA)
Bruce Dobkin (UCLA)
Alcino Silva (UCLA)
Andrew Clarkson (Univ Otago)
Roman Giger (Michigan)
Michael Levine (UCLA)
Michael Sofroniew (UCLA)
Carlos Portera-Cailliau (UCLA)
Michael Levine (UCLA)
Peyman Golshani (UCLA)
Istvan Mody (UCLA)
Clifford Woolf (Boston Childrens)
Larry Benowitz (Boston Childrens)
Einor Ben Assayag (Telv Aviv Med Ctr)
Esther Shoham (Hebrew Univ)

UCLA is here

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