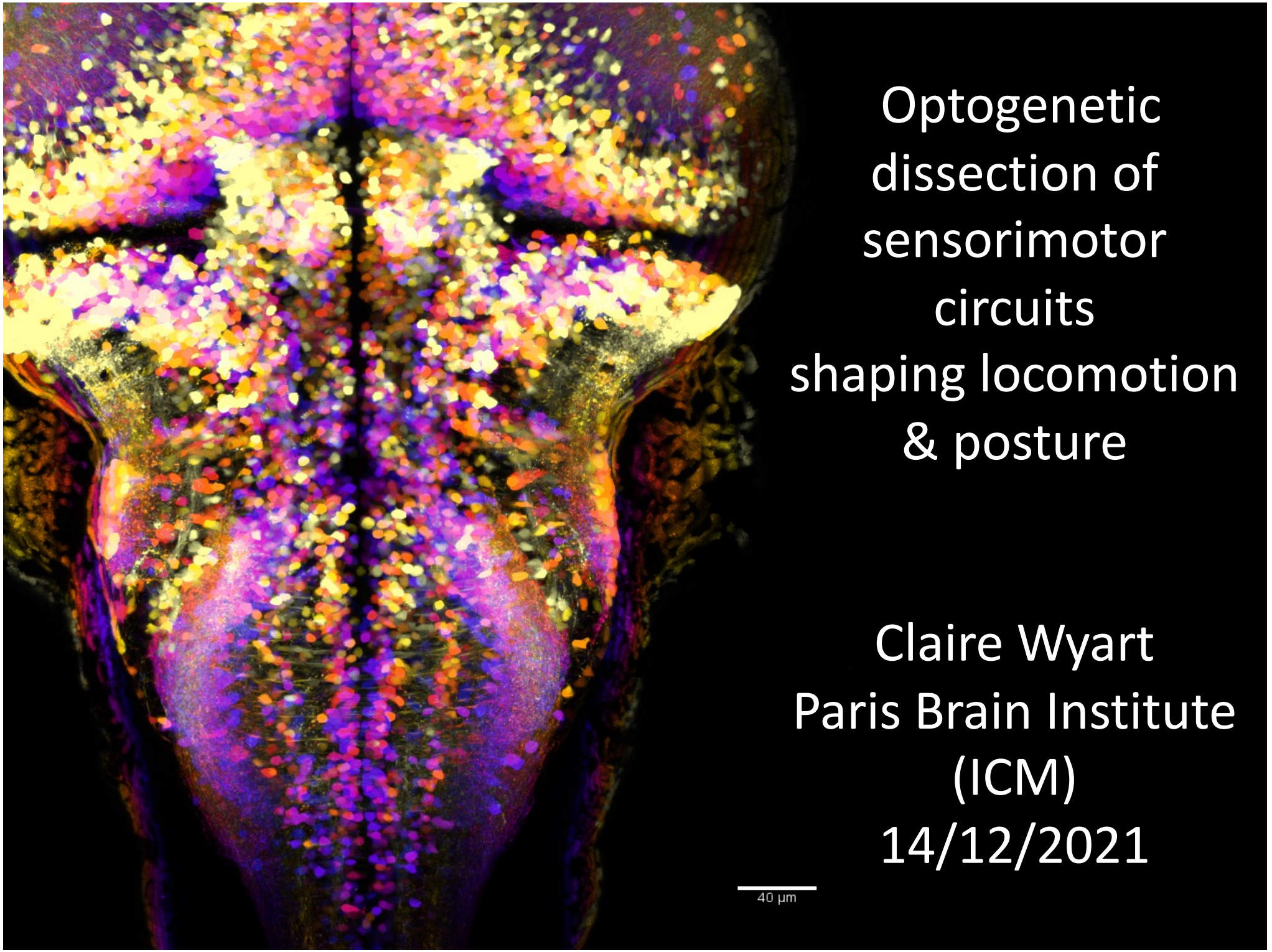
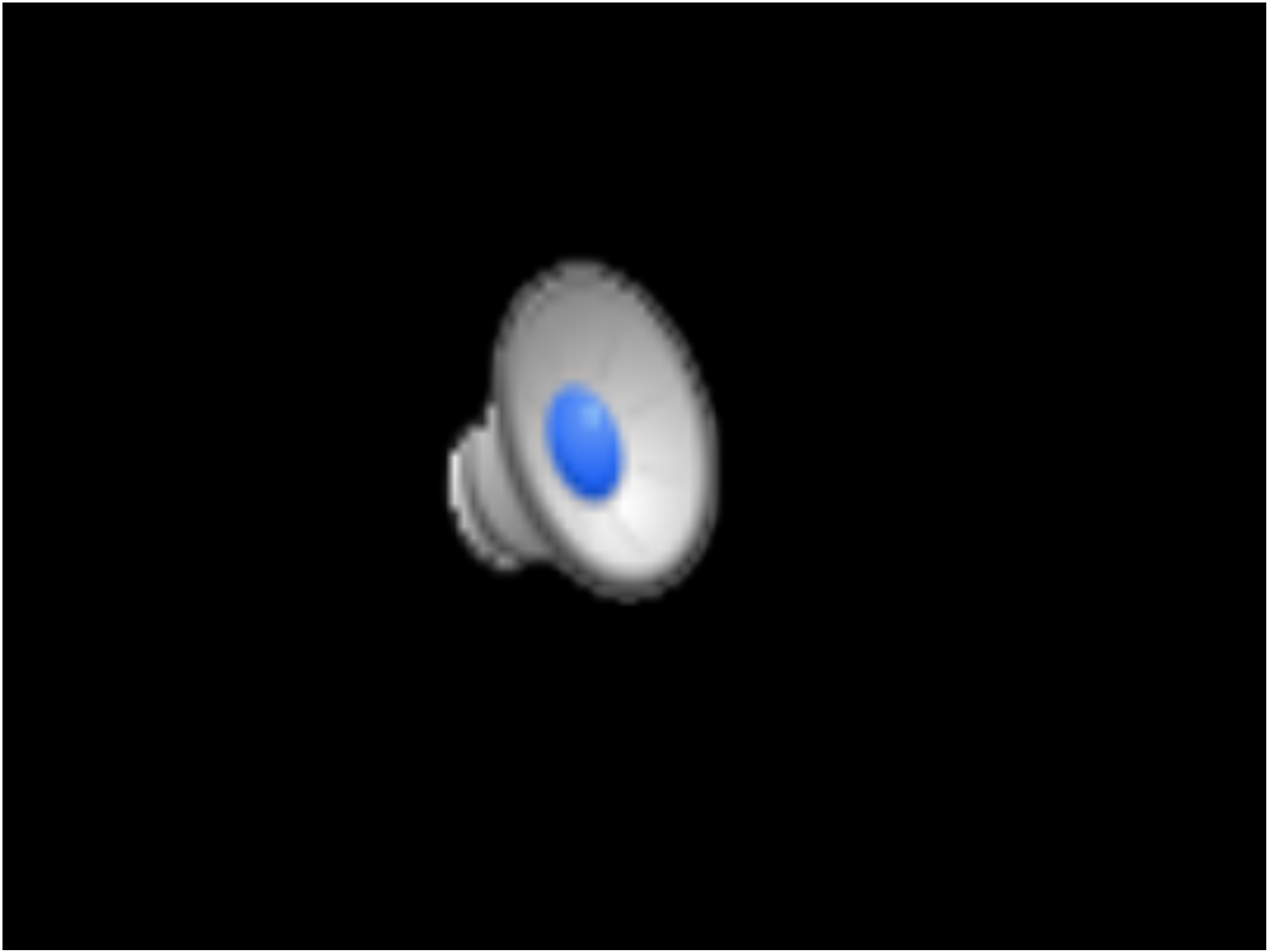


Optogenetic dissection of sensorimotor circuits shaping locomotion & posture

Claire Wyart
Paris Brain Institute (ICM)

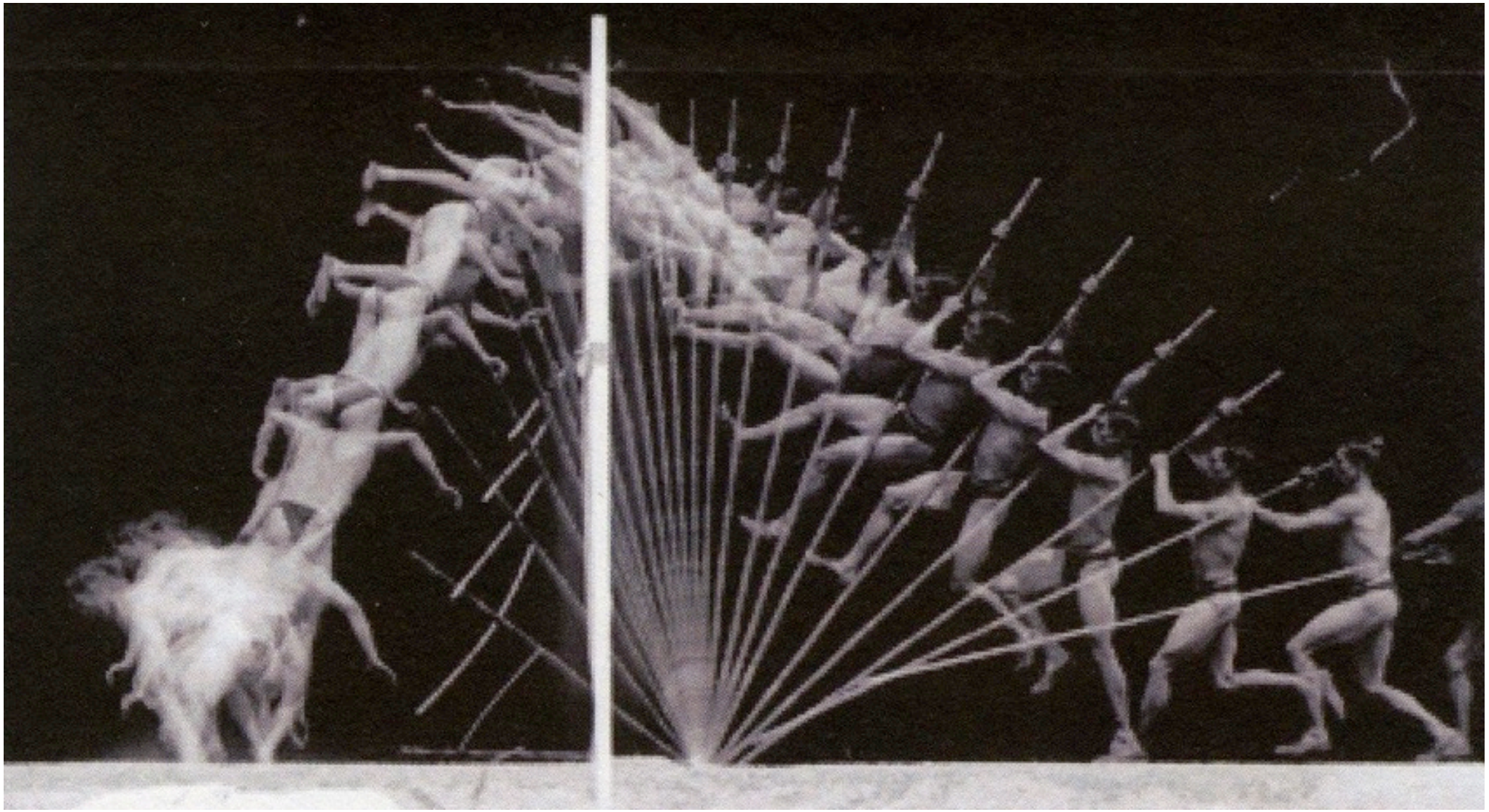
14/12/2021



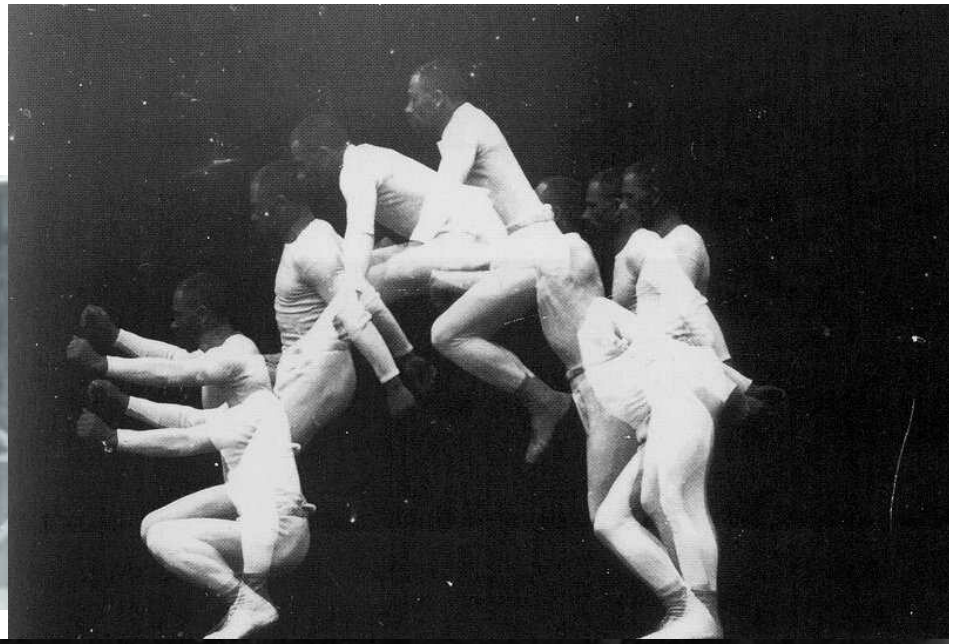
With an imaging shotgun?

Étienne-Jules Marey, 1830-1904

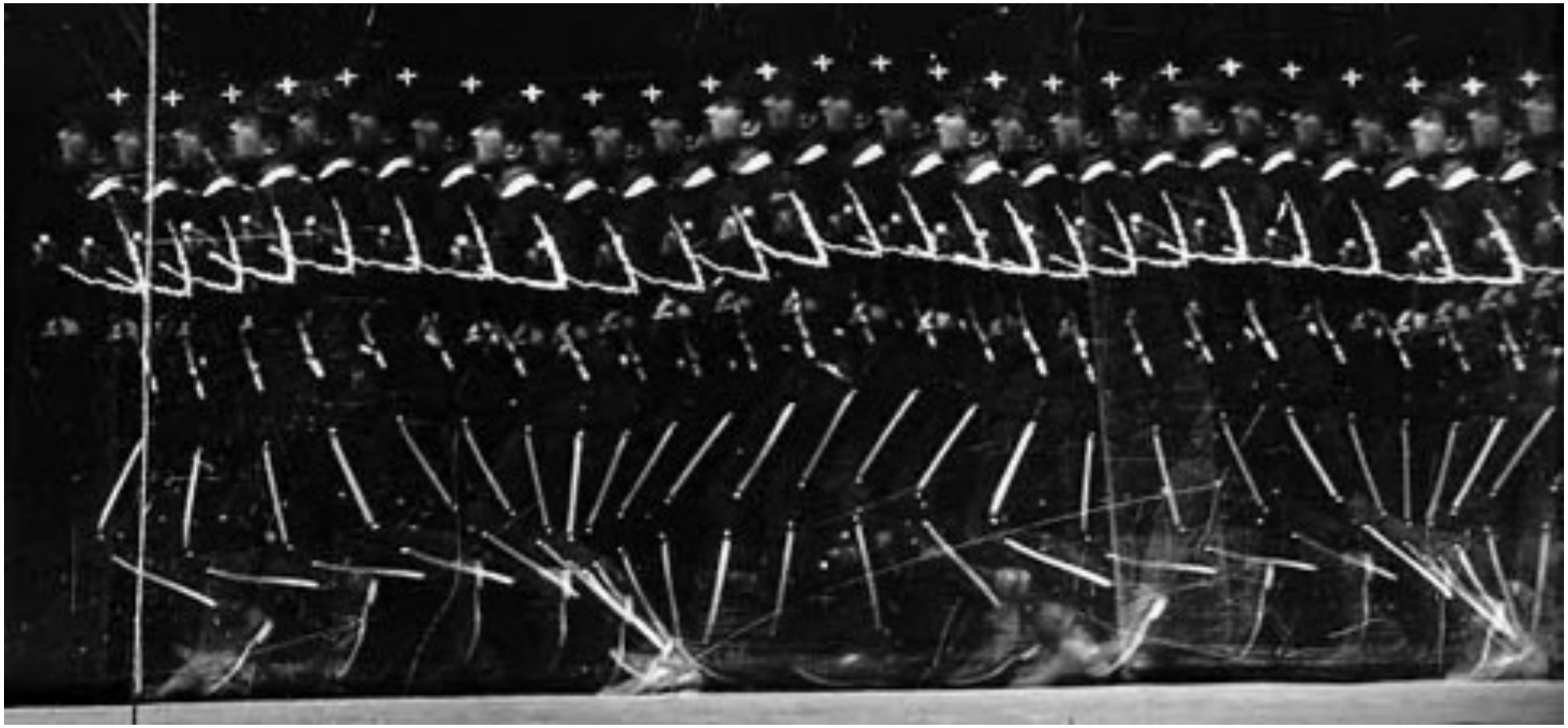
Shooting without killing:
12 x per second



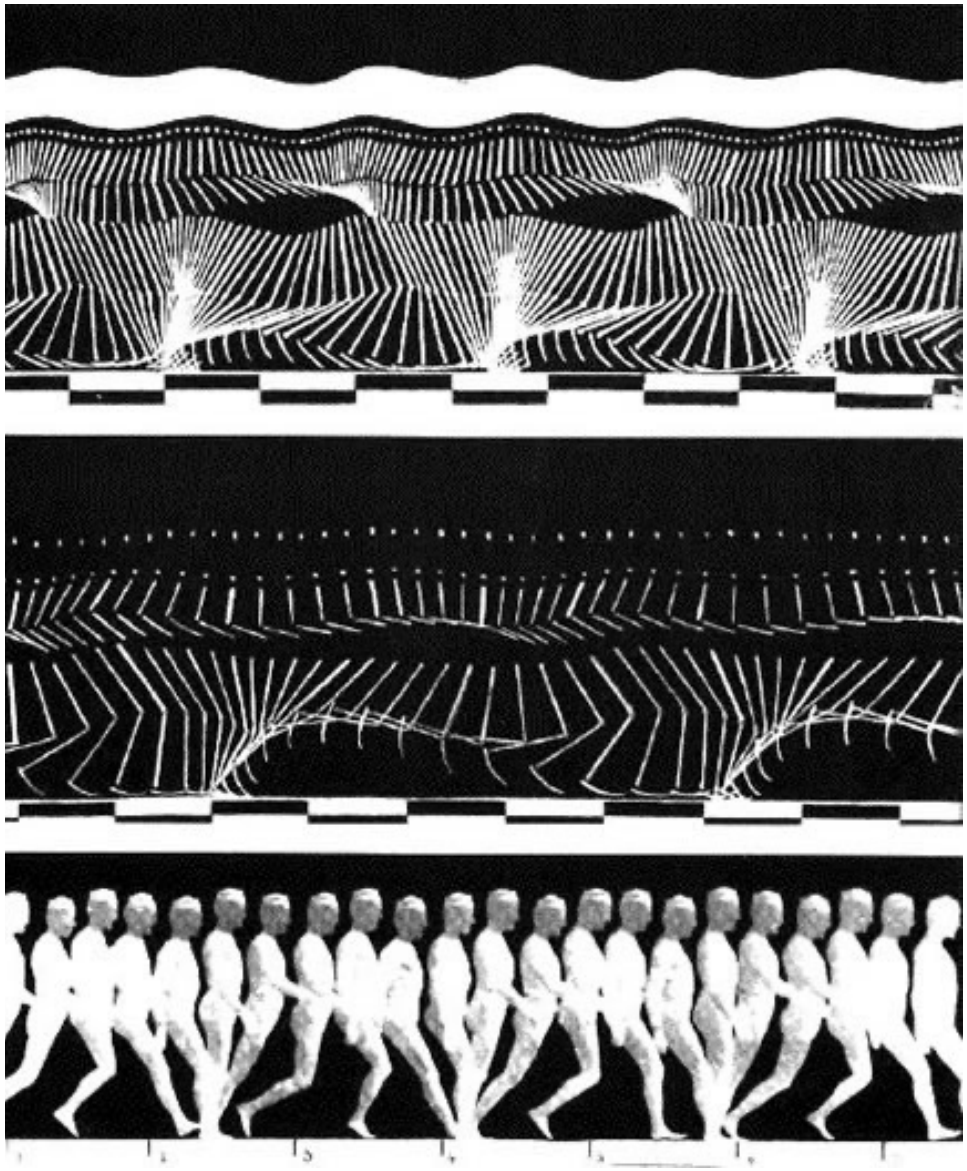
Body on black background



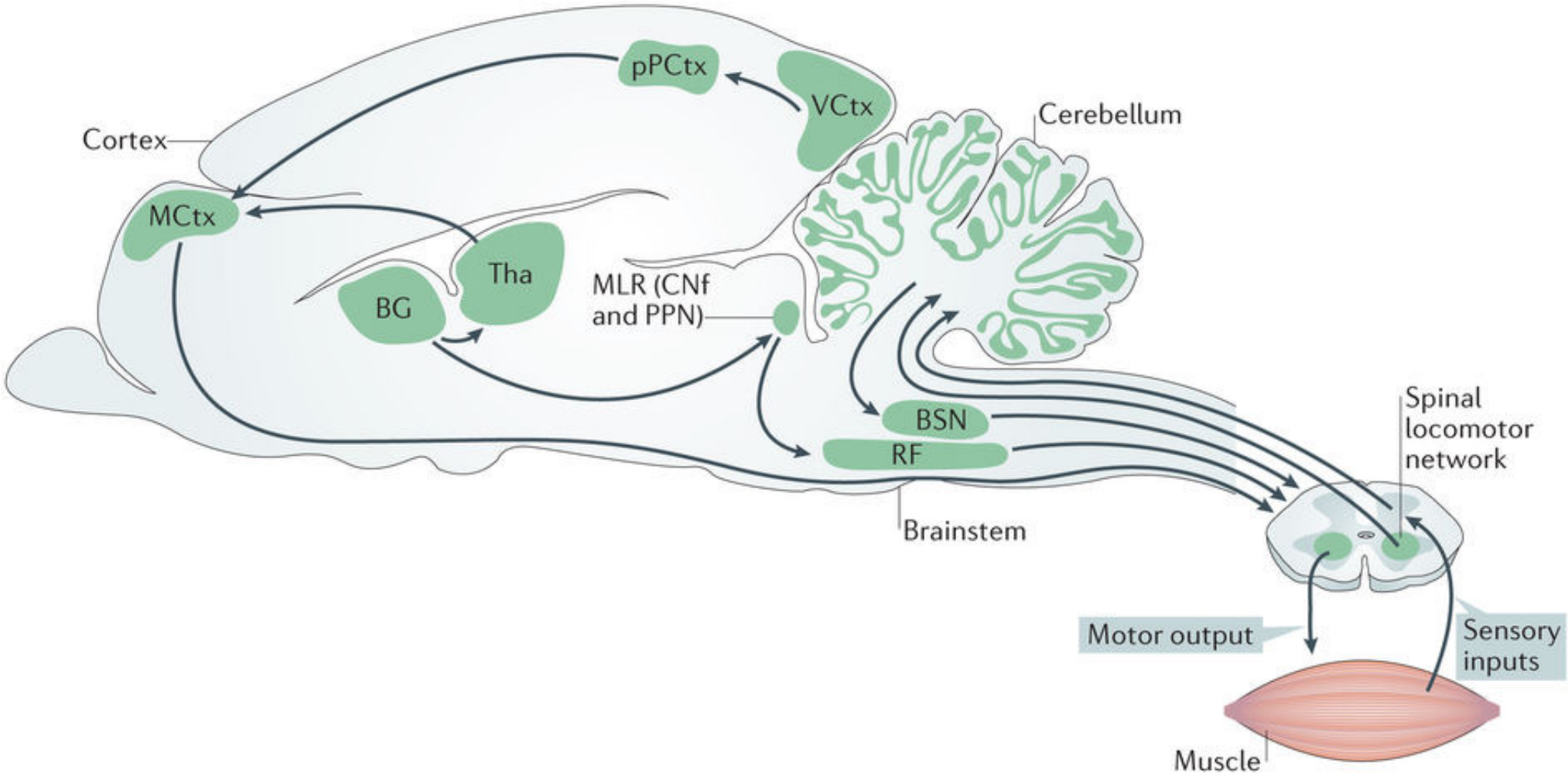
The trace left by the movement remains, even if the movement faded away



Light on movement: Chronophotography



Classical view of motor control in vertebrates



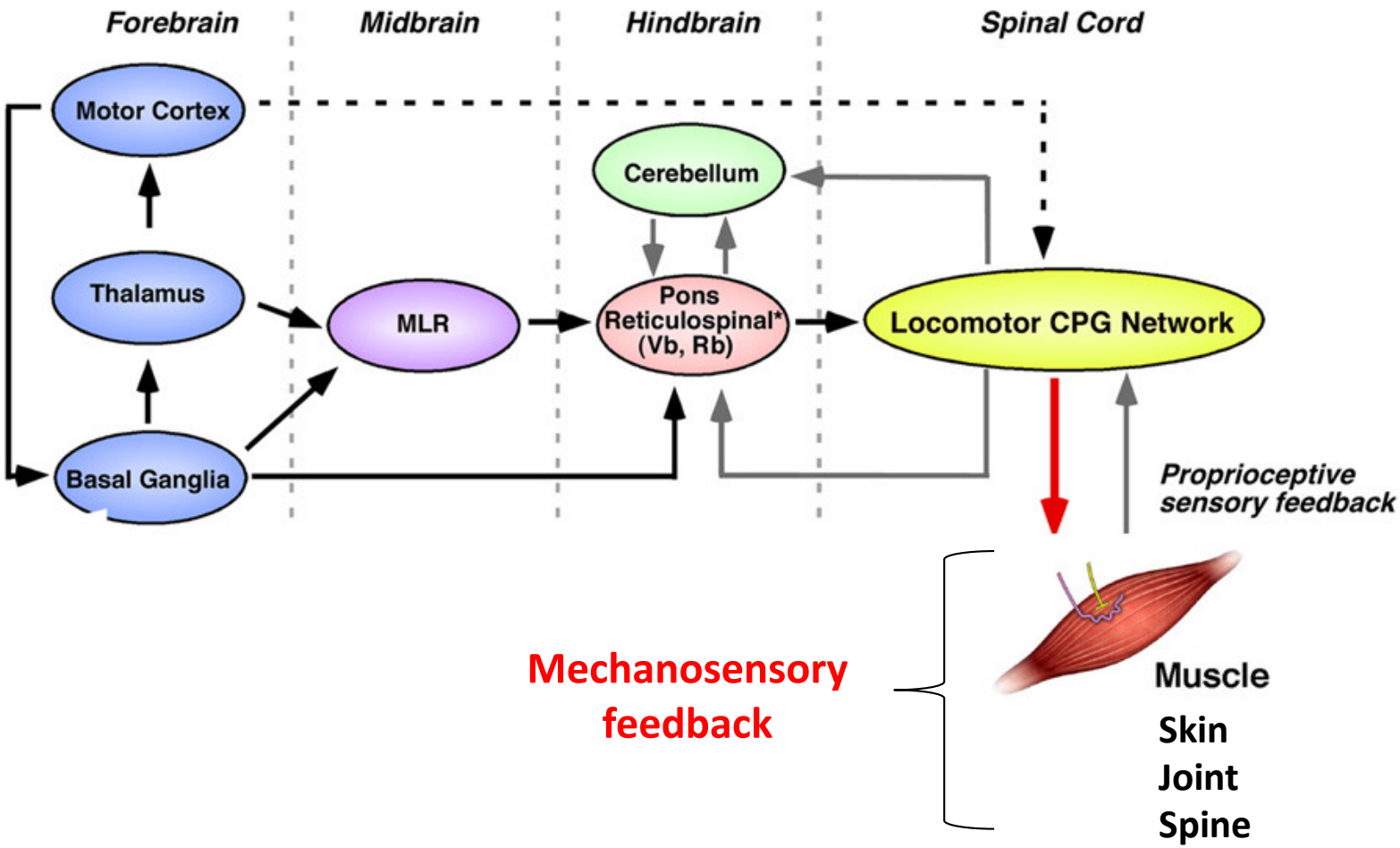
Kiehn 2018

Nature Reviews | Neuroscience

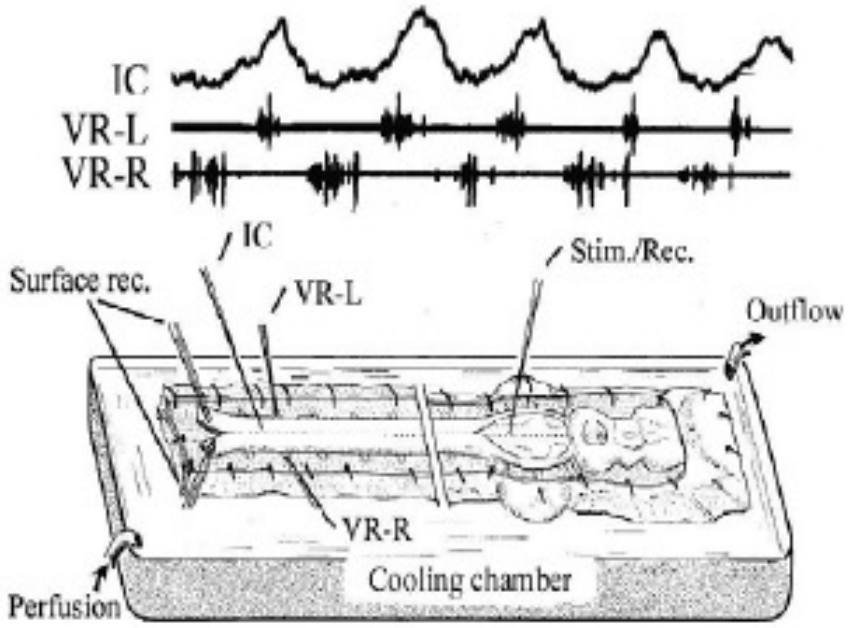
Decerebrated cat on a rolling mat changes gait according to rolling speed

Dr Graham Brown, 1882-1965

Classical view of motor control in vertebrates

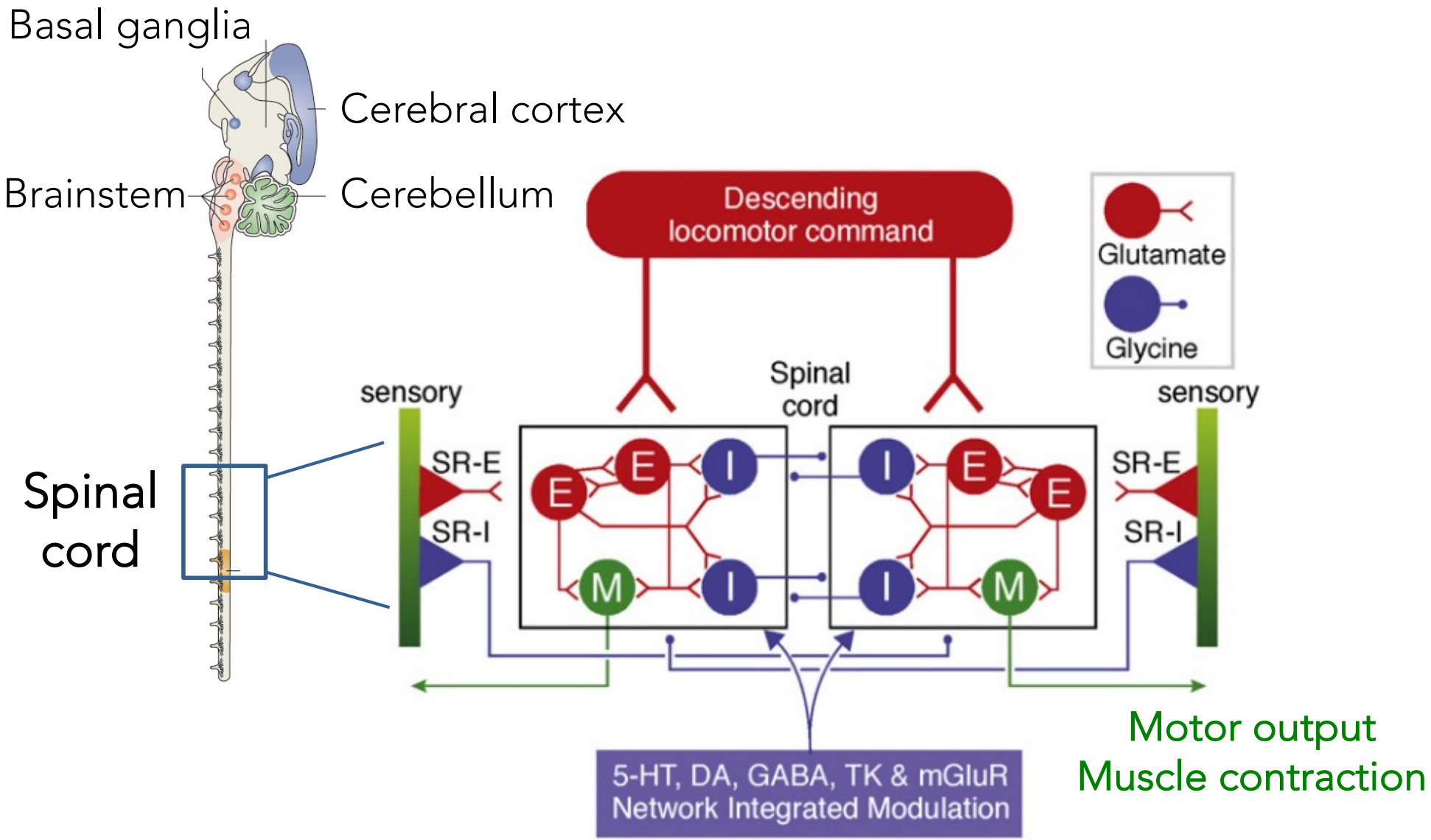


Most of our knowledge comes from « Fictive locomotion »



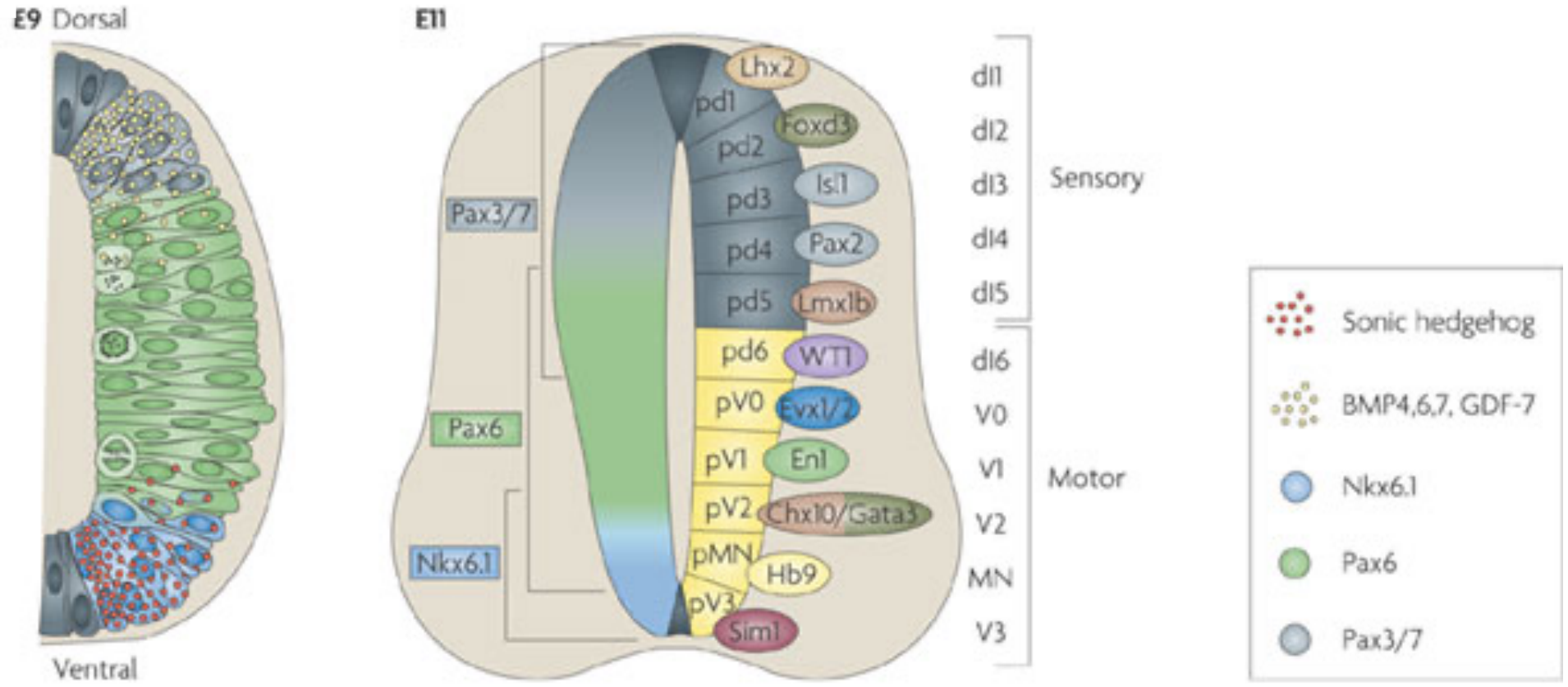
Sten Grillner & Collaborators

Spinal cord hosts central pattern generators (CPGs)

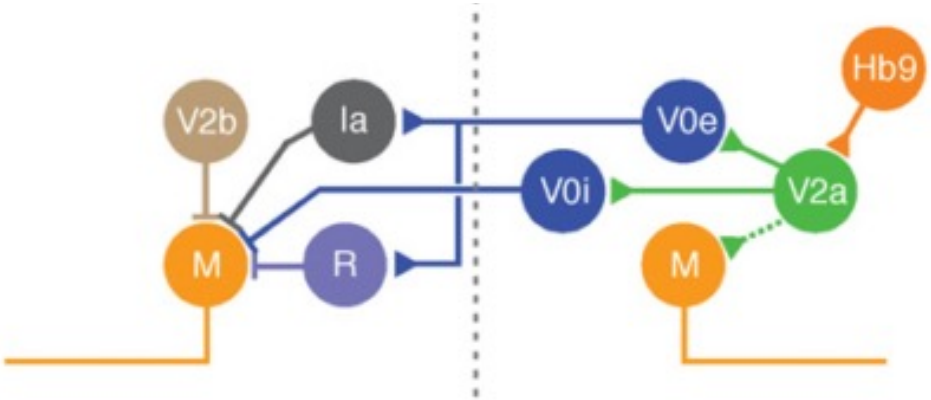


Adapted from Grillner and Jessell, 2009

The diversity of spinal neurons – from genetics to physiology

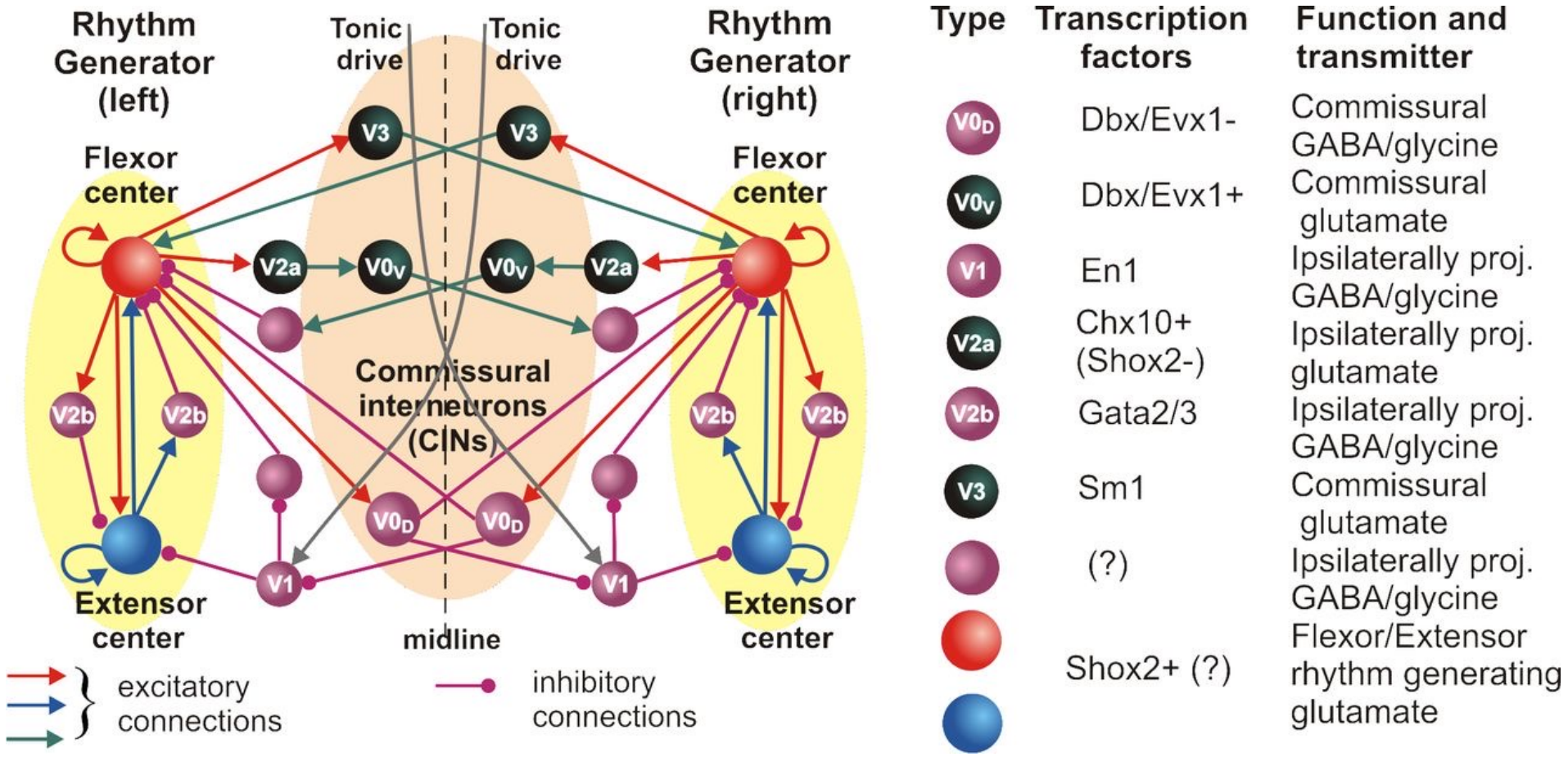


Functionnal Diagram & connectivity



Goulding 2007;
Grillner and Jessell 2009

Current model for spinal CPGs



Rybak et al., 2015

Organisation of the online course

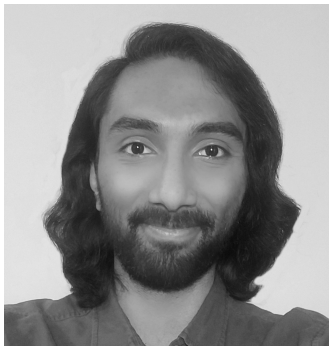
- **Step 1: Kinematics of single locomotor bouts : role of mechanosensory feedback**

Claire Wyart, Paris Brain Institute

- **Step 2: Segmentation of bout sequences**

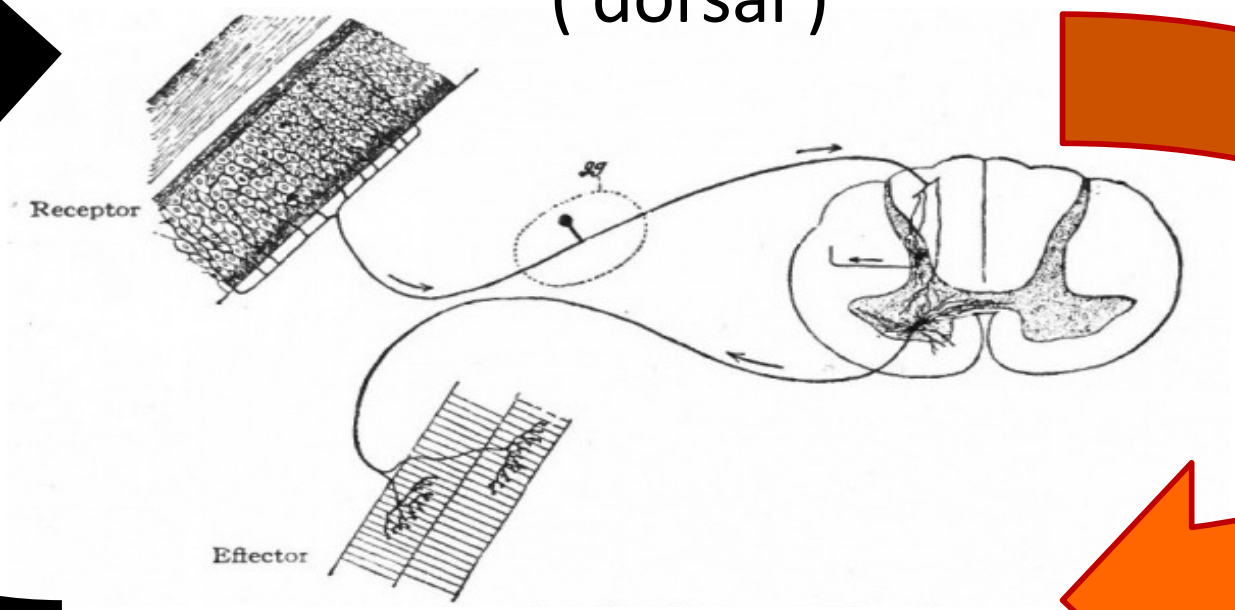
Gautam Reddy, Harvard University

Gautam Sridhar, Paris Brain Institute



How does mechanosensory feedback shape locomotion?

Sensory
(dorsal)



Motor
(ventral)

Charles Sherrington

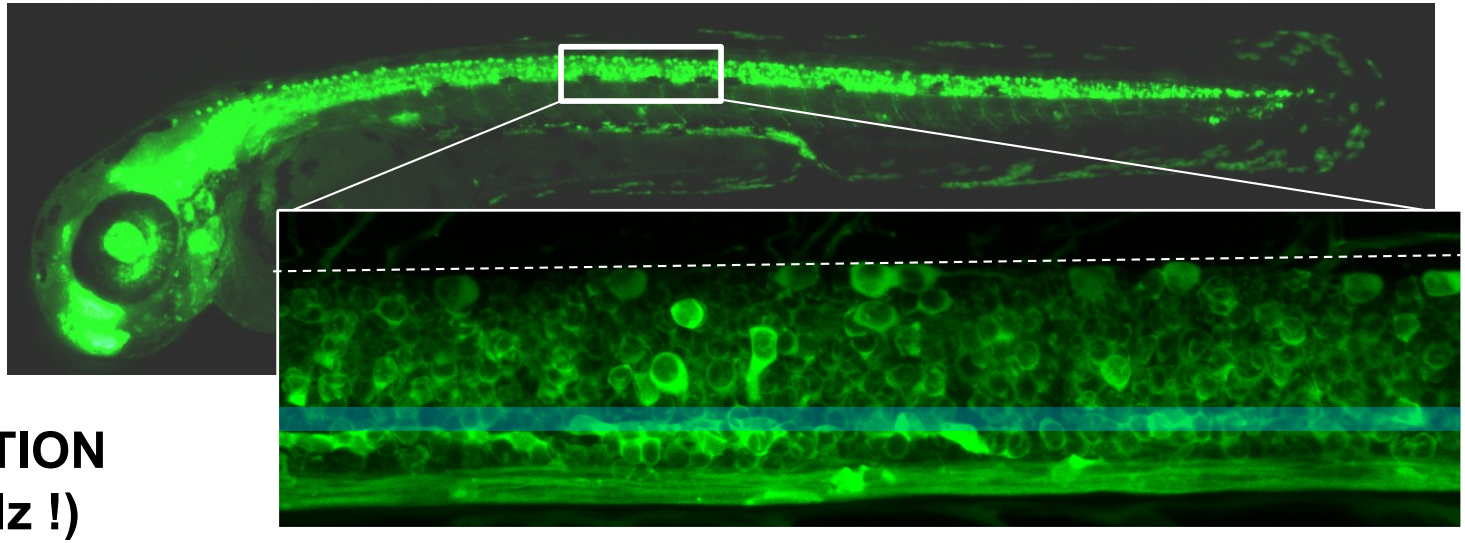
Problem :

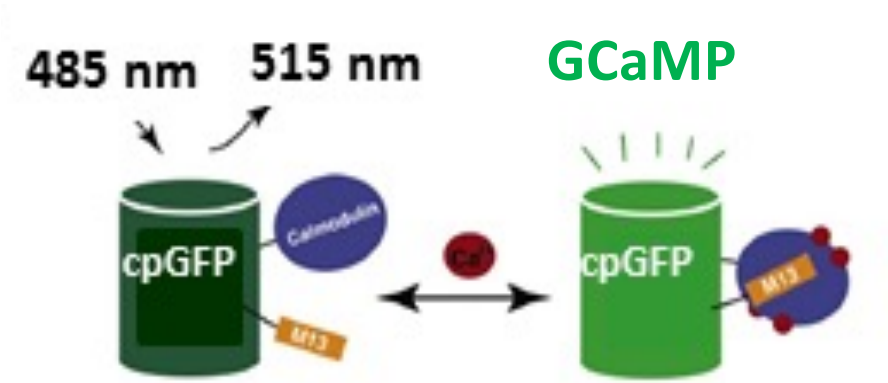
Single cell electrophysiology requires mechanical stability

What about optics?

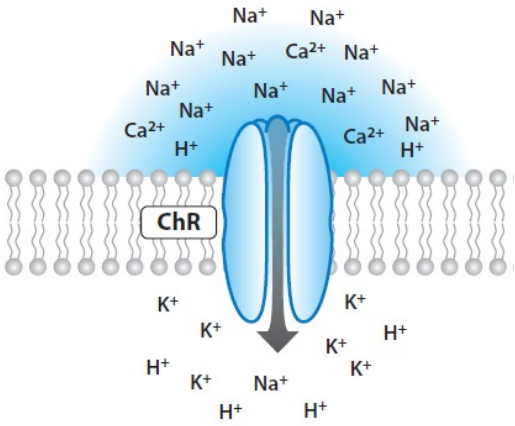
Probing sensory feedback in zebrafish larva

- **TRANSPARENT**
- **GENETIC MODEL**
- **INTACT**
- **PHYSIOLOGY**
- **SIMPLE LOCOMOTION**
  (… but fast 15-100Hz !)



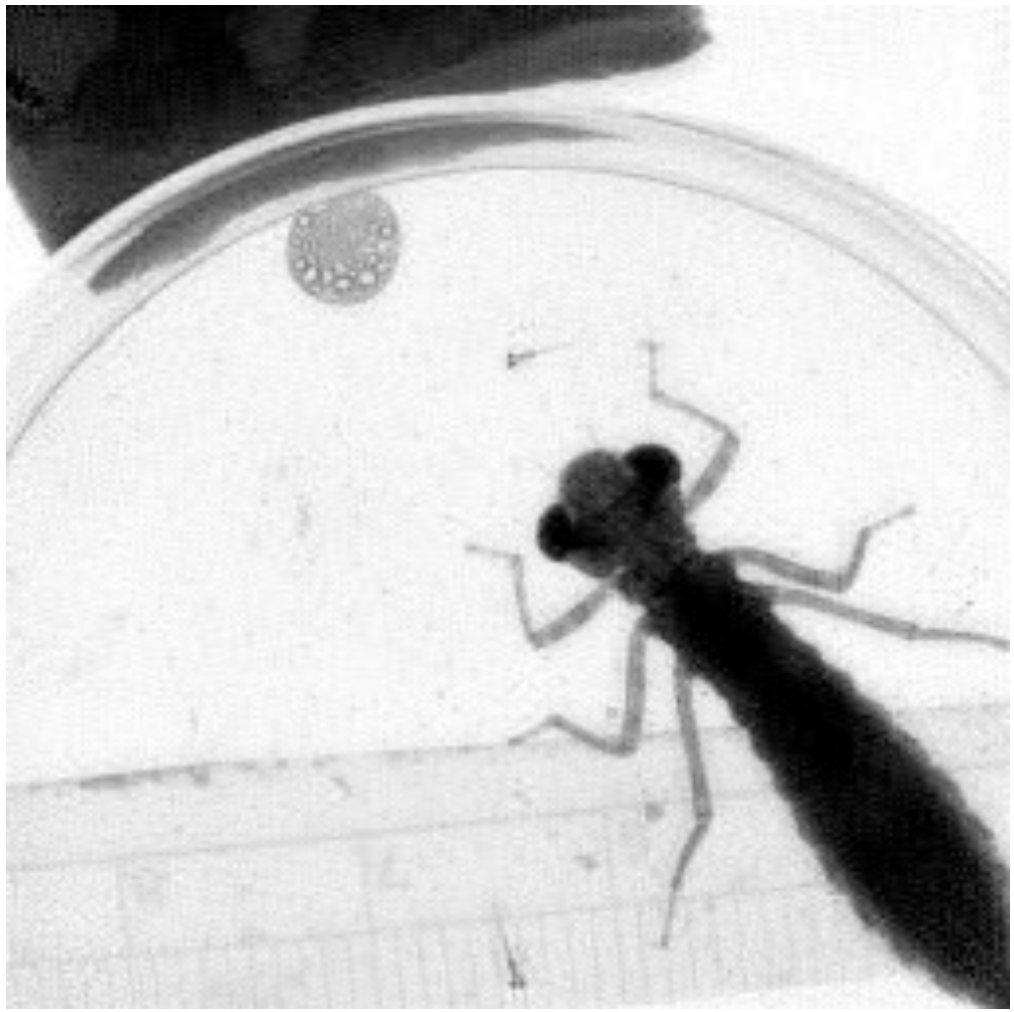


Nakai et Looger



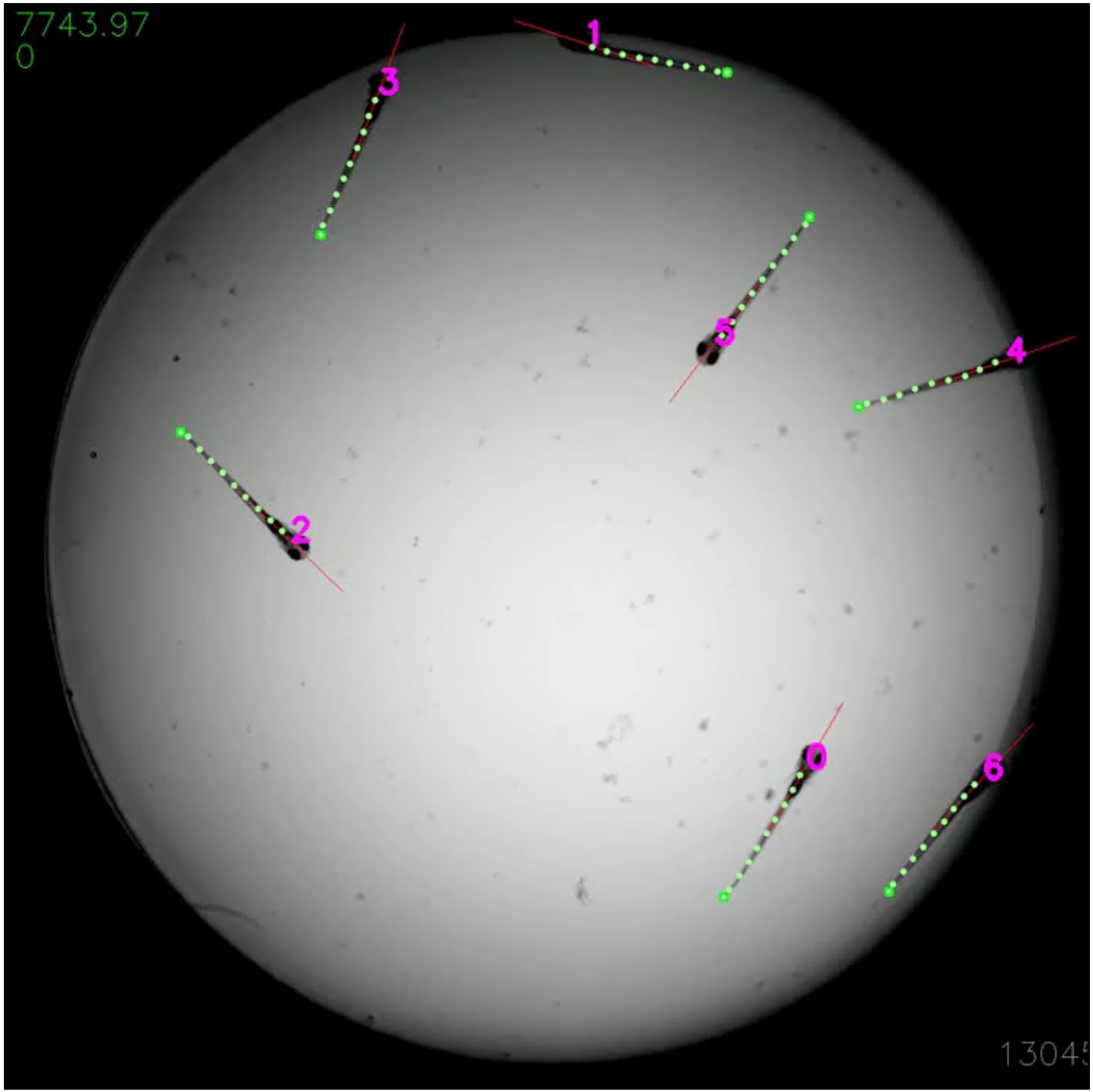
Stereotypical locomotor behaviors

Escape response (Fetcho lab)



Prey capture (Baier lab)

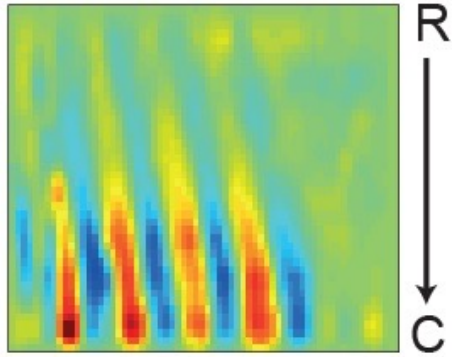
Gathan et al., 2005

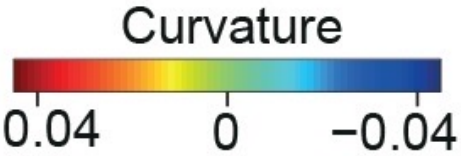


Mirat et al. Frontiers in Neural Circuits 2013

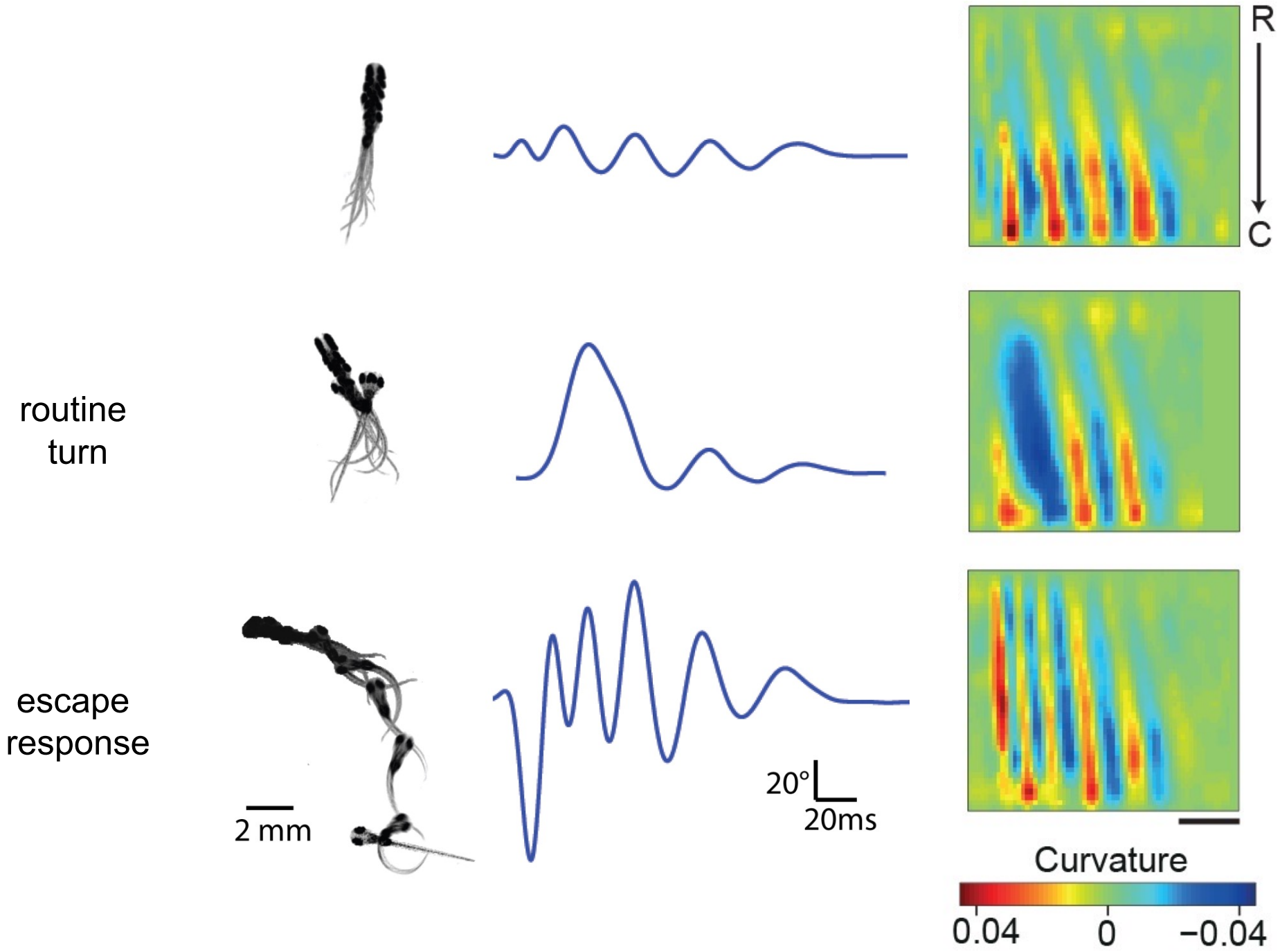
ZebraZoom, discriminating maneuvers in 3 categories

slow forward swim



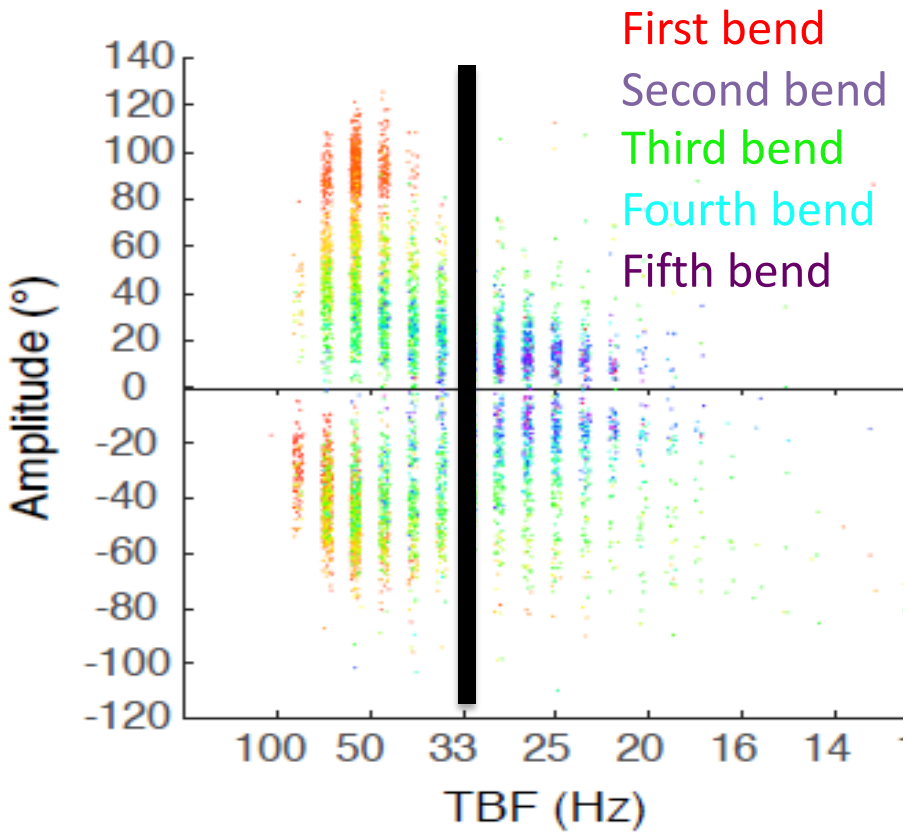


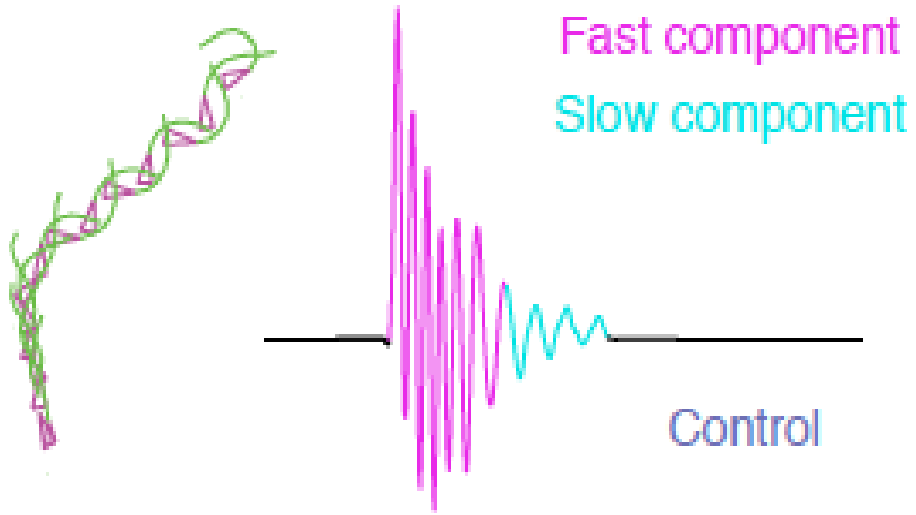
ZebraZoom, discriminating maneuvers in 3 categories



Stereotyped acousto-vestibular escapes

- Amplitude and Frequency are highly correlated
- Transition from fast regime to slow regime

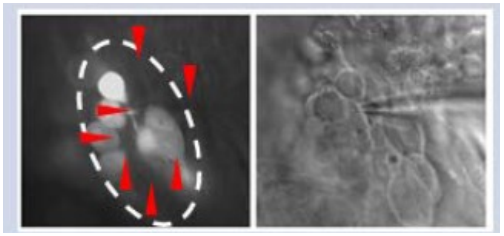




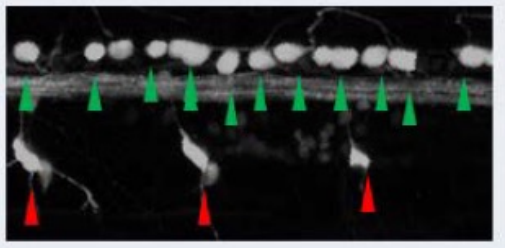
Knafo, Fidelin et al., eLife 2017

Genetic targeting of zebrafish mechanoceptors

Lateral Line

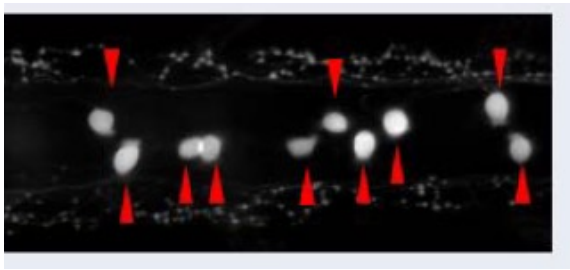


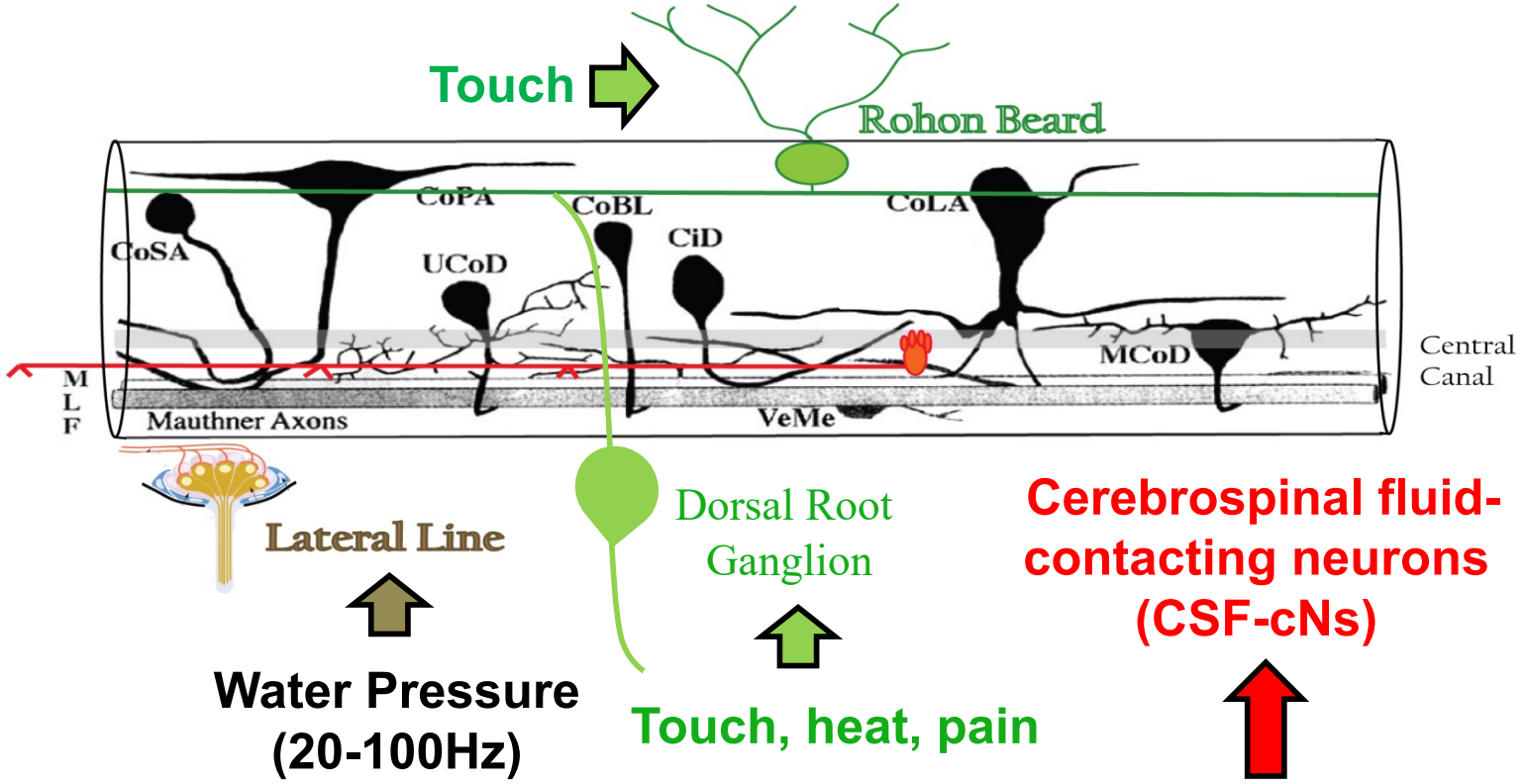
Rohon Beard



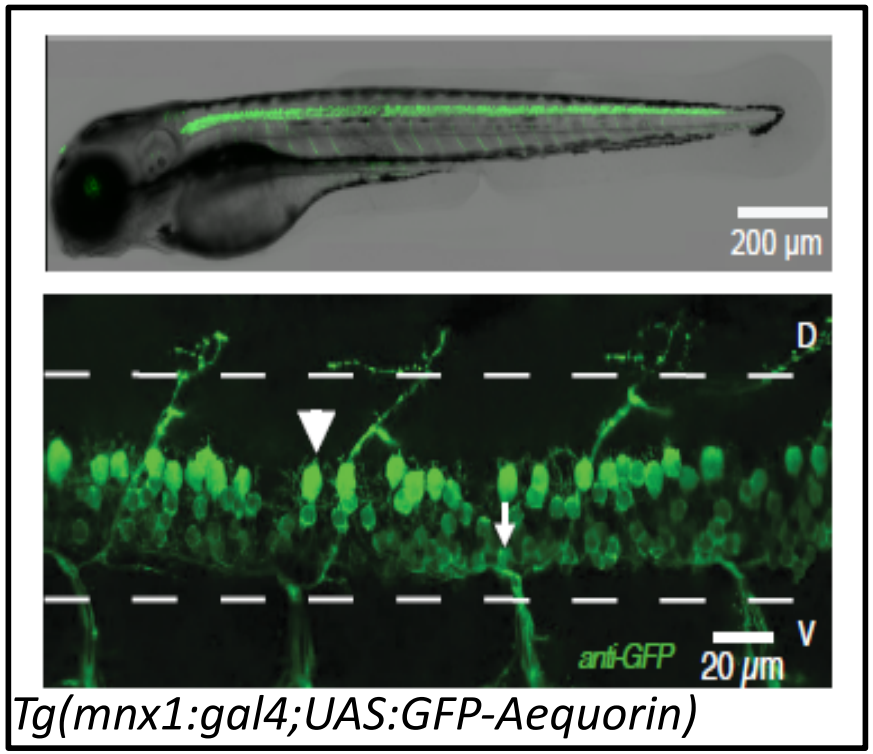
Dorsal Root Ganglion

CSF contacting neurons

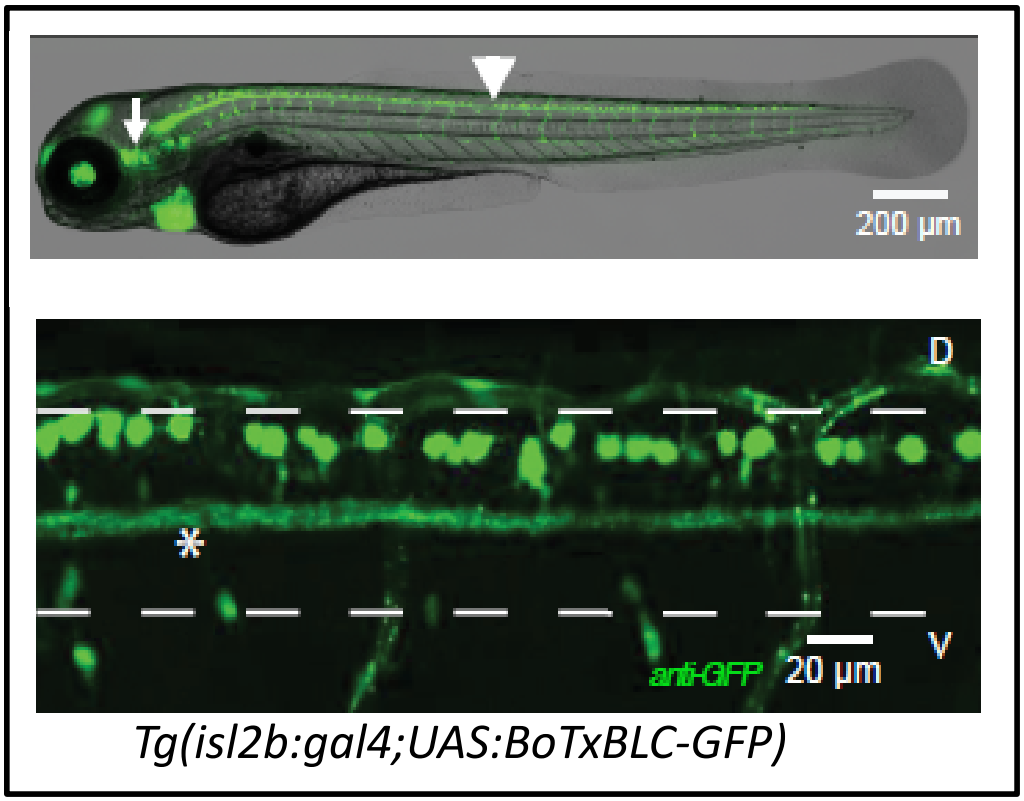




Zebrafish larva: genetic targeting & optical access



Tg(mnx1:gal4;UAS:GFP-Aequorin)



Tg(isl2b:gal4;UAS:BoTxBLC-GFP)

How does sensory feedback…

1➔ Impact recruitment of the motor pool?

2➔ Contribute to active locomotion?

3➔ Mediate these effects in the spinal cord?

Light on mechanosensory feedback during active locomotion

I. How to estimate recruitment of neurons in the spinal cord of moving animals?

II. How do sensorimotor loops contribute to locomotion & posture?

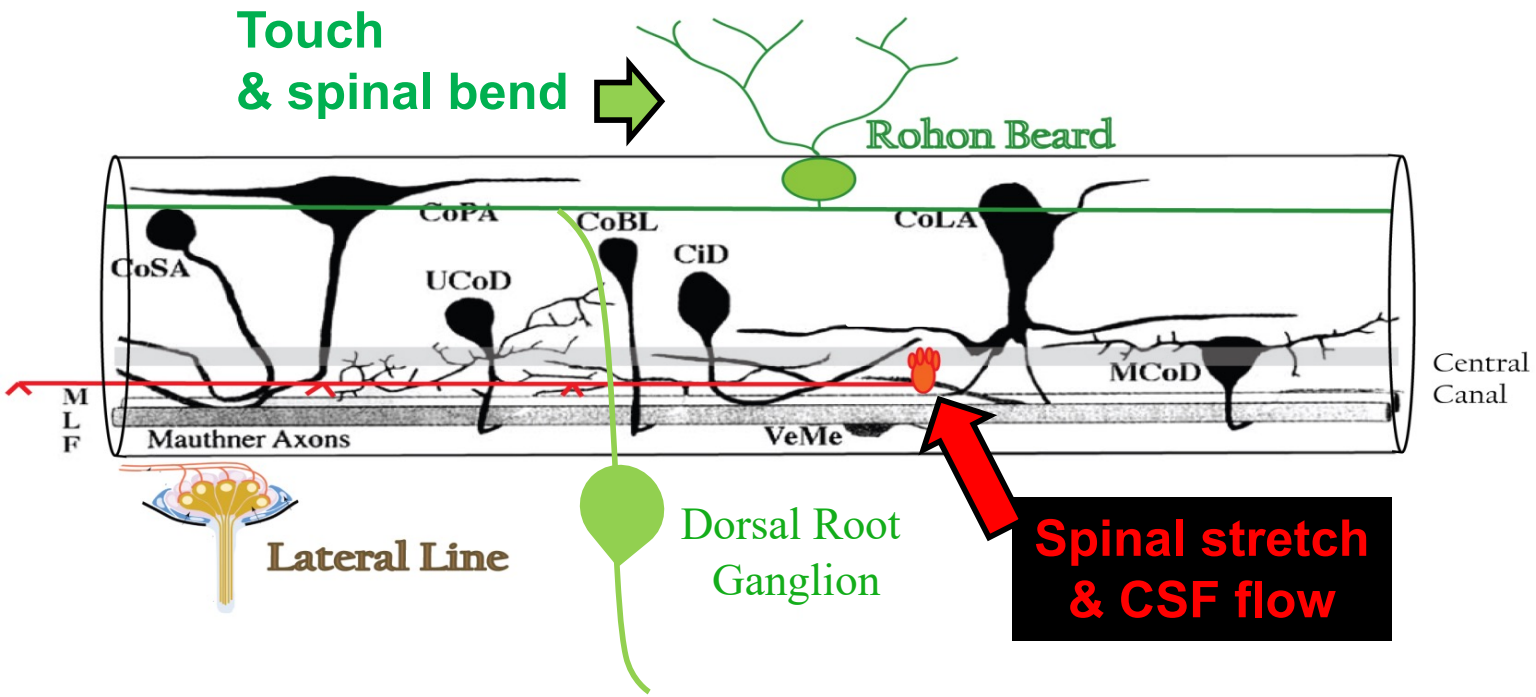
=> Predictions on relevant neuronal circuits ?

III. Manipulating sensorimotor circuits with optogenetics

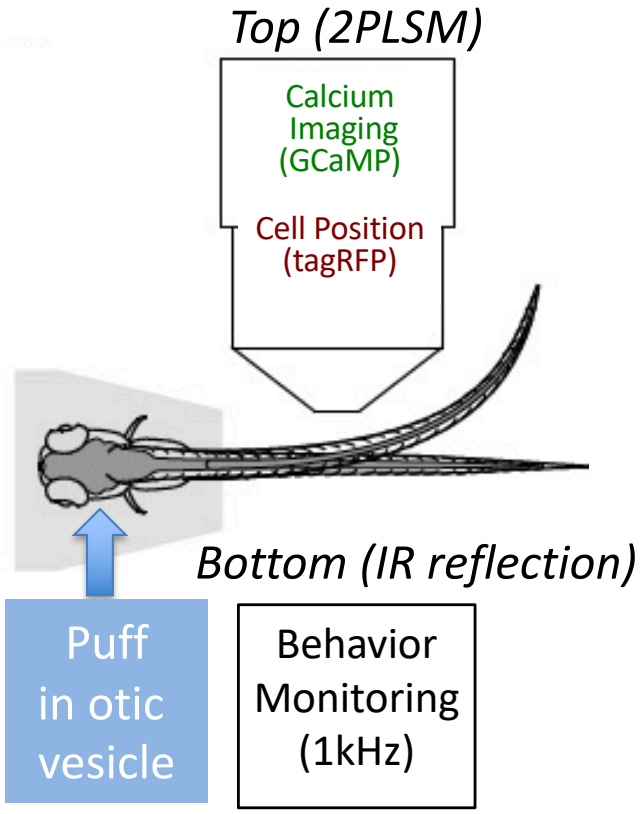
=> Single cell-connectivity mapping

I. How are motor & sensory neurons recruited during motion *in vivo*?

Bioluminescence monitoring & calcium imaging reveals the recruitment of spinal neurons in motion



Capturing the recruitment of mechanosensory neurons during motion: curvature detection

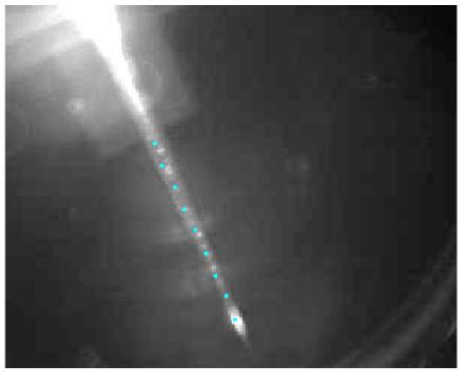


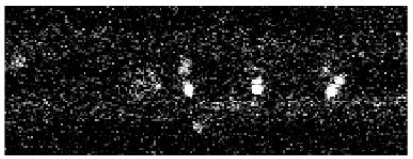
Behavior

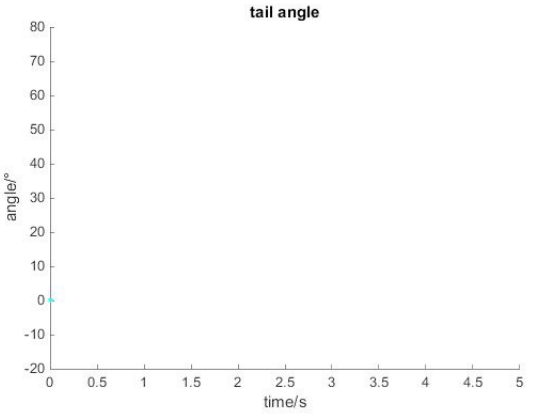
GCaMP6f

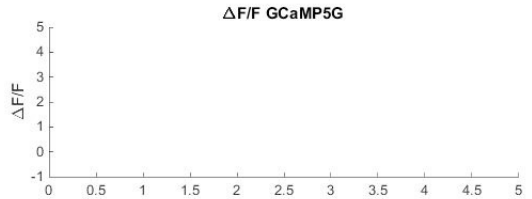
tagRFP

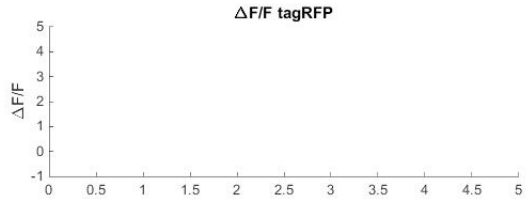
ratio

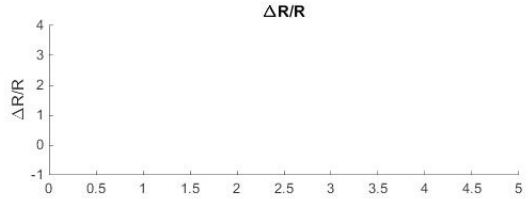






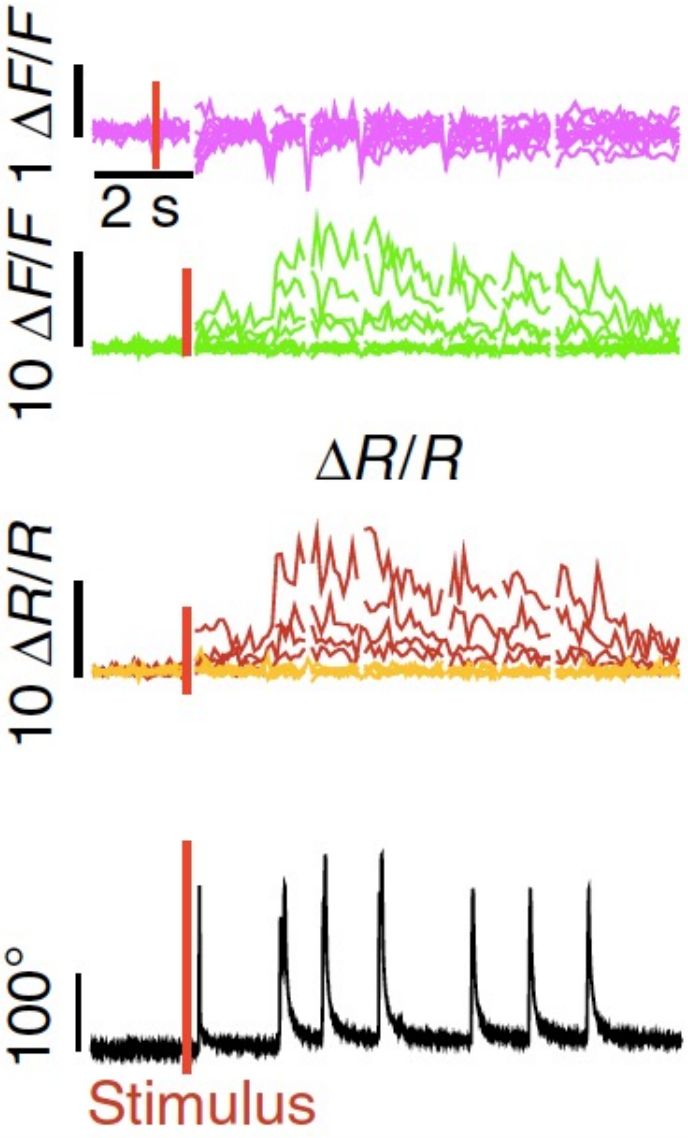






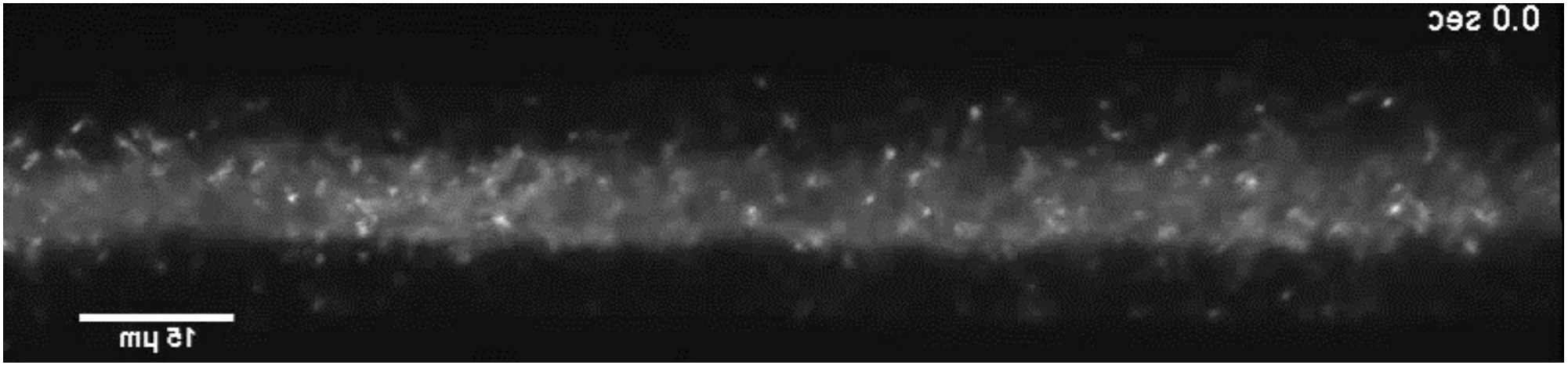
Böhm, Prendergast et al., Nature Communications *2016*

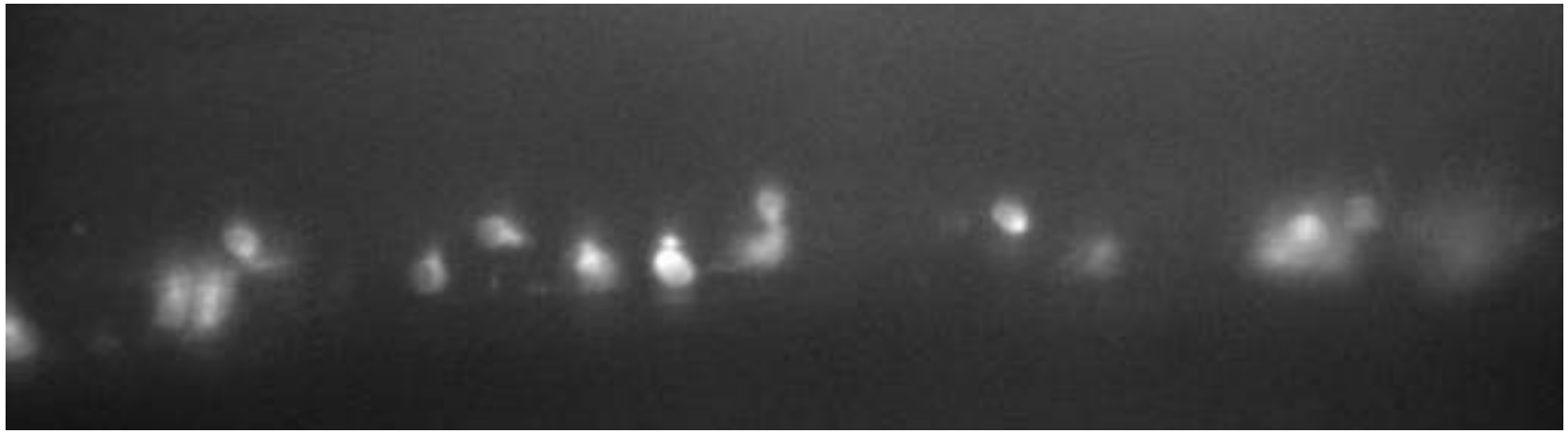
CSF-cN recruitment during spinal stretch (active contraction)



Detection of CSF flow during muscle contractions

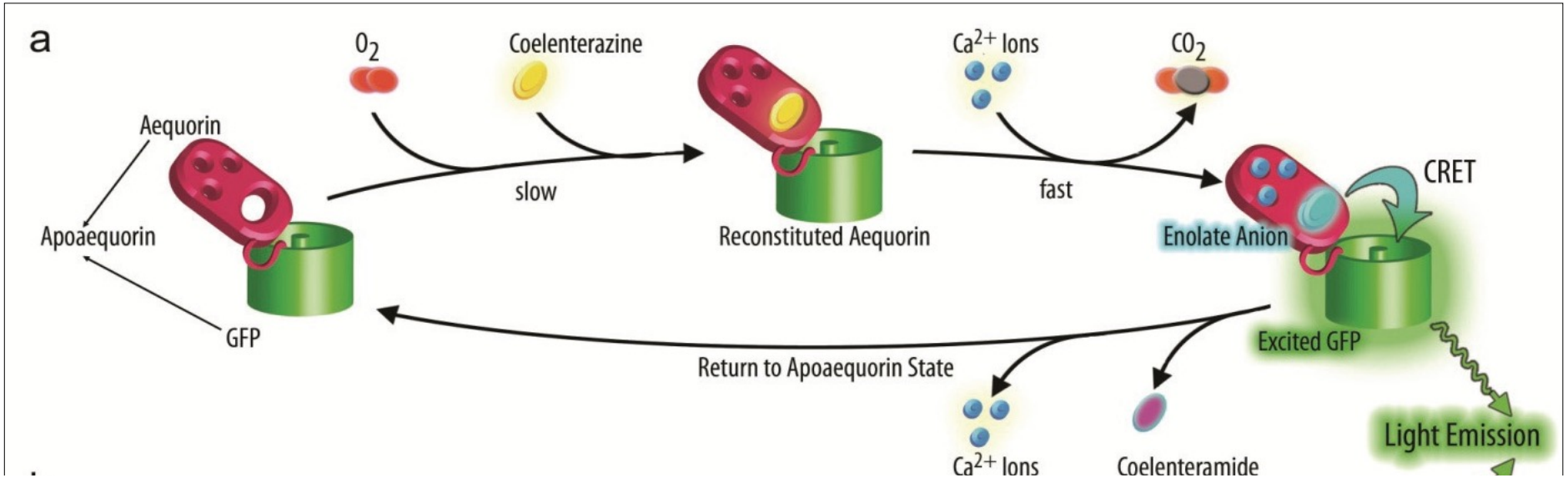
Rostral **Caudal**





Thouvenin *et al.,* eLife 2020

Bioluminescence monitoring of neuronal activity

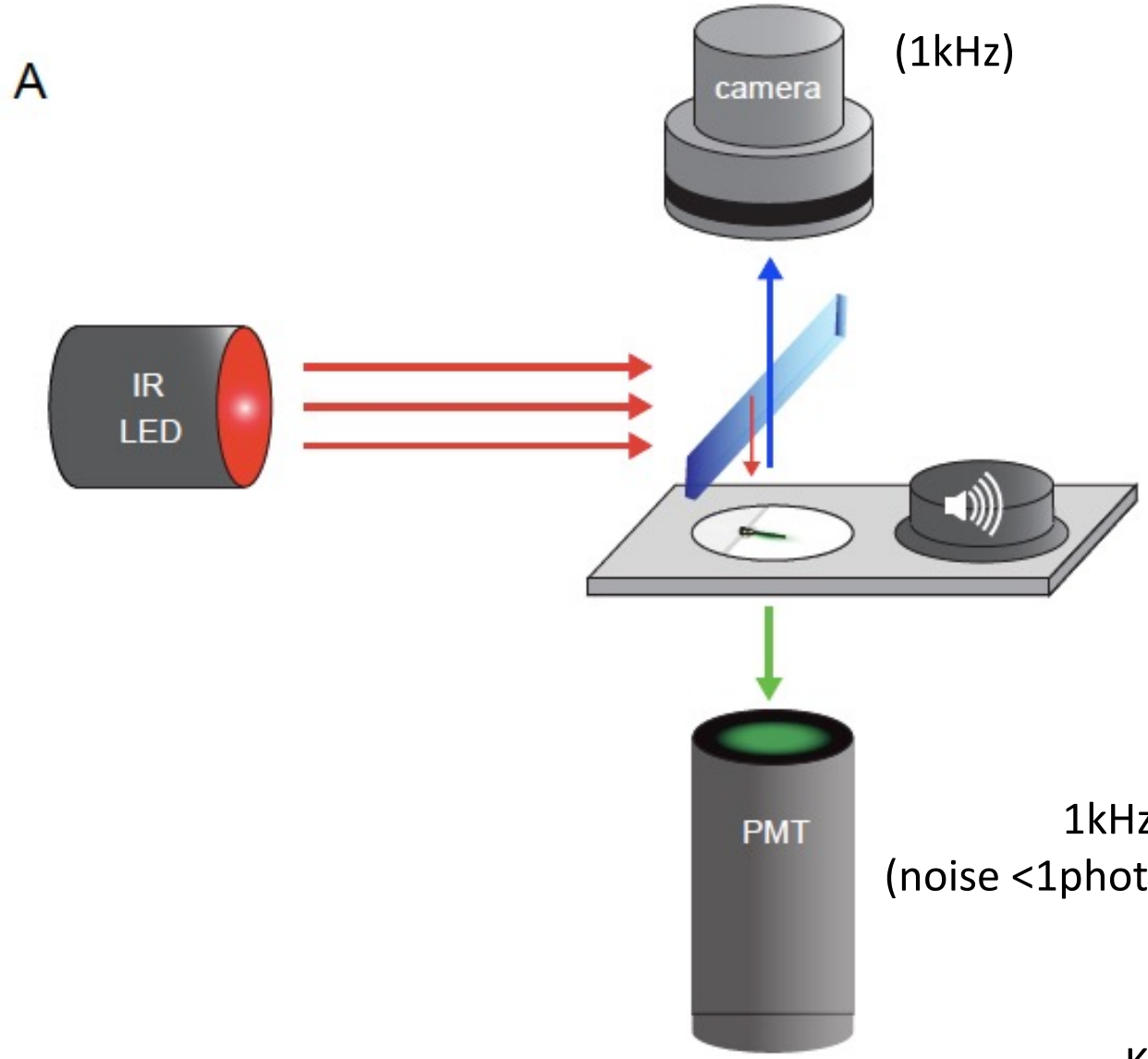


Baubet *et al.*, 2000 [Philippe Brûlet]

Kd = 10 μM

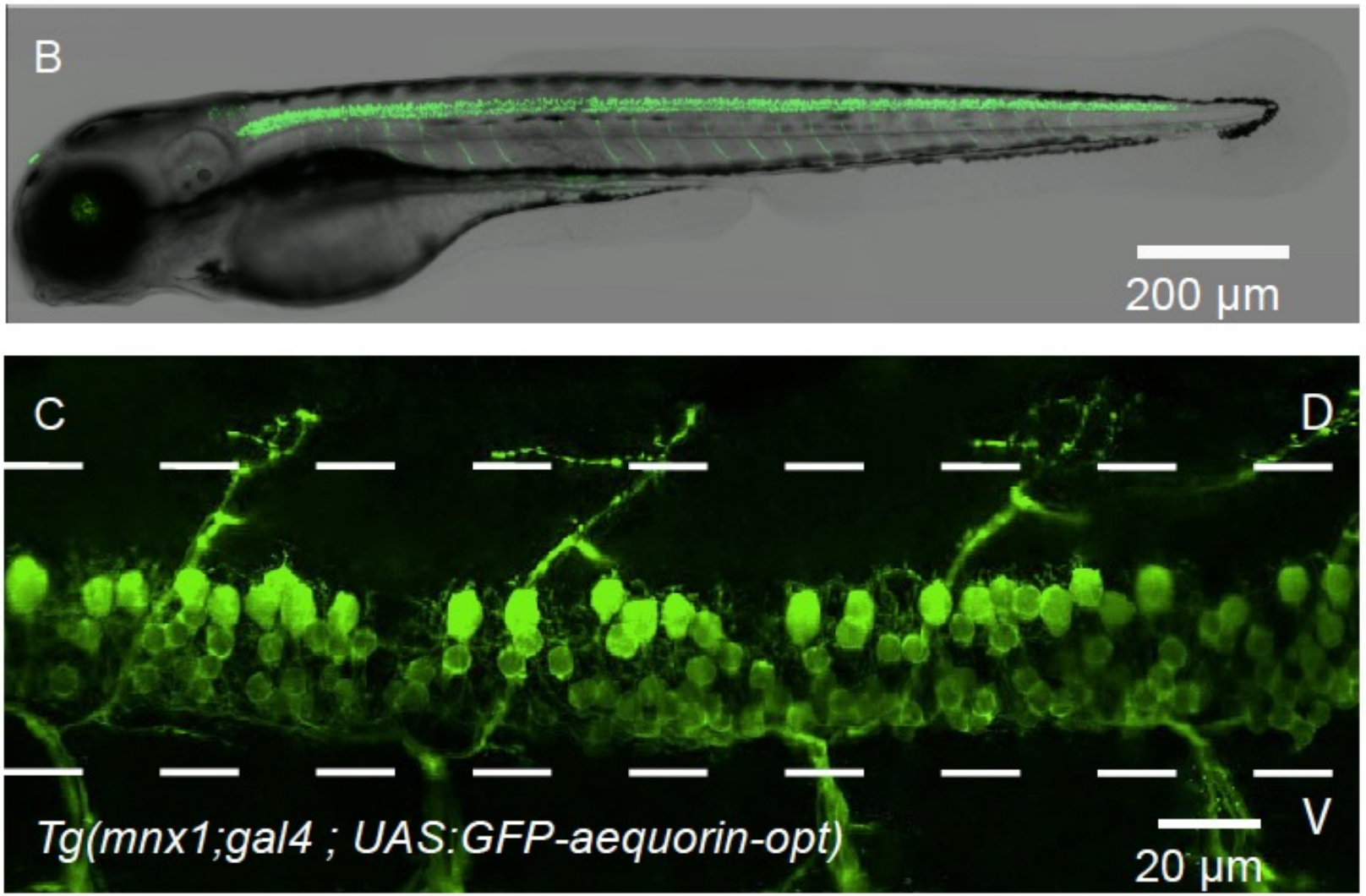
GFP Fusion increases quantum yield

Simultaneous behavior analysis and bioluminescence photon counting



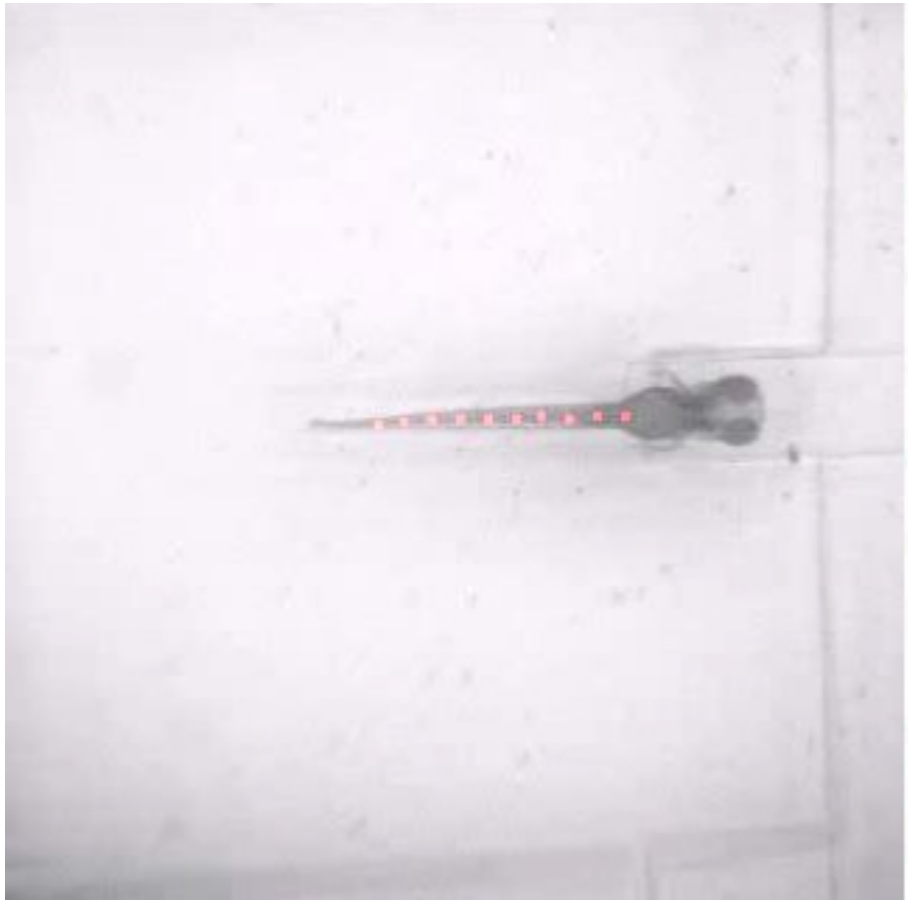
Knafo, *et al.*, eLife 2017

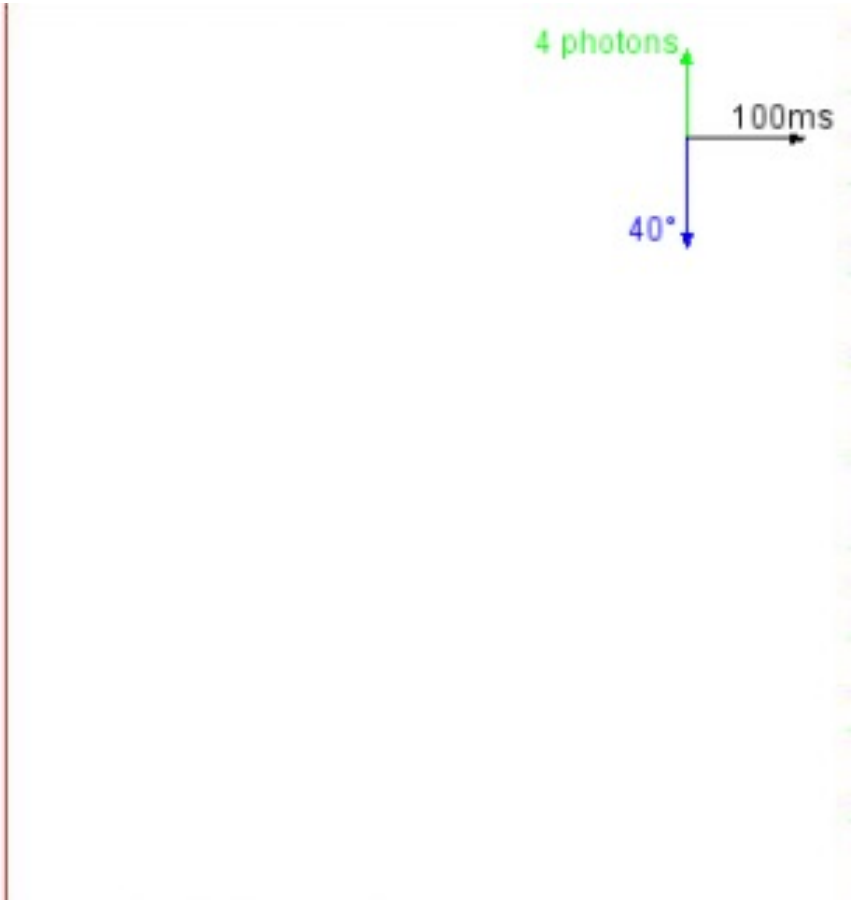
Restricted to neurons, no expression in muscle fibers



Knafo, *et al.*, eLife 2017

GFP-Aequorin on motor neurons

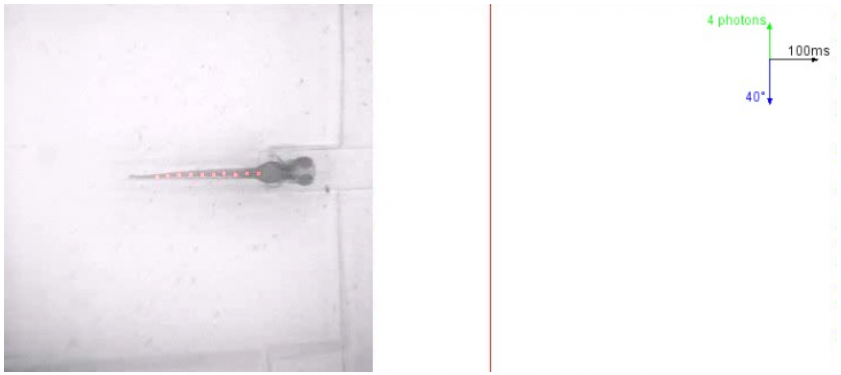




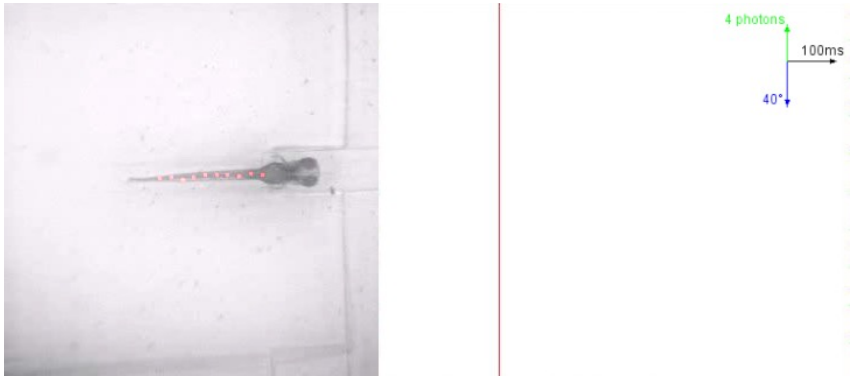
Knafo, *et al.*, eLife 2017

Synchronous recording of motoneurons bioluminescence & behavior

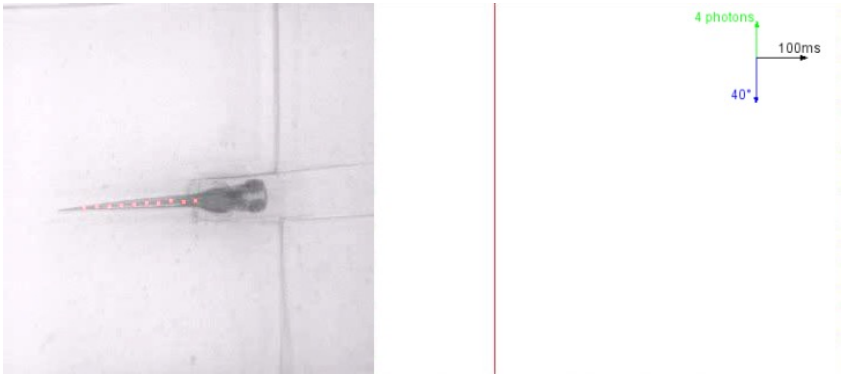
Escape
65.7%



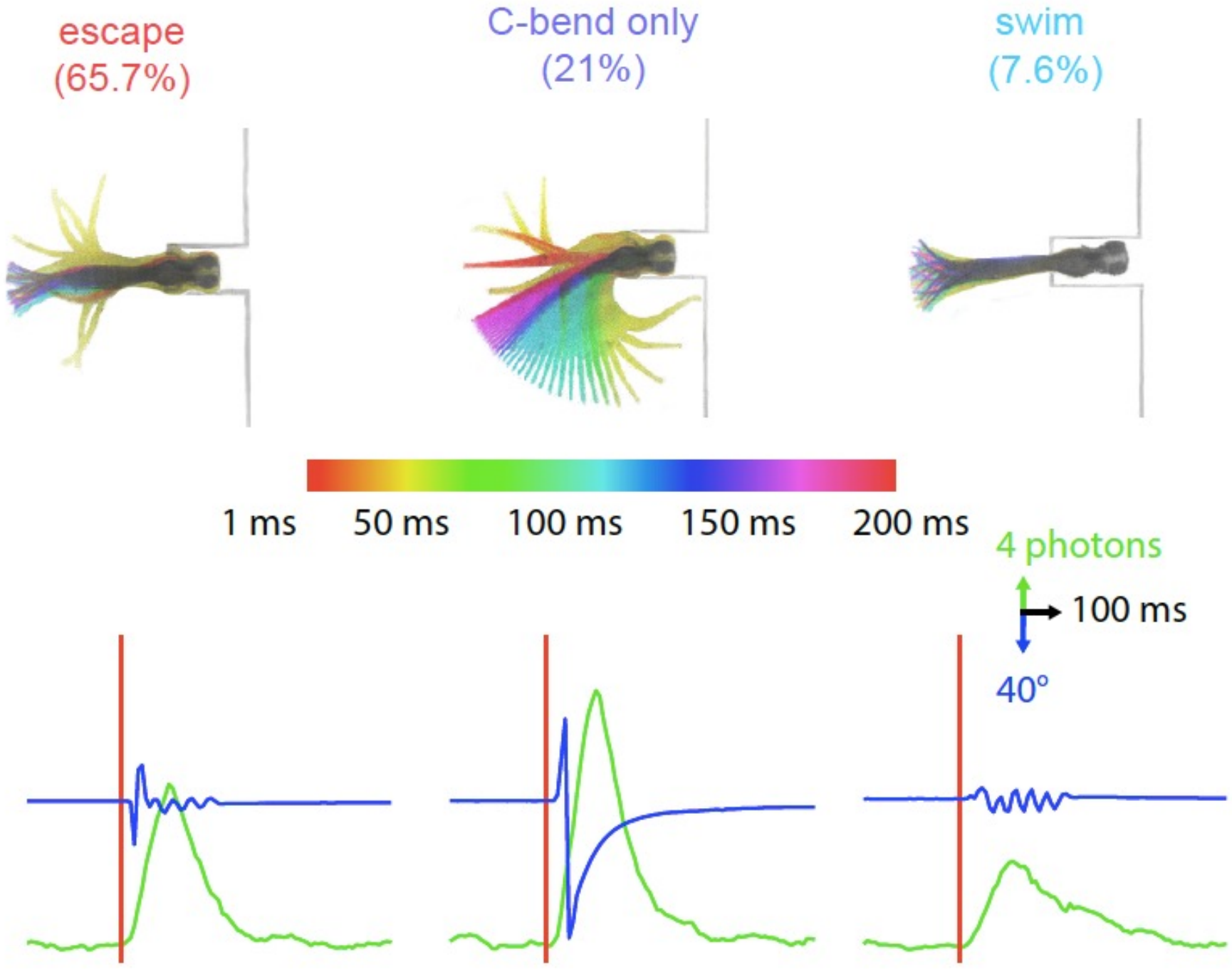
C bend
21%



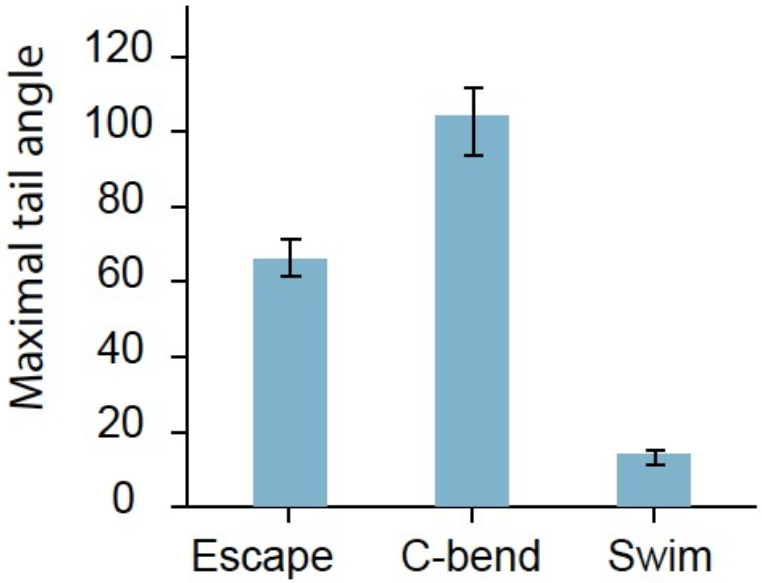
Slow
Swims
7.7%

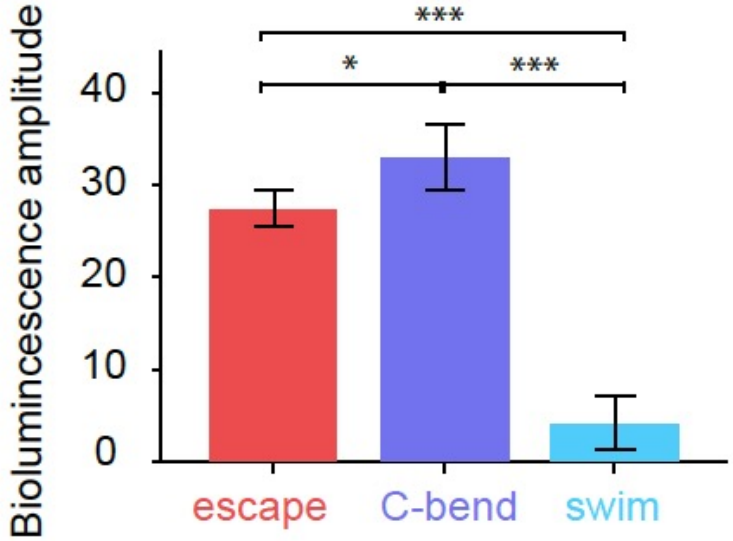


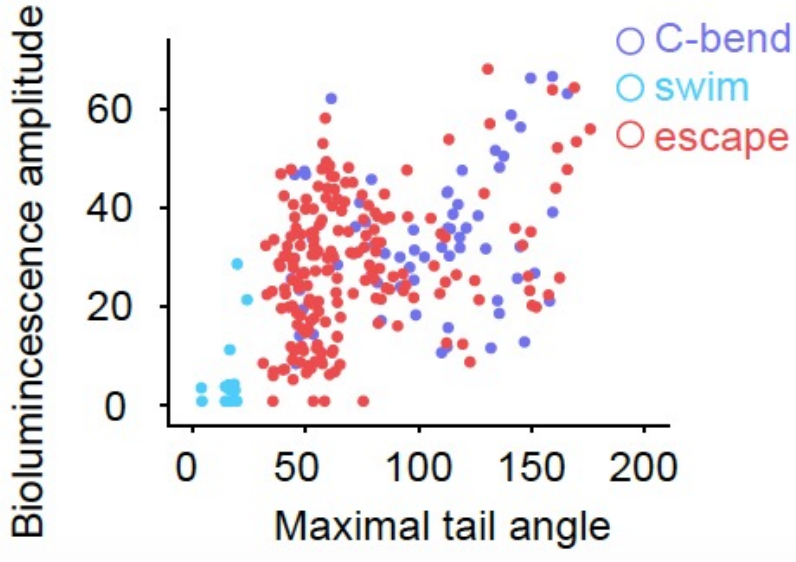
Acoustic stimulus
10 ms long, 500 Hz sine wave


escape
(65.7%)
C-bend only
(21%)
swim
(7.6%)
1 ms
50 ms
100 ms
150 ms
200 ms
4 photons
100 ms
40°

Bioluminescence signals from motor neurons match the amplitude of tail bend

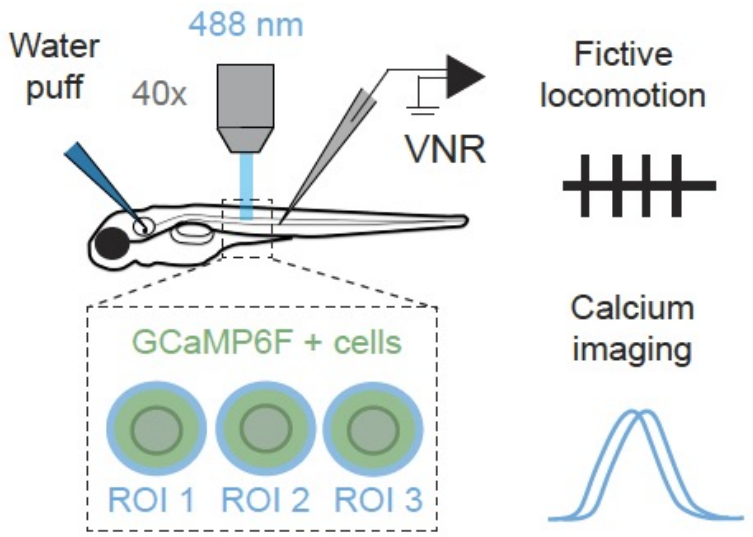


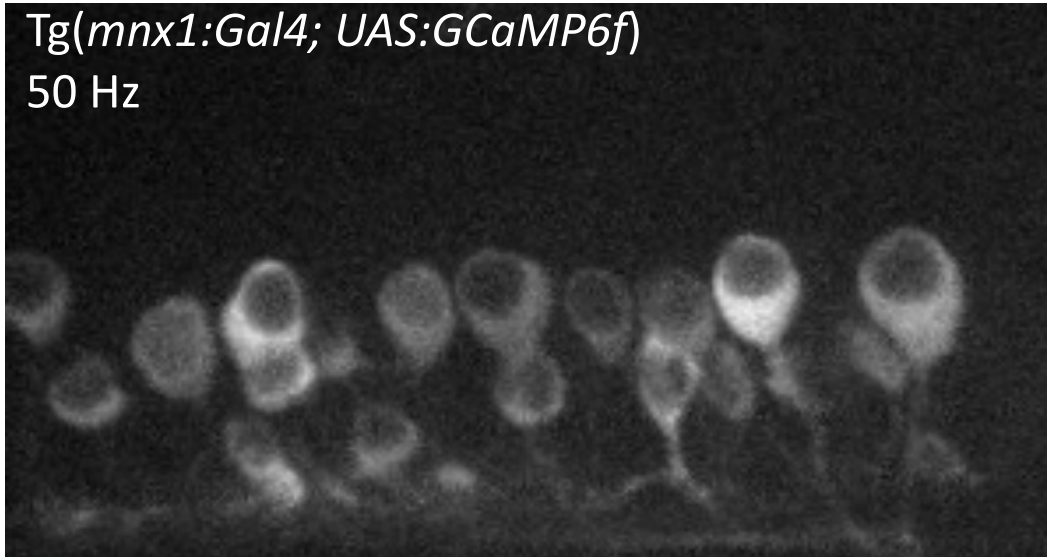


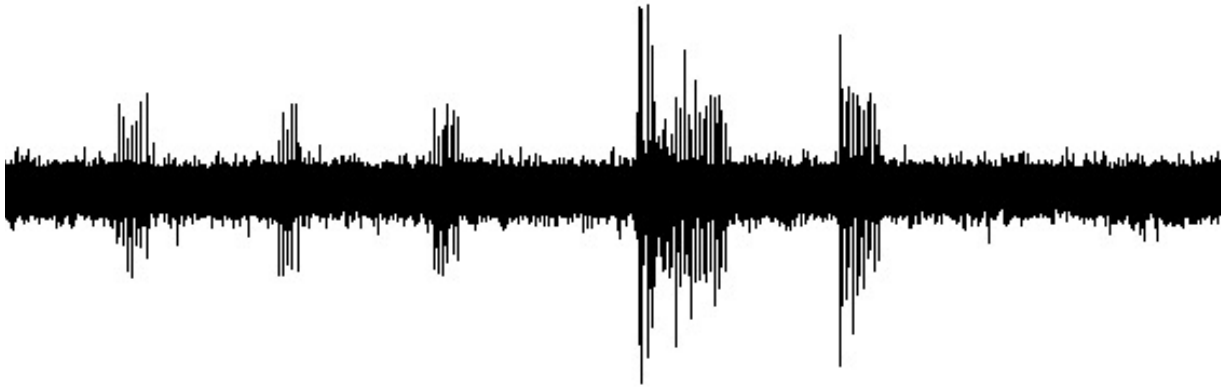


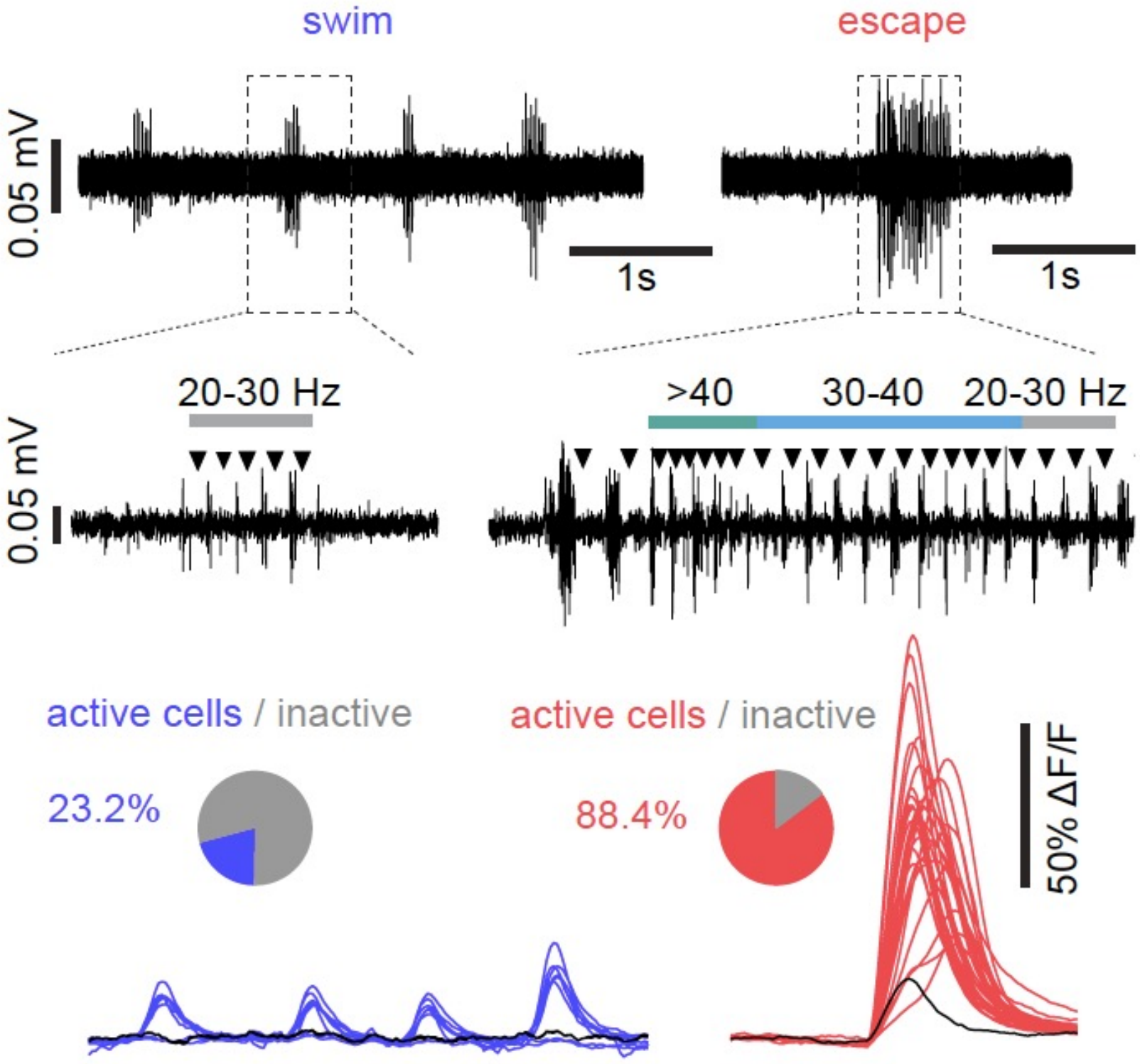
Knafo, *et al.*, eLife 2017

GCaMP imaging in fictive confirms the larger recruitment of MNs at different frequencies

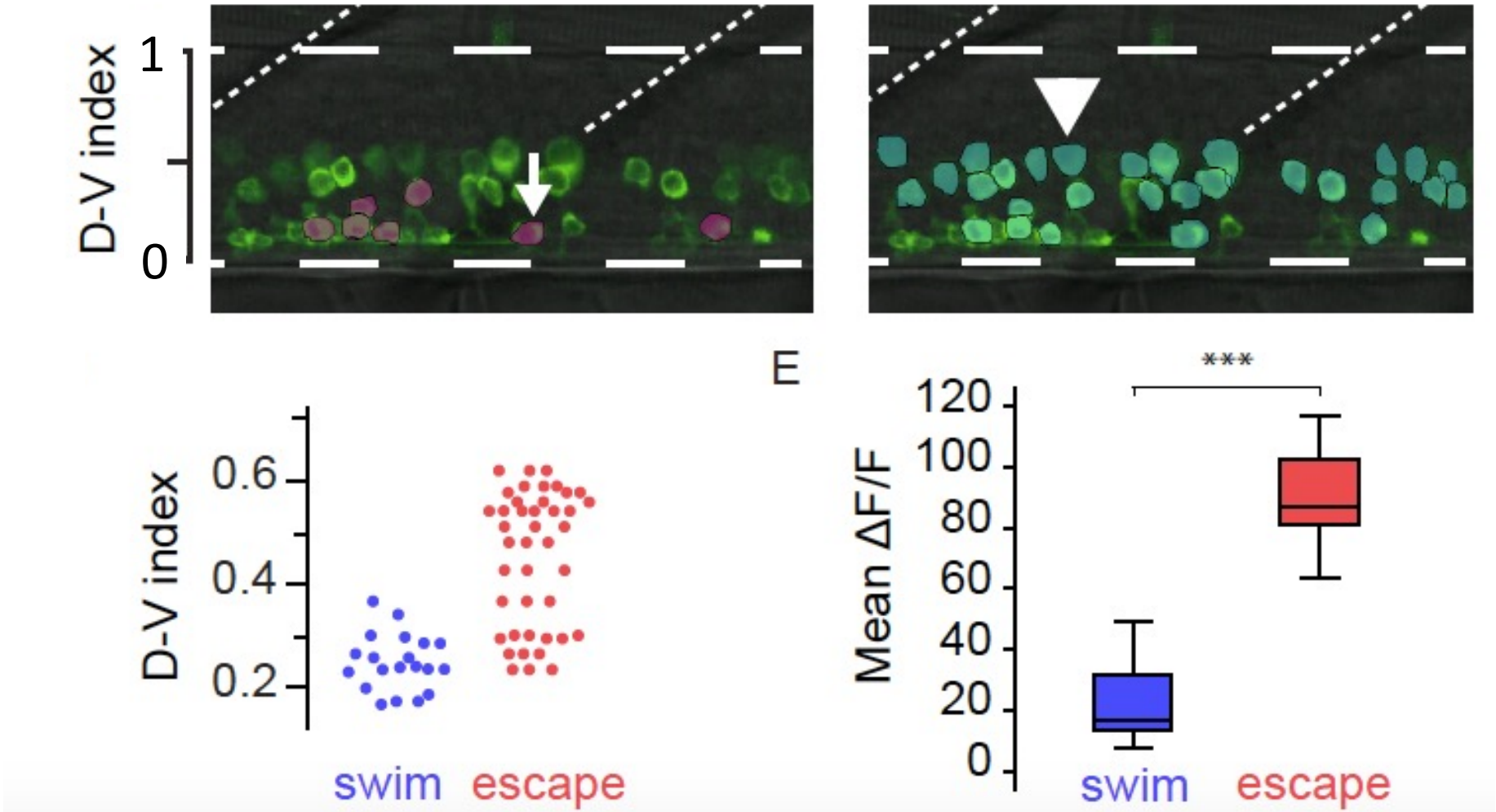




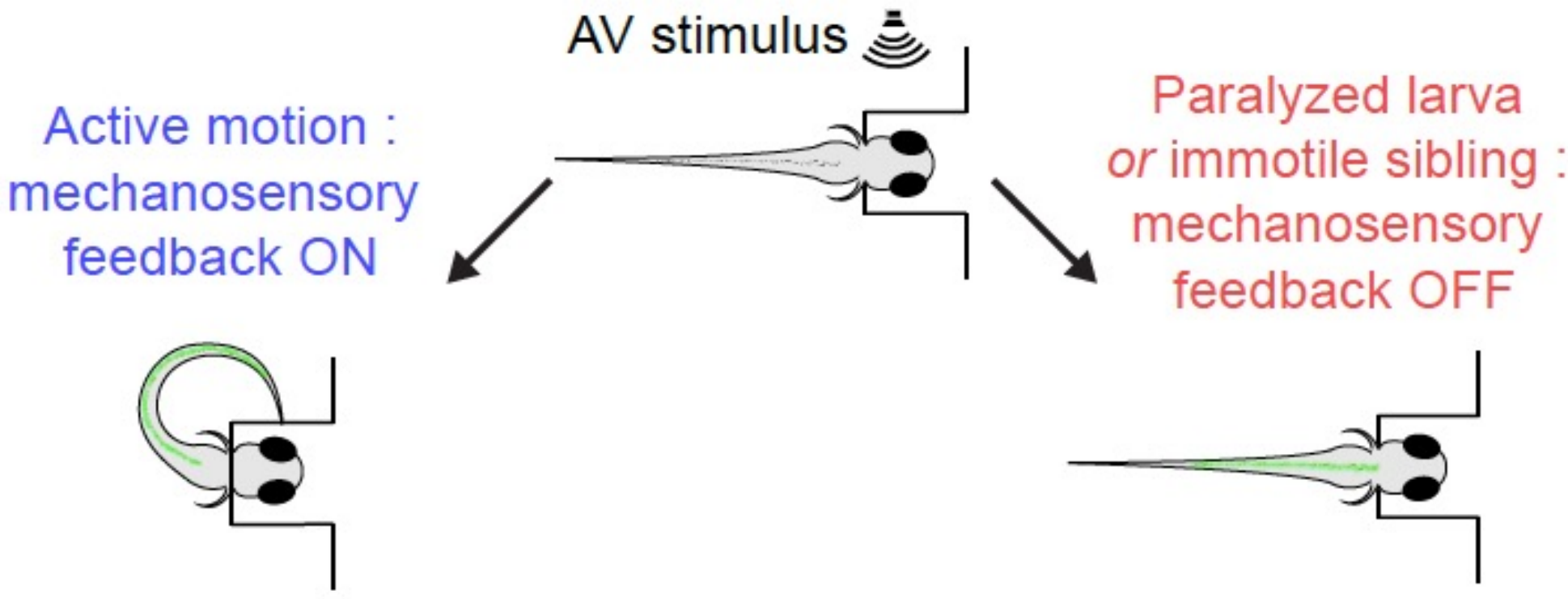



swim
escape
0.05 mV
1s
1s
20-30 Hz
>40
30-40
20-30 Hz
0.05 mV
active cells / inactive
23.2%
active cells / inactive
88.4%
50% ΔF/F

Frequency-dependent recruitment of motor neurons



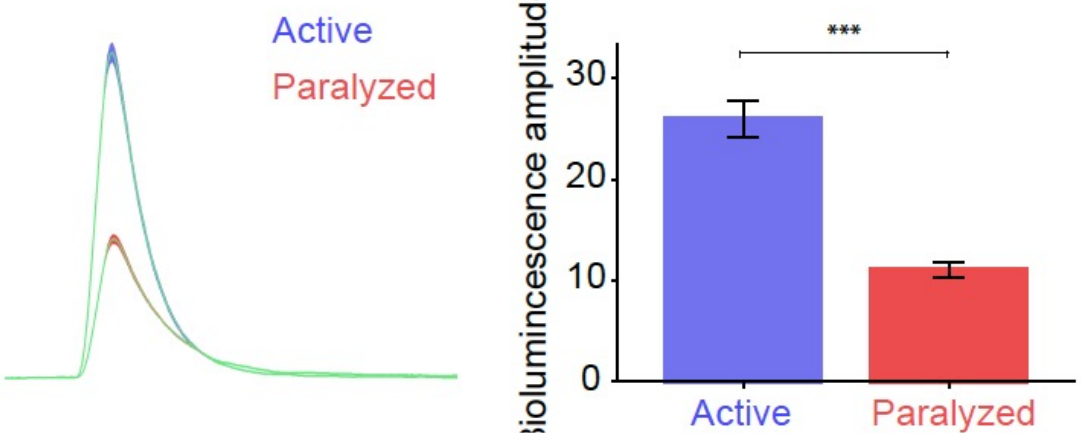
Probing the contribution of mechanosensory feedback on motor neuron recruitment



Knafo, *et al.*, eLife 2017

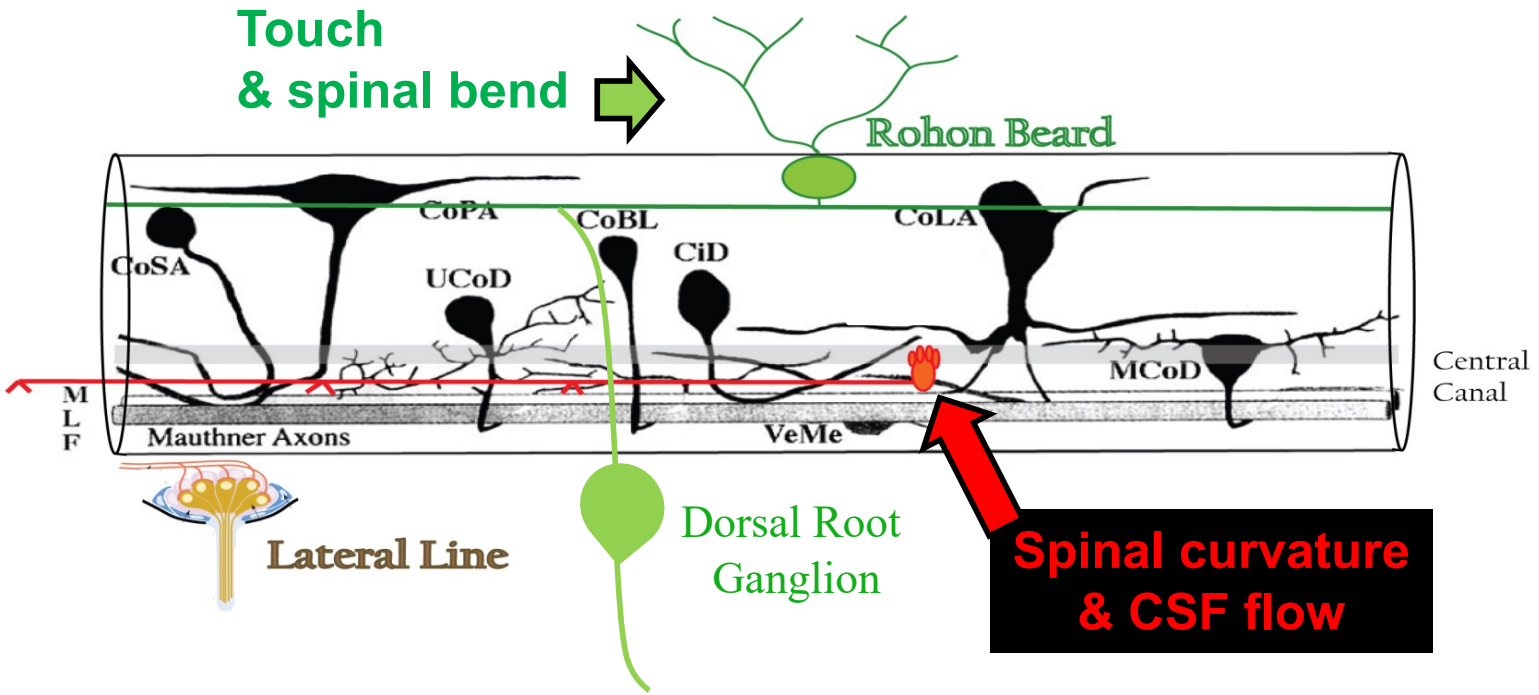
Mechanosensory feedback enhances motor neuron recruitment

Pharmacology
(pancuronium bromide)



Knafo, *et al.*, eLife 2017

Bioluminescence monitoring & calcium imaging reveals the recruitment of spinal neurons in motion

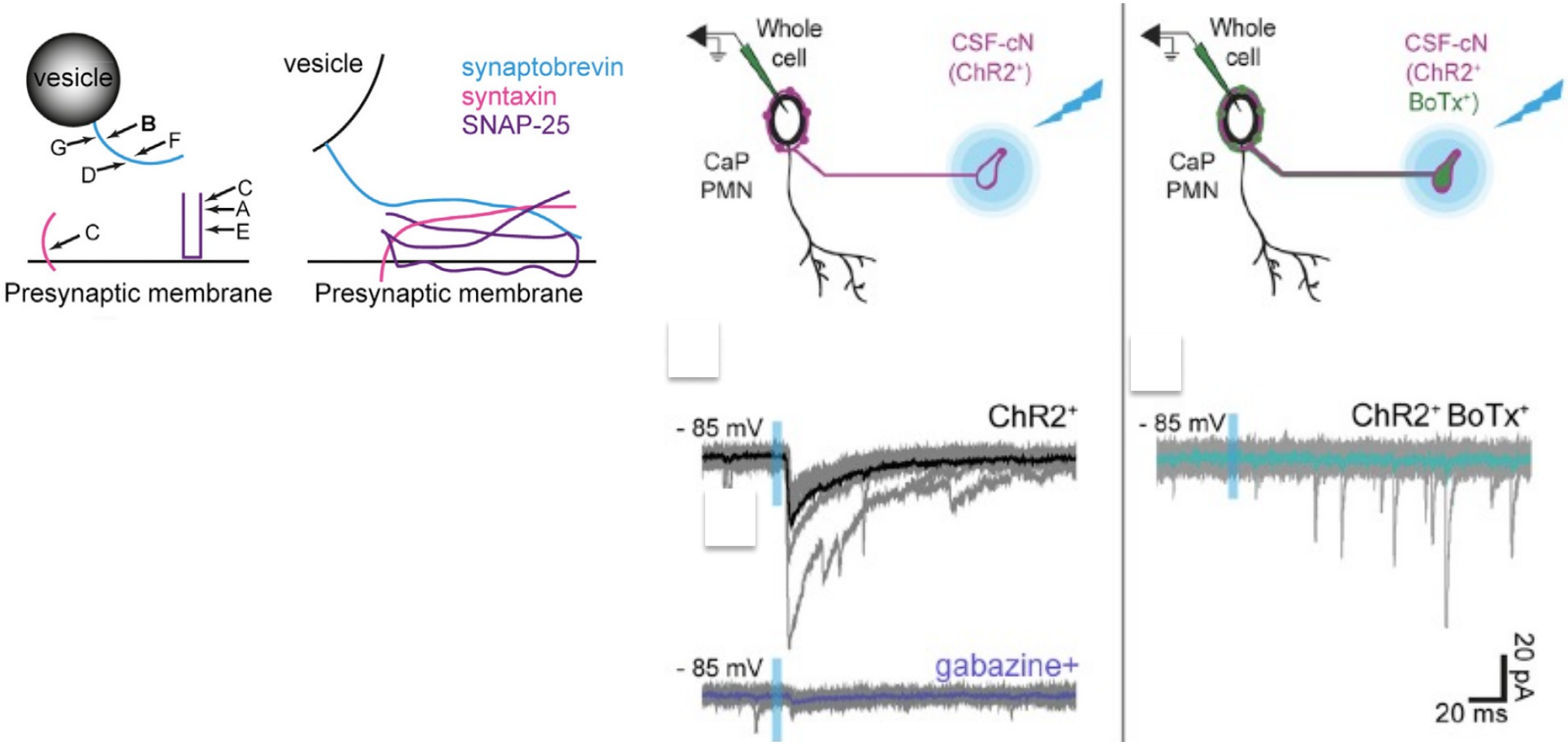


Enhance recruitment of motor neurons:

- increased Speed of movements?
- increased Amplitude of movements?

II. How do sensory feedback shape locomotion & posture ?

Silencing output of neurons with Botulinum Toxin

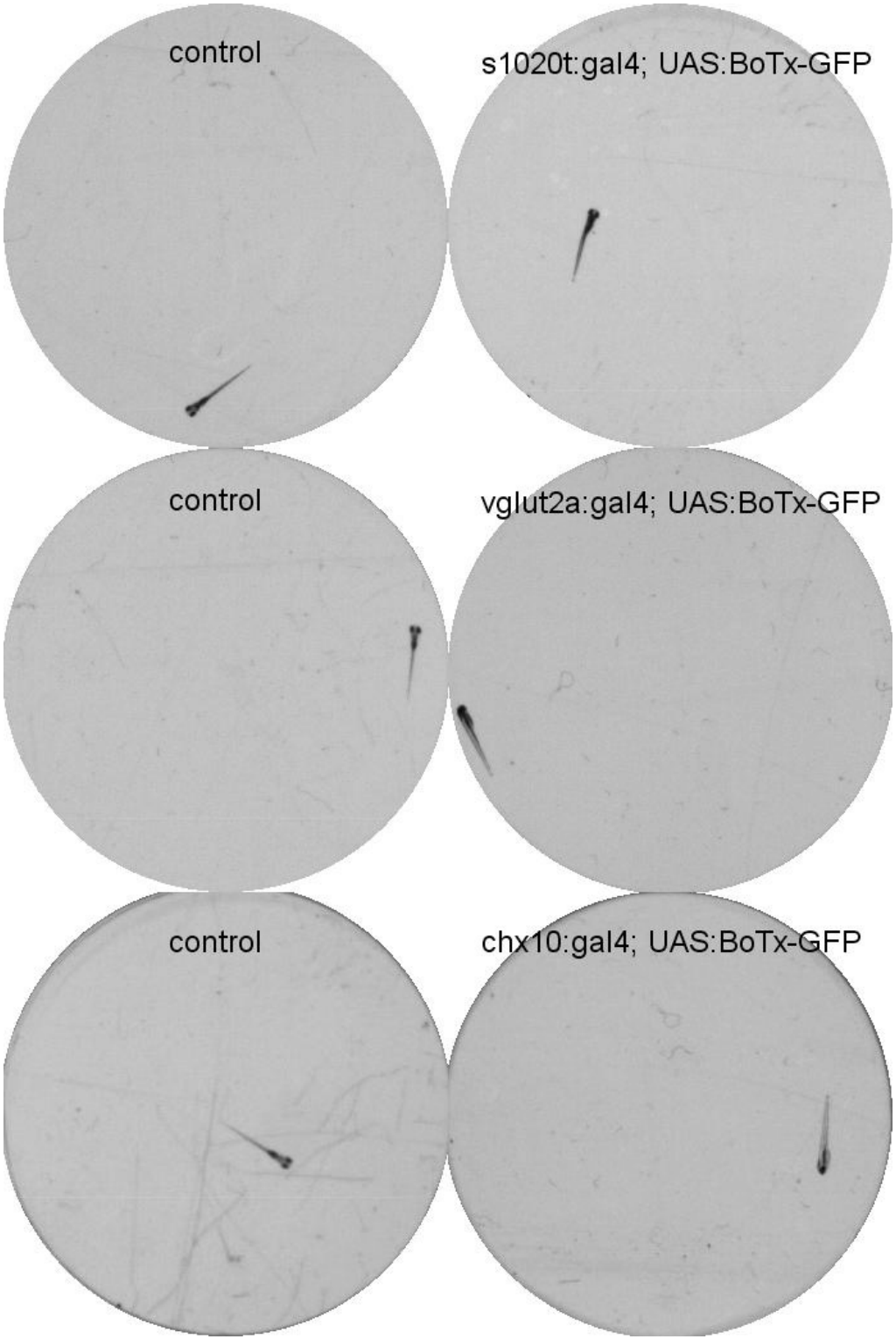


Sternberg, Severi *et al.*, Current Biology 2016

Silence output of motoneuron:
Paralysis

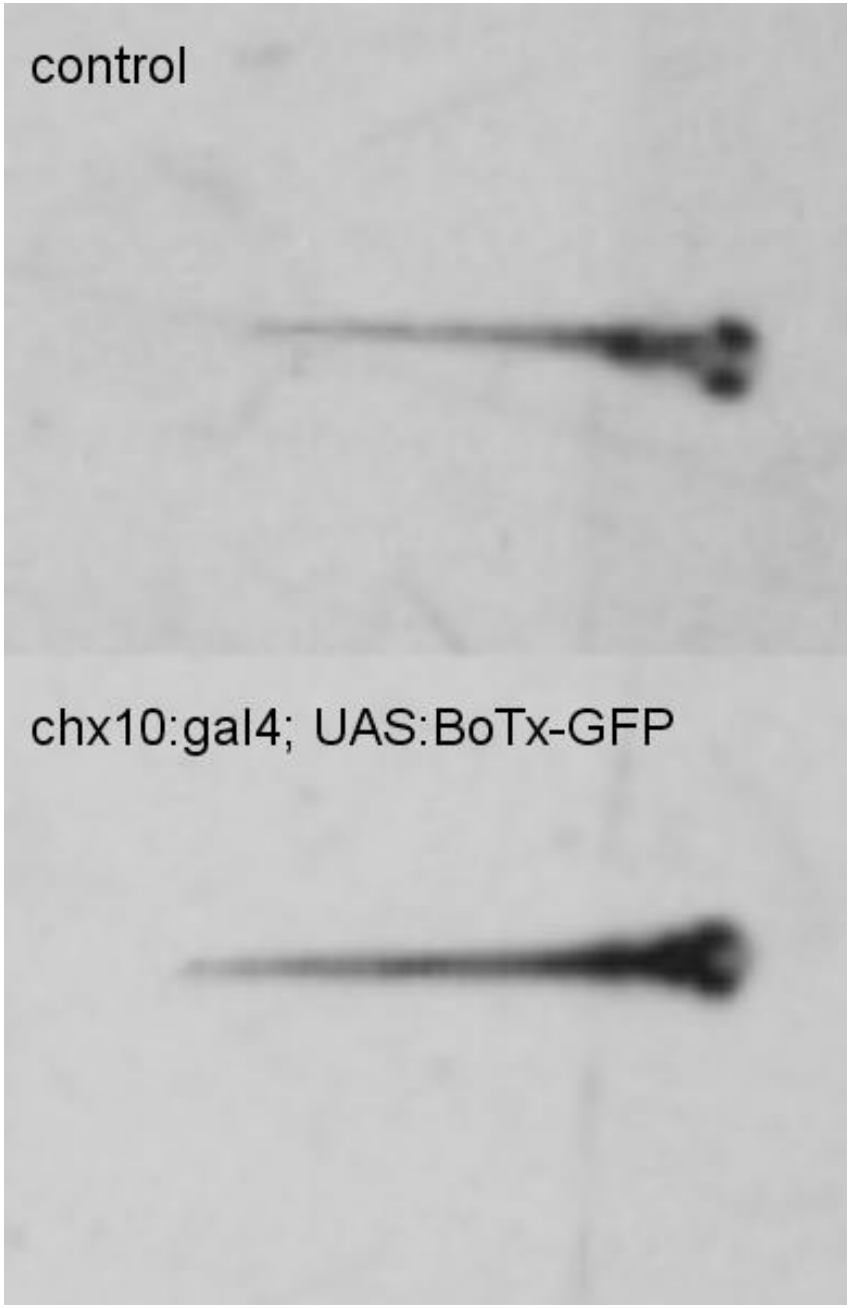
Silence output of glutamatergic premotor neurons:
Paralysis

Silence outputs
of V2a premotor interneurons:
Reduced frequency and amplitude

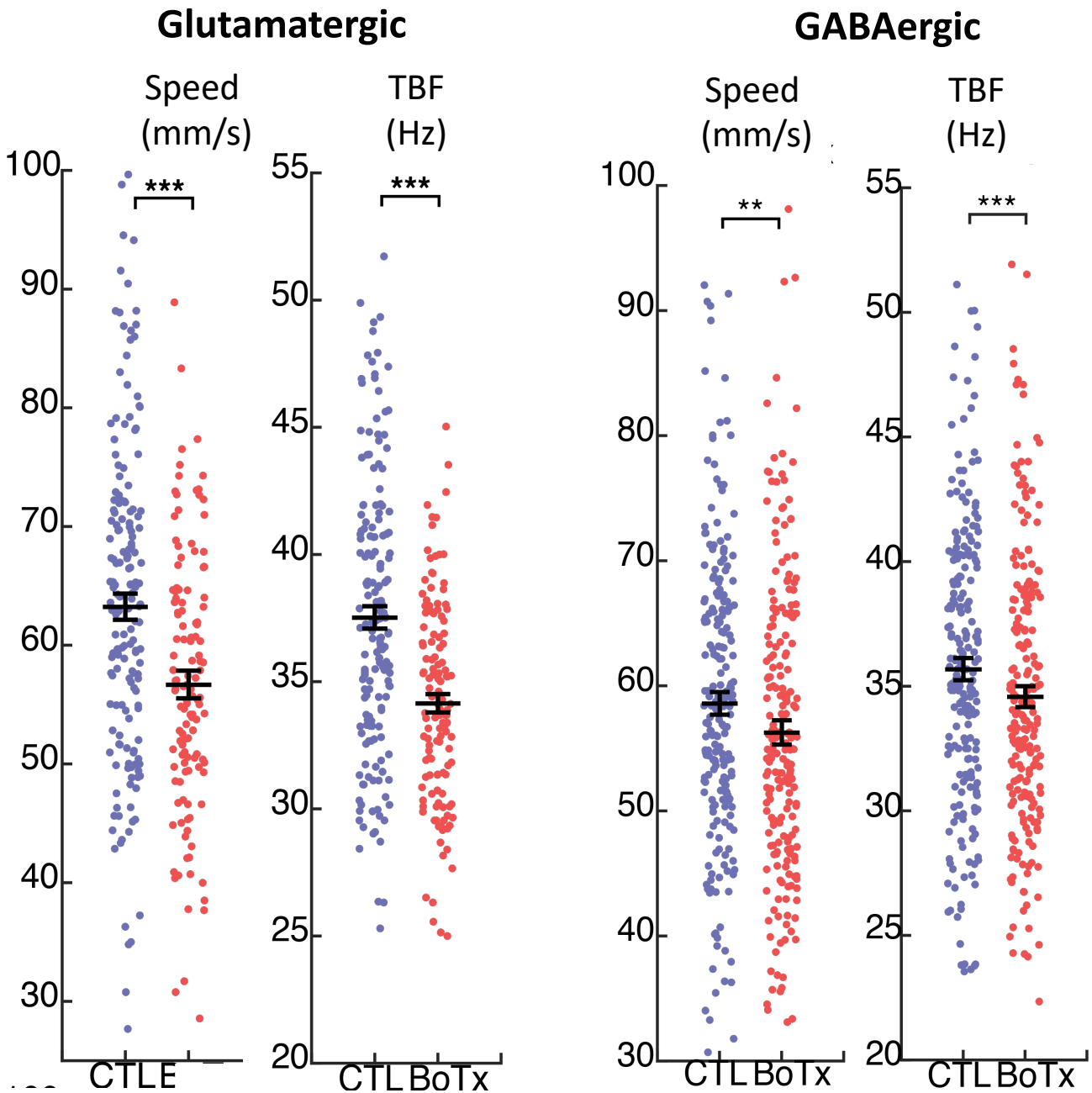


Silence outputs
of V2a interneurons:

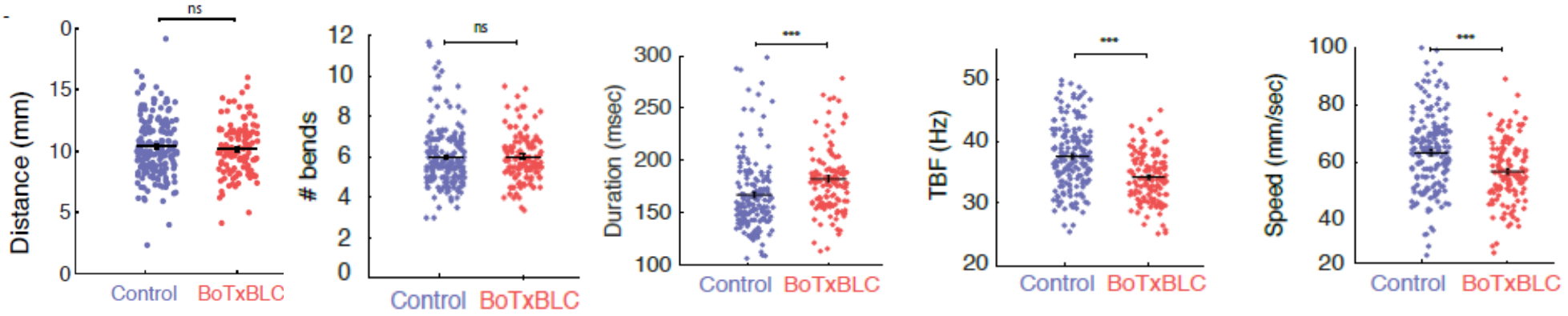
Reduced
Locomotor Frequency,
Number of Oscillations
and Amplitude

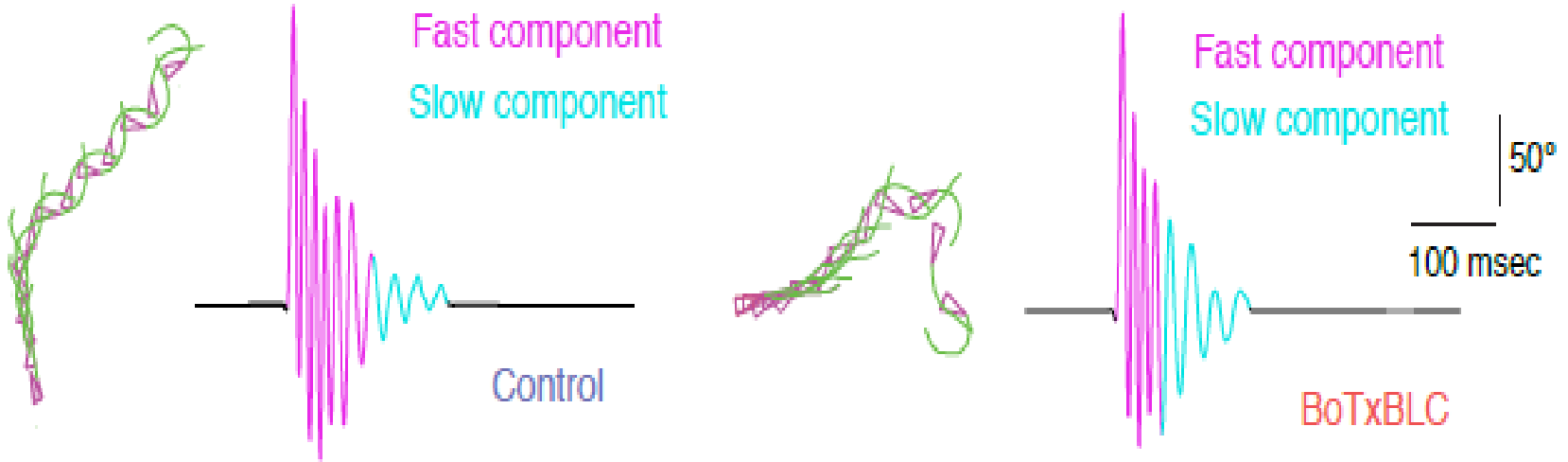


Silencing mechanosensory feedback reduces speed



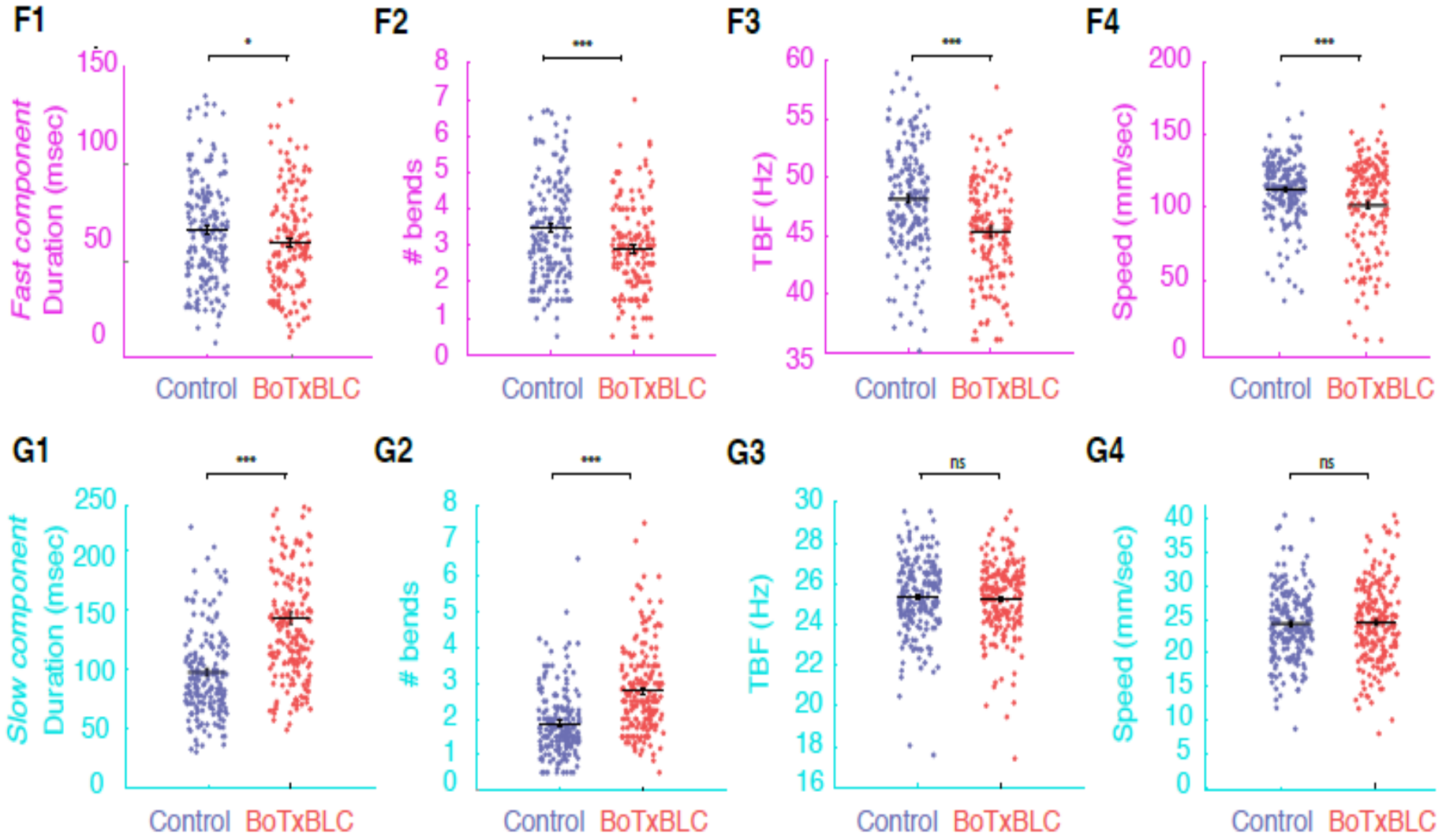
Mechanosensory feedback boosts locomotor speed



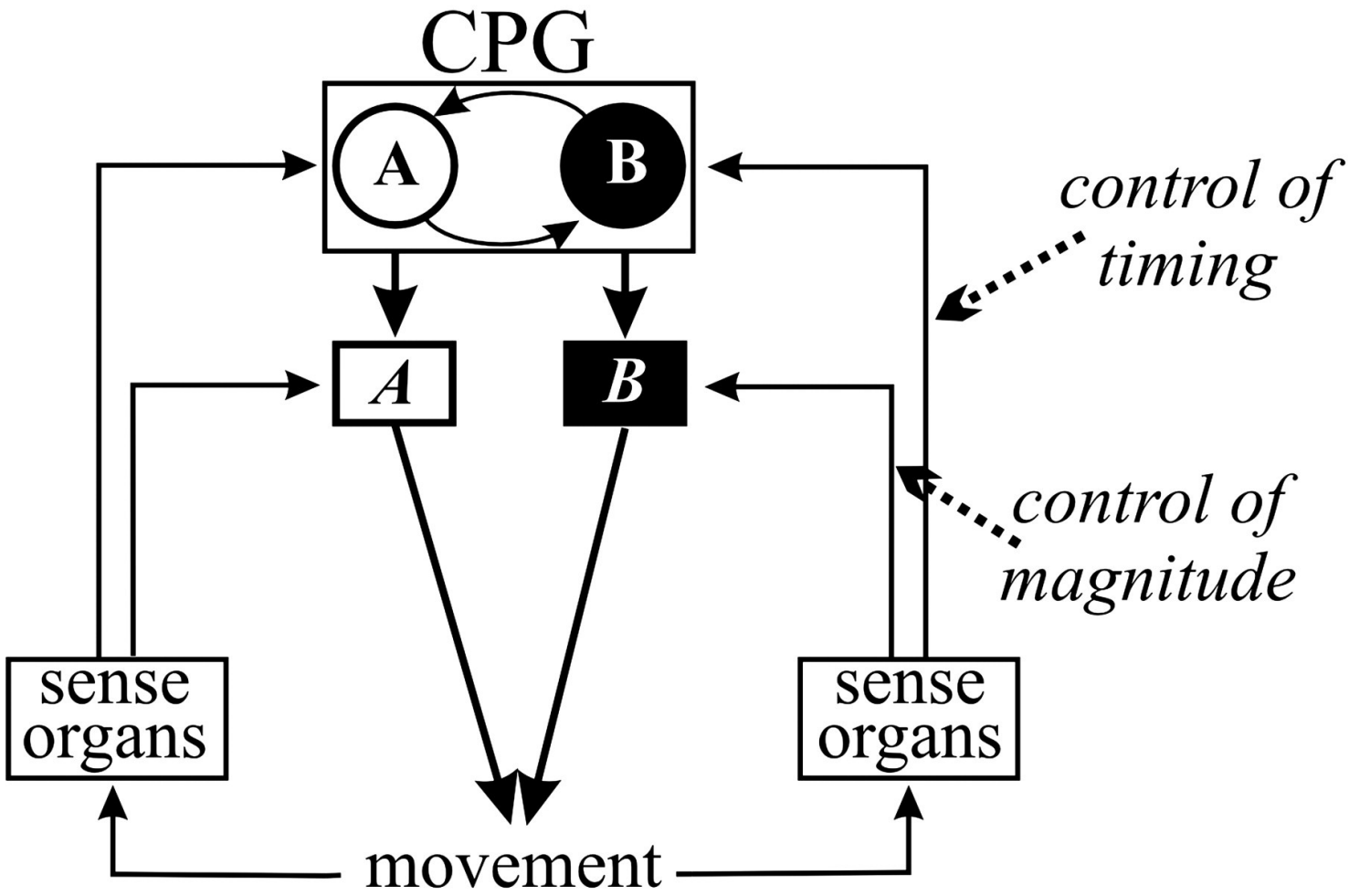


Knafo*, Fidelin* et al., eLife 2017

Speed-dependent modulation of locomotion



Predictions on the effects of mechanosensory feedback from electrical stimulations



Real and fictive locomotion induced by drugs

Electromyography
in a swim mill

Wallen and Williams 1984

Entrainment of the motor pattern with imposed movements

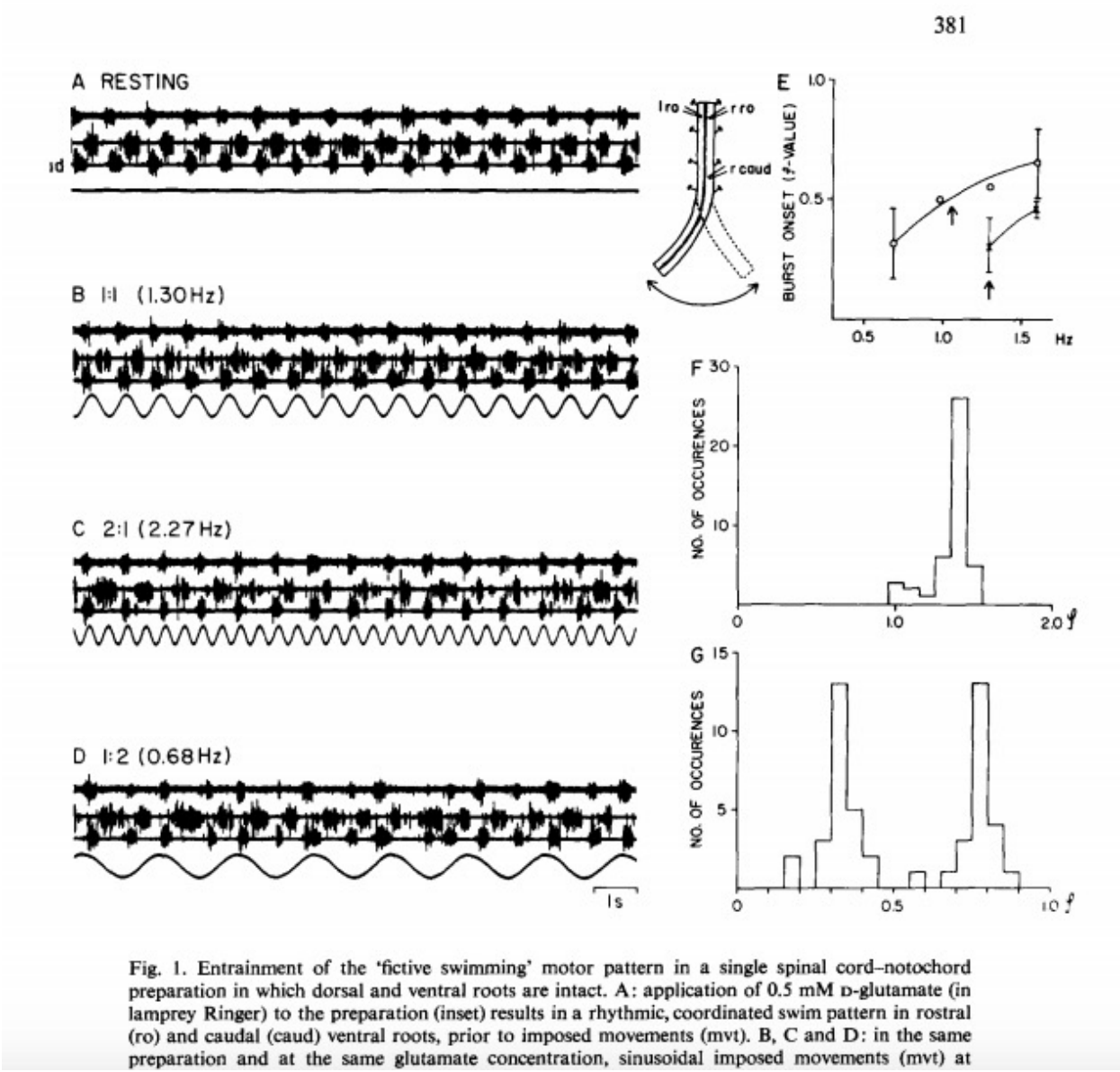
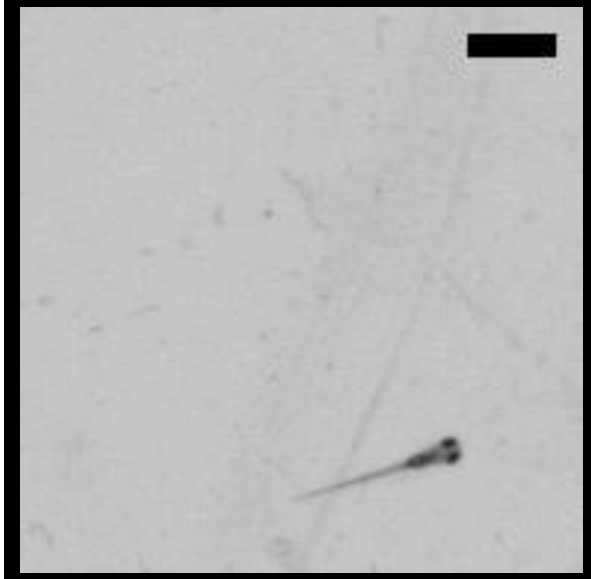
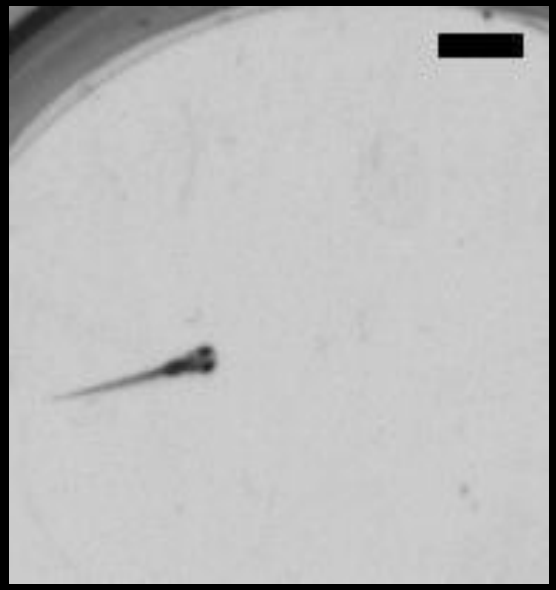


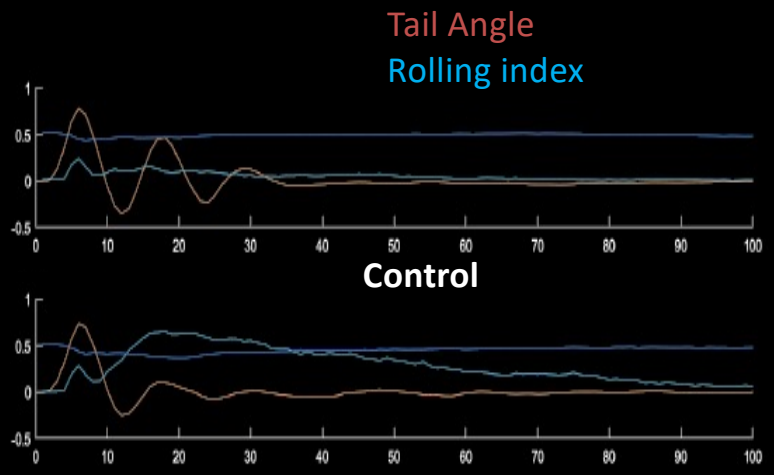
Fig. 1. Entrainment of the 'fictive swimming' motor pattern in a single spinal cord–notochord preparation in which dorsal and ventral roots are intact. A: application of 0.5 mM D-glutamate (in lamprey Ringer) to the preparation (inset) results in a rhythmic, coordinated swim pattern in rostral (ro) and caudal (caud) ventral roots, prior to imposed movements (mvt). B, C and D: in the same preparation and at the same glutamate concentration, sinusoidal imposed movements (mvt) at

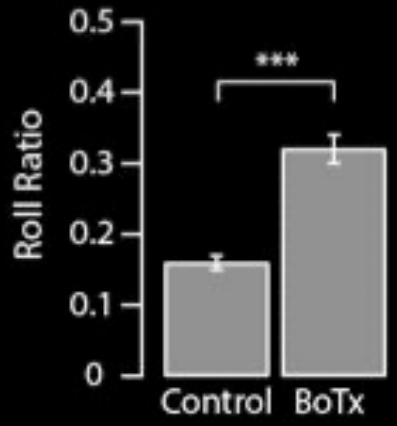
Grillner et al., 1981

Silencing CSF neurons affects balance during active motion





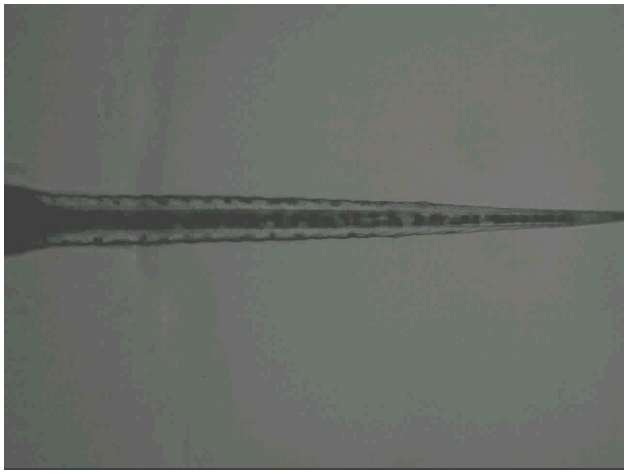


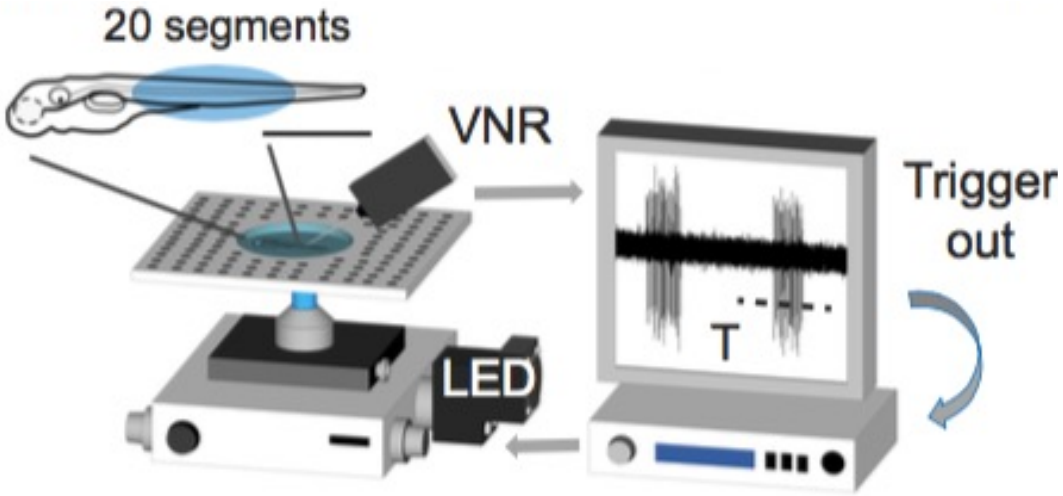


Hubbard *et al.,* Current Biology 2016
Wu *et al.,* Current Biology 2021

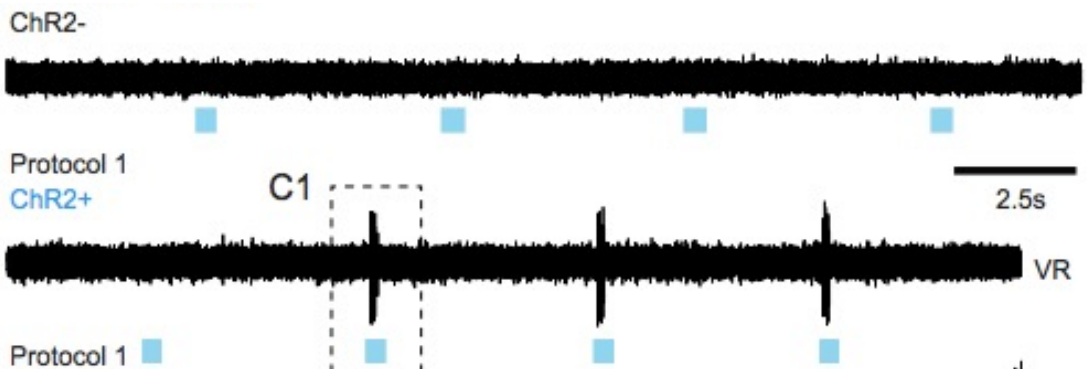
State-dependent modulation of locomotion arousal

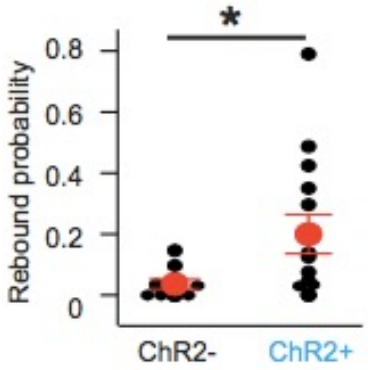
Induction of slow locomotion at rest





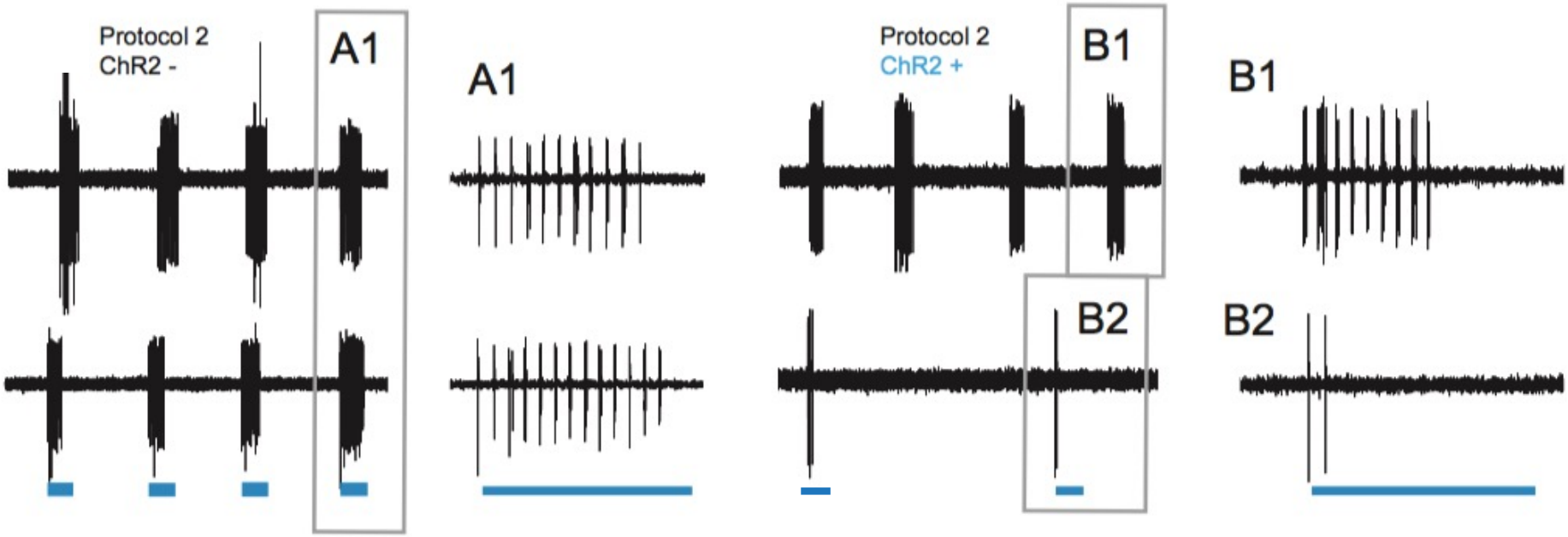
Wyart et al., Nature 2009





Fidelin et al., Current Biology 2015

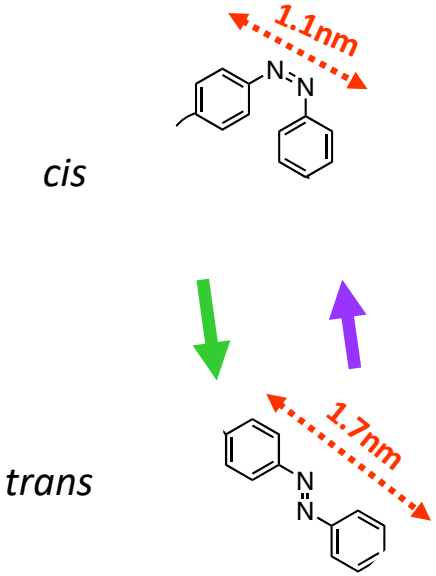
State-dependent modulation of locomotion arousal: silencing of ongoing activity



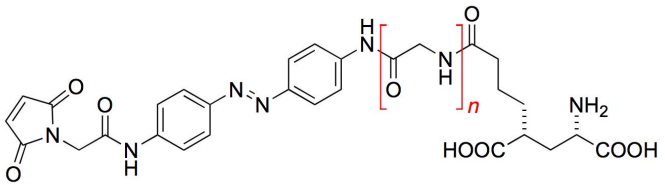
Fidelin et al., Current Biology 2015

III. How to map connectivity of sensory neurons in the spinal cord?

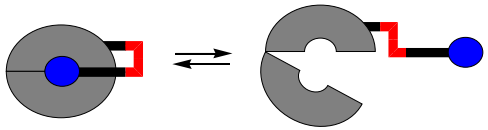
Permeability : Controlling receptors with light & photoswitches



Azobenzene:
nanoscale chemical photoswitch

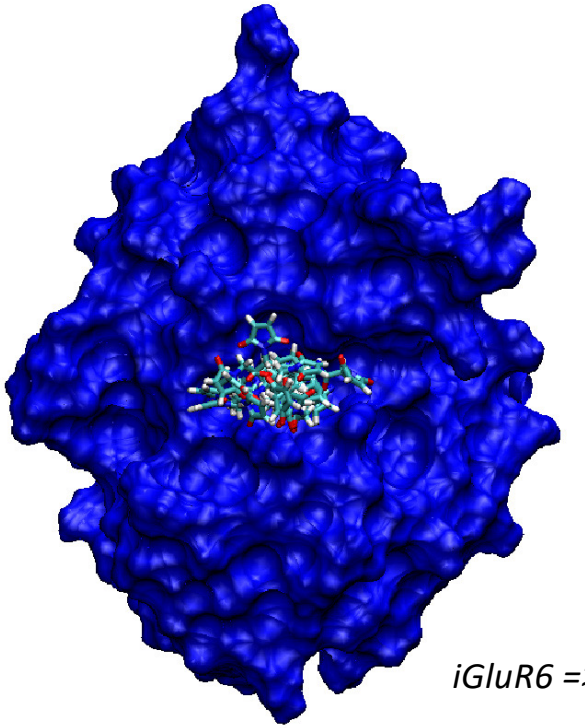


MAG
(Maleamide Azobenzene Glutamate)



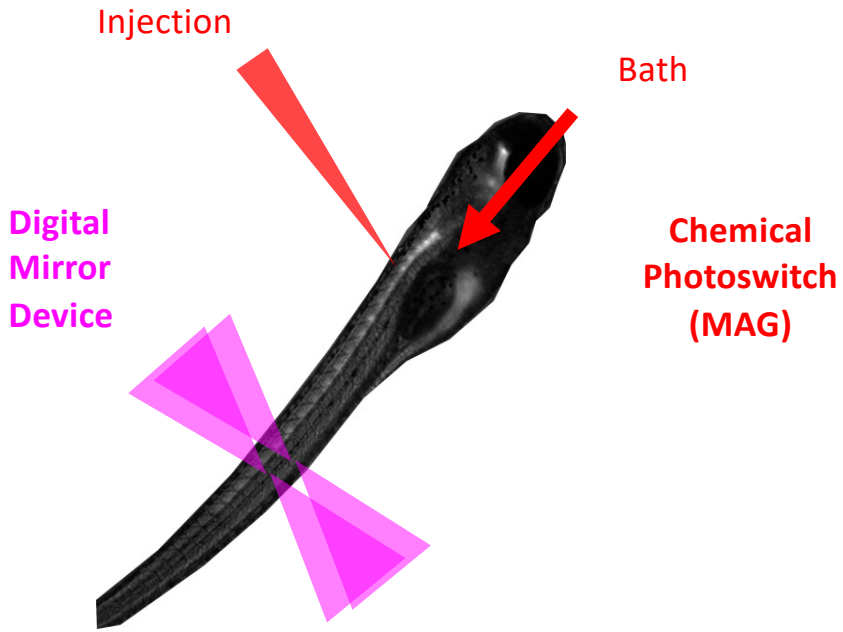
Joined effort: Trauner, Isacoff

Permeability



iGluR6 => LiGluR

“Chemical Optogenetics”

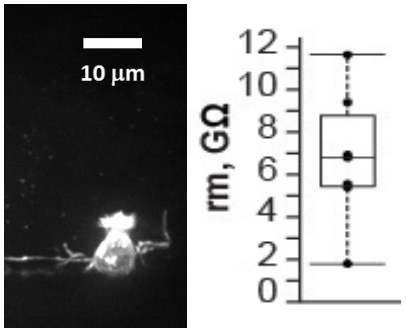


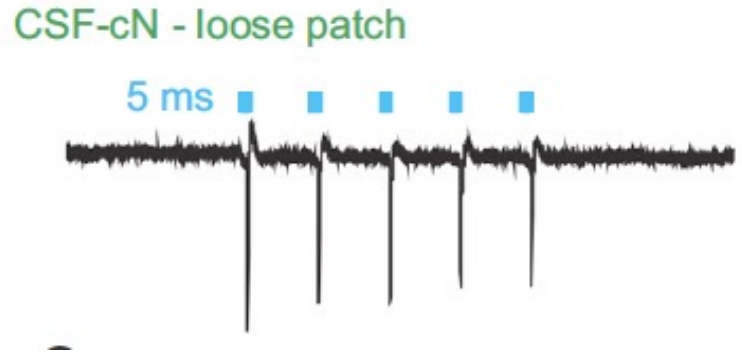
Day 5 : zebrafish larva is highly permeable

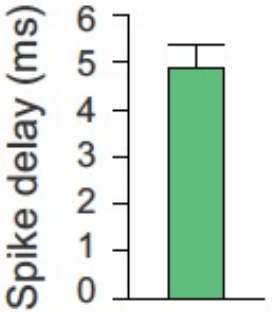
Goroszita P, Numano R. and Volgraf M.
Ehud Isacoff, Dirk Trauner

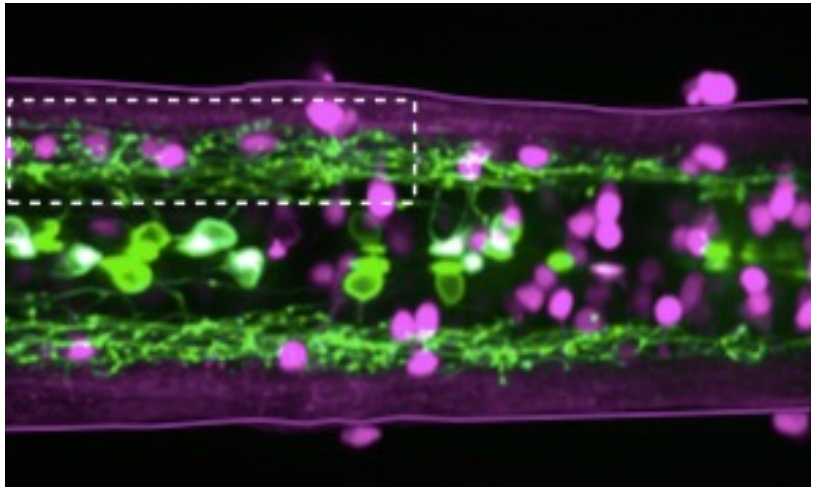
Szobota *et al.,* Neuron 2007
Wyart *et al.,* Nature 2009

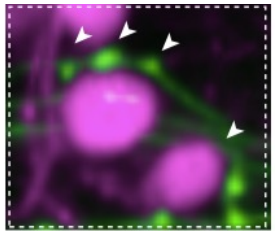
Optogenetic-mediated connectivity mapping *in vivo*

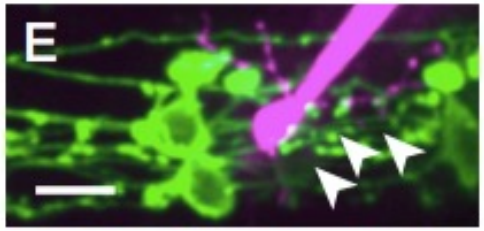


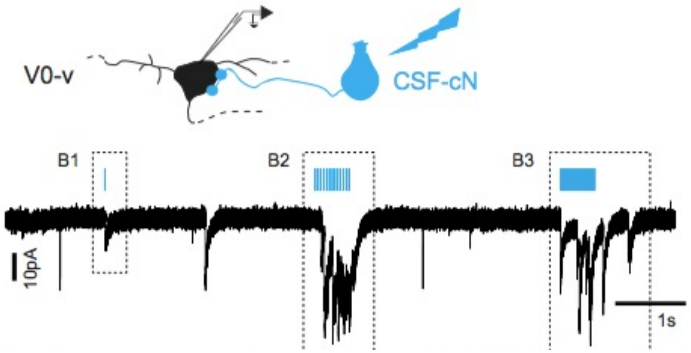








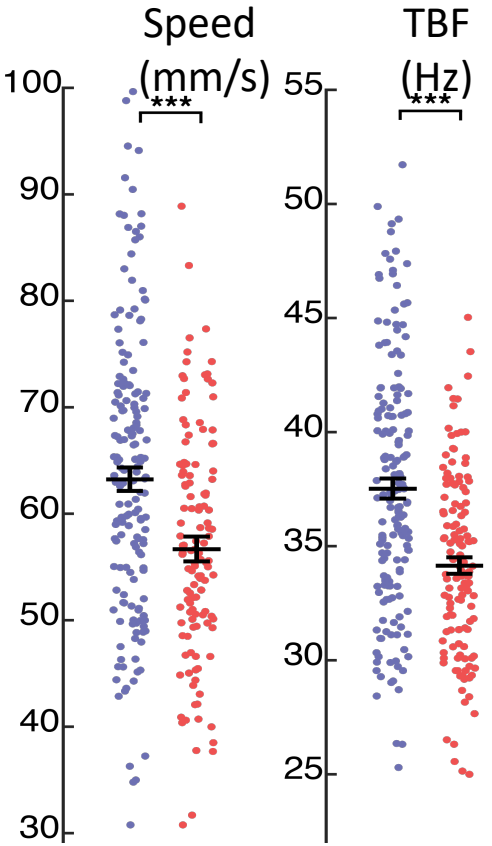




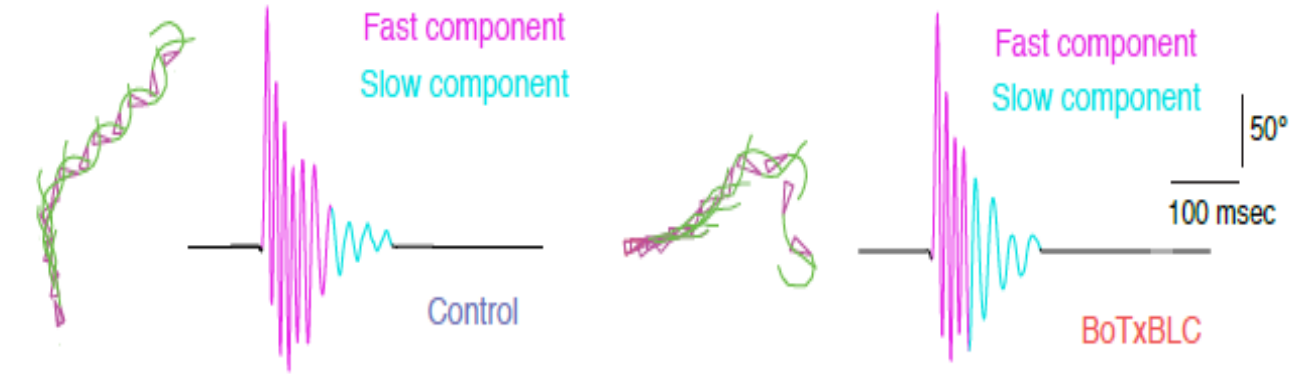
Fidelin et al., Current Biology 2015

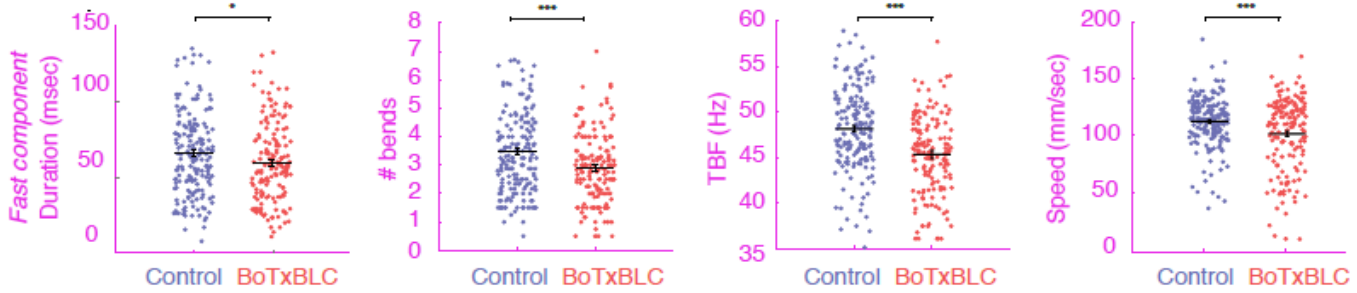
How does sensory feedback modulate Locomotion speed?

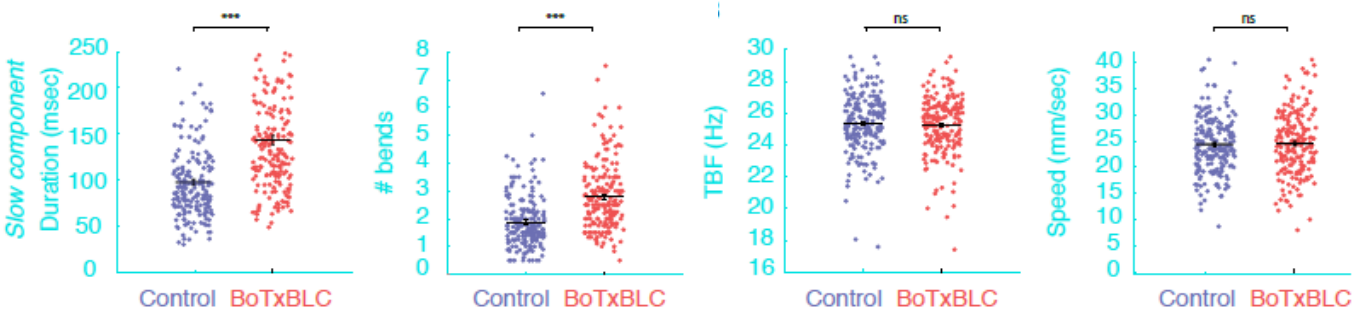
Silencing mechanosensory feedback reduces speed



Control - BoTxLC-GFP

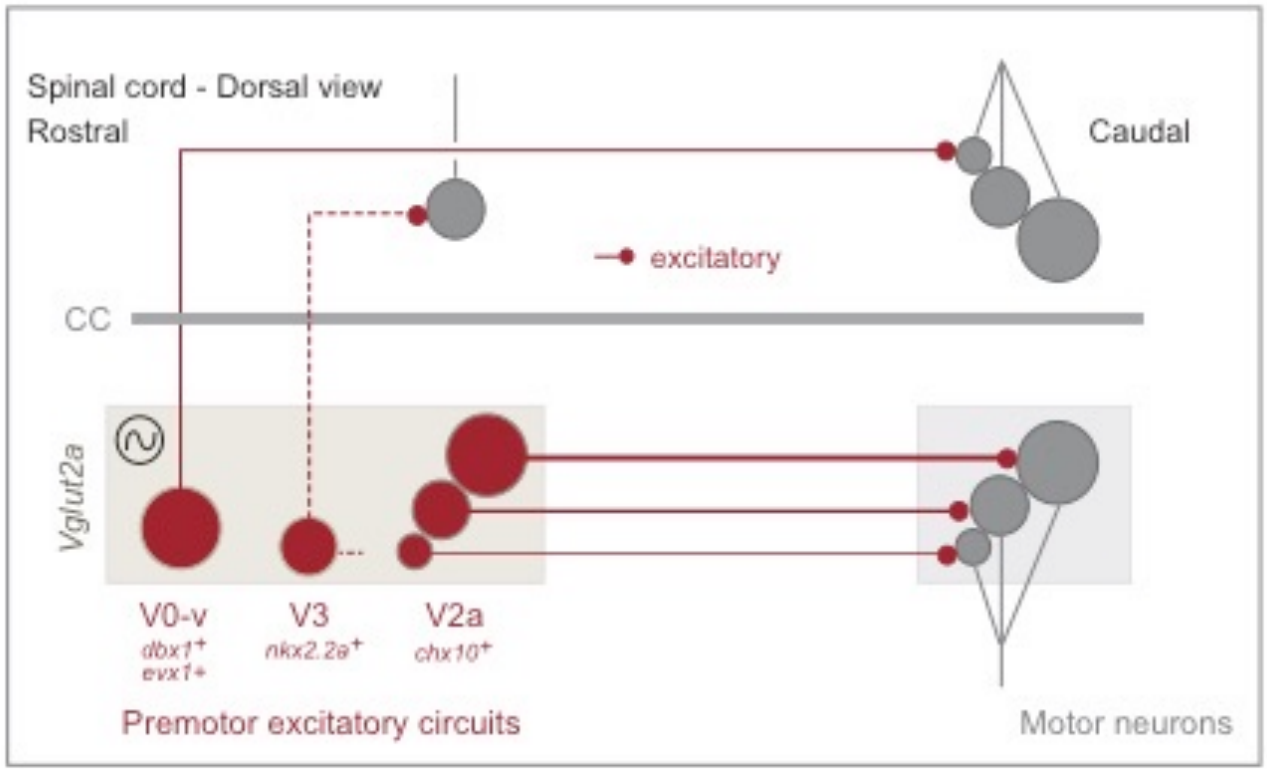


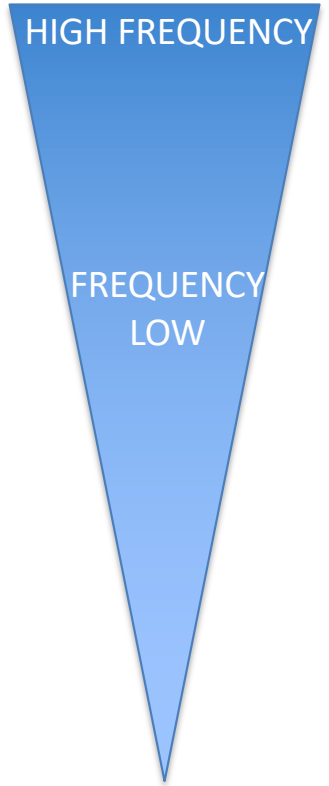




Knafo *et al.*, eLife 2017

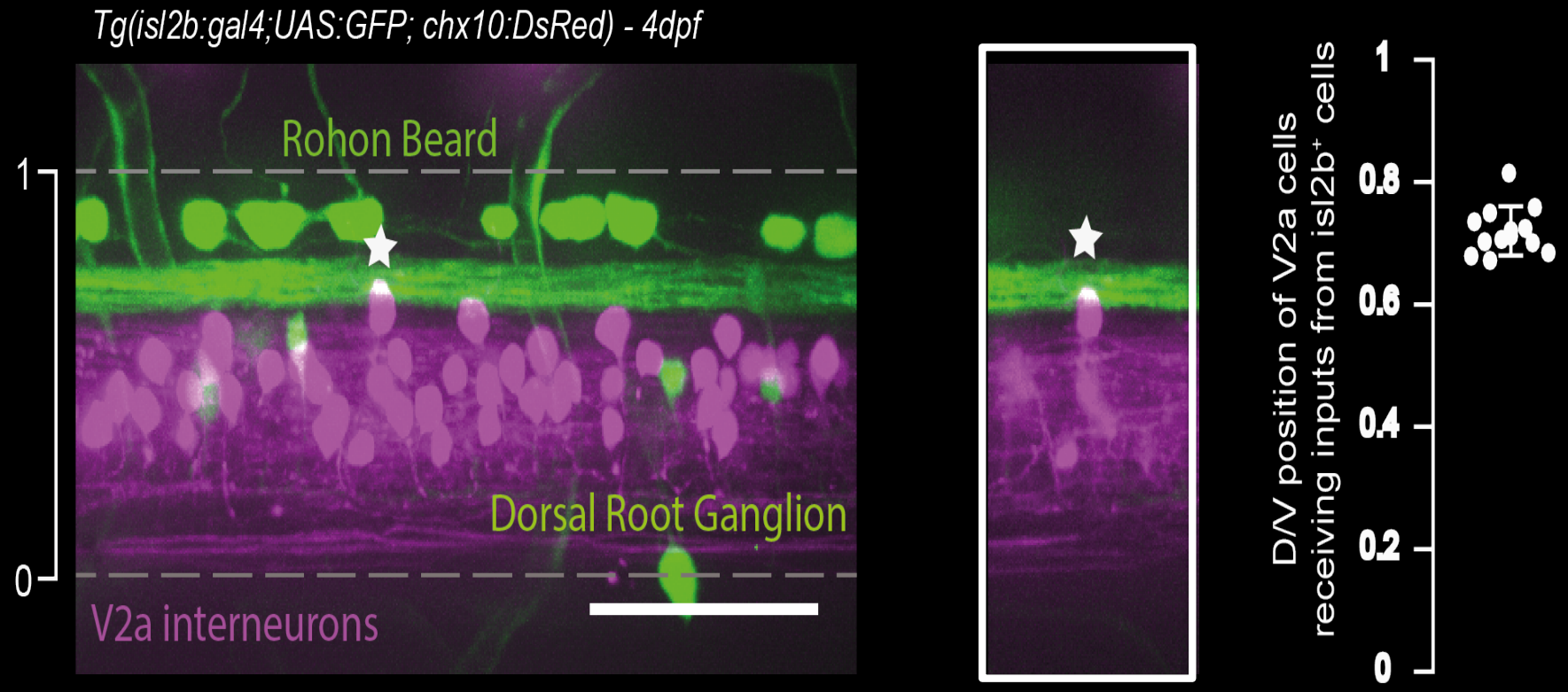
The excitatory drive onto motor neurons in vertebrates





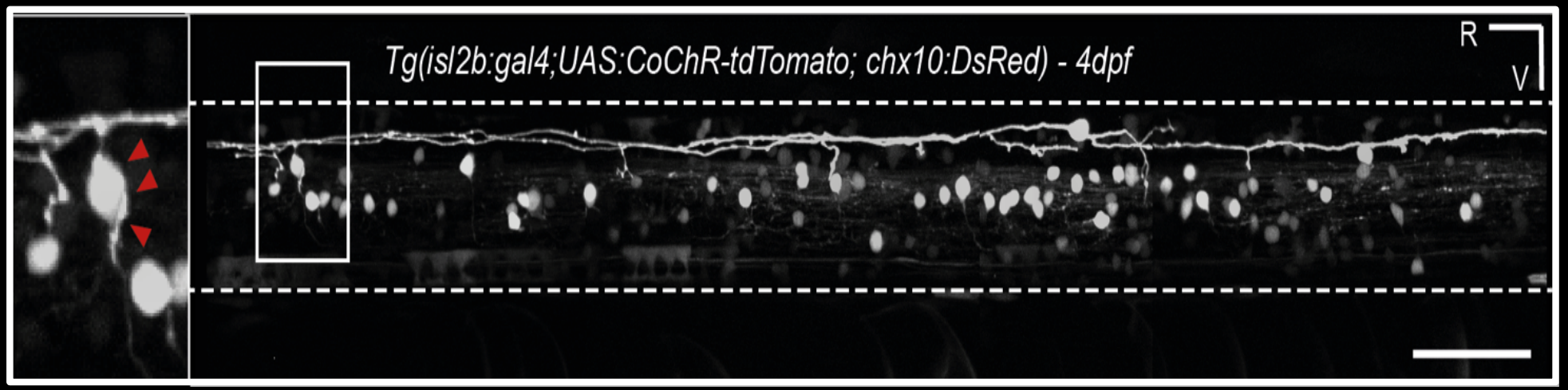
Single cell electrophysiology: Fetcho, McLean, Higashijima, El Manira, …

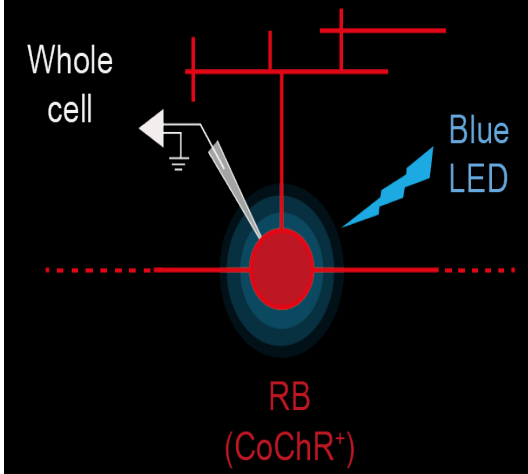
Anatomical projections from mechanosensory neurons onto V2a neurons

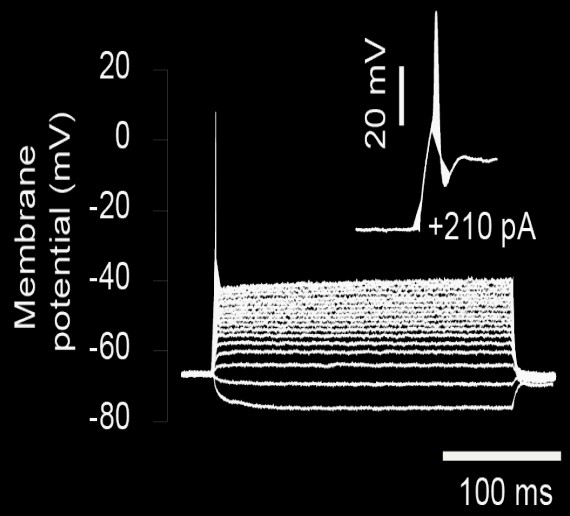


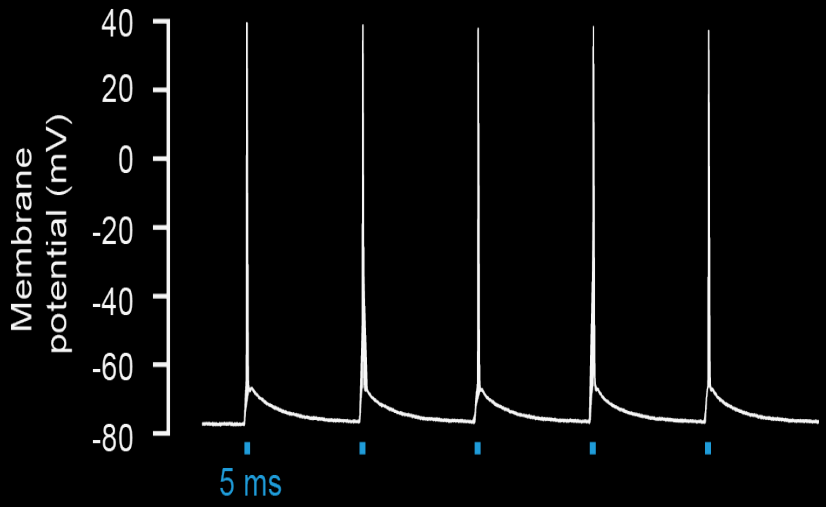
Kevin Fidelin

Testing connections from MS neurons onto V2a involved in fast locomotion



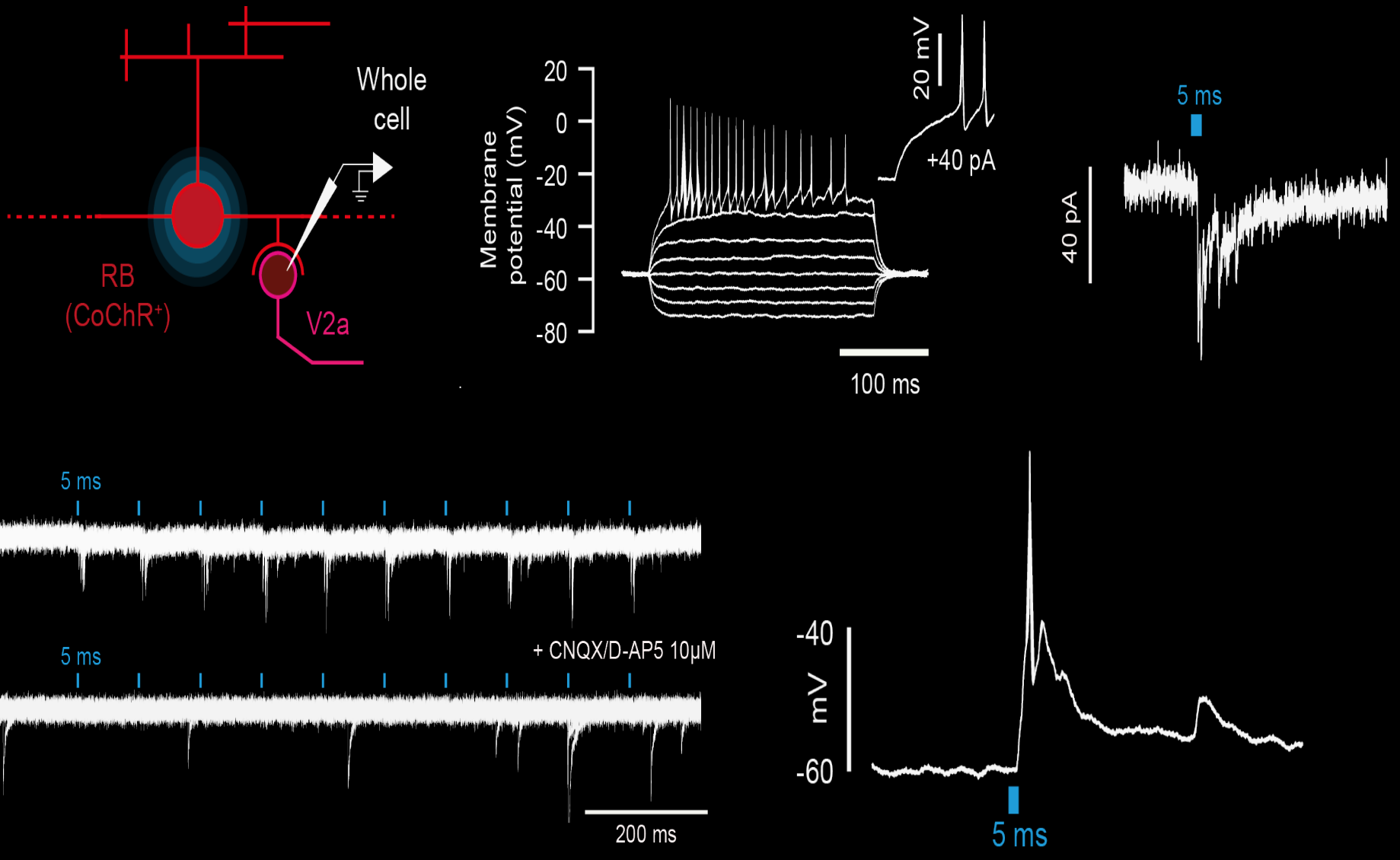






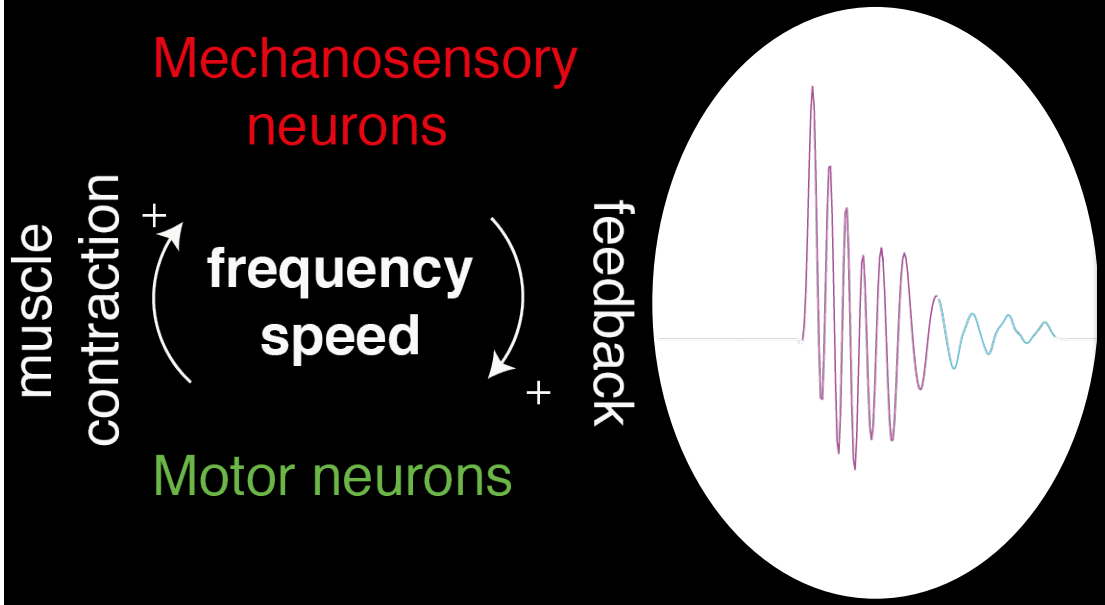
Knafo, Fidelin *et al.*, eLife 2017

Short latency EPSCs elicited by mechanosensory neurons in V2a neurons



Kevin Fidelin

Effect of glutamatergic mechanosensory feedback



Acknowledgements

Urs Böhm
Lydia Djenoune
Kevin Fidelin
Jeff Hubbard
Jenna Sternberg
Olivier Thouvenin
Steven Knafo

Andy Prendergast
Yasmine Cantaut-Belarif
Adeline Orts Del'Imagine
Feng Quan
Julian Roussel
Olivier Mirat
Martin Carbo-Tano
Mathilde Lapoix

Apply we are hiring !

Optics & hardware engineer
Postdoc in electrophysiology

Contact: claire.wyart@icm-institute.org

Spinal Sensory Signalling

Organisation of the online course

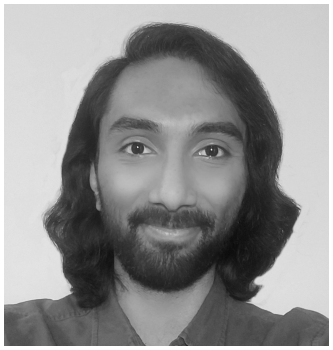
- **Step 1: Kinematics of single locomotor bouts : role of mechanosensory feedback**

Claire Wyart, Paris Brain Institute

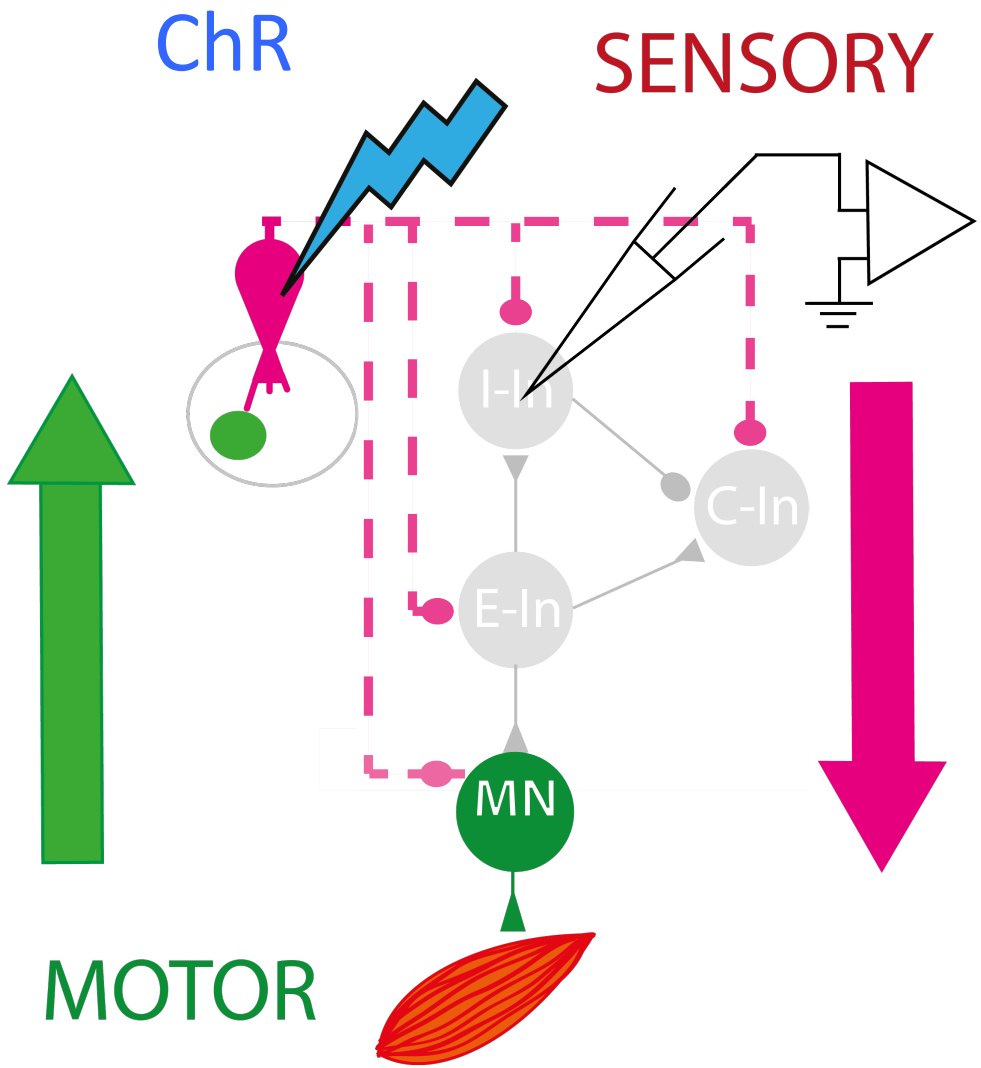
- **Step 2: Segmentation of bout sequences**

Gautam Reddy, Harvard University

Gautam Sridhar, Paris Brain Institute



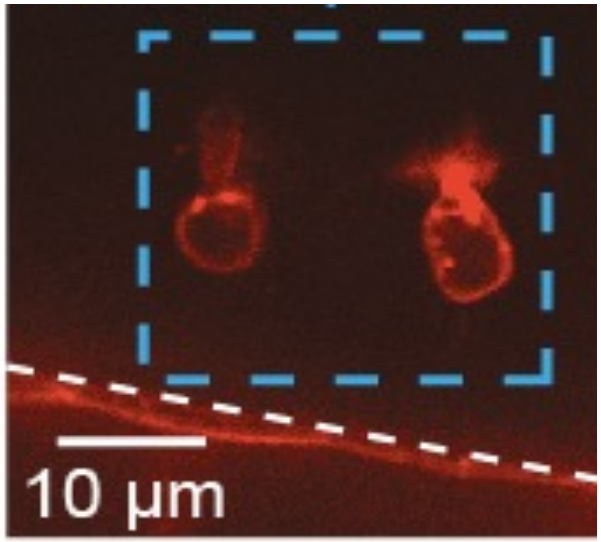
ChR-mediated mapping of connectivity of CSF-contacting neurons in the spinal cord



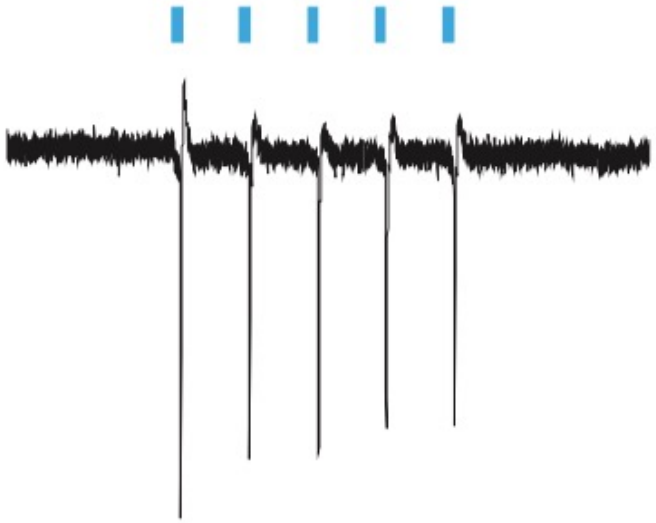
Kevin Fidelin
Jeff Hubbard
Caleb Stokes

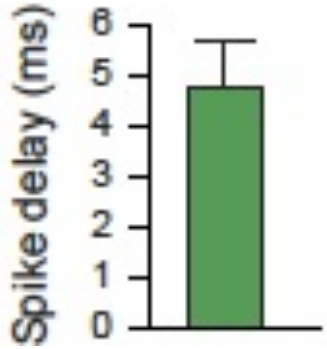
Full field stimulation (LED)

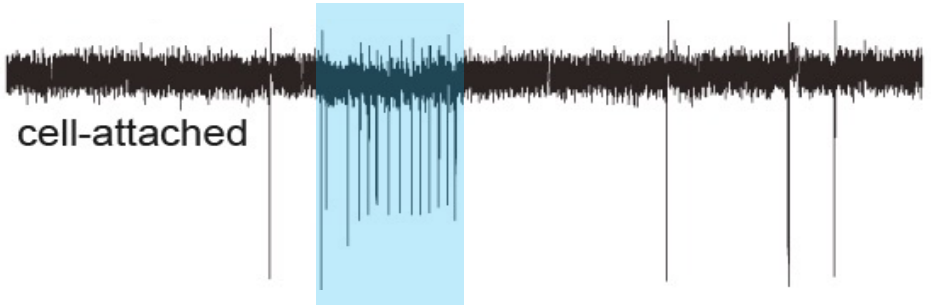
ChannelRhodopsin-mCherry



Loose patch of CSF-cNs

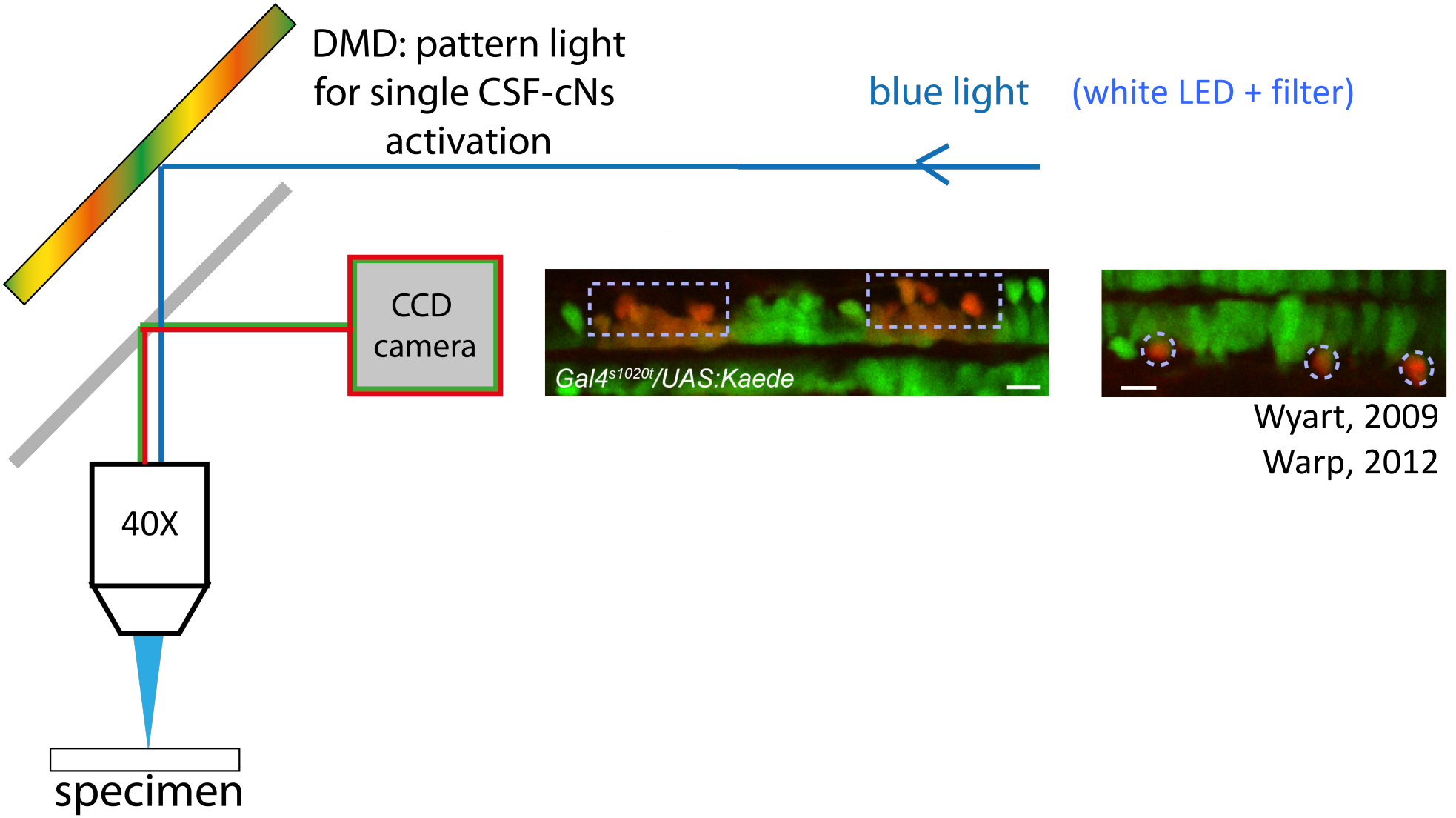






Kevin Fidelin & Caleb Stokes

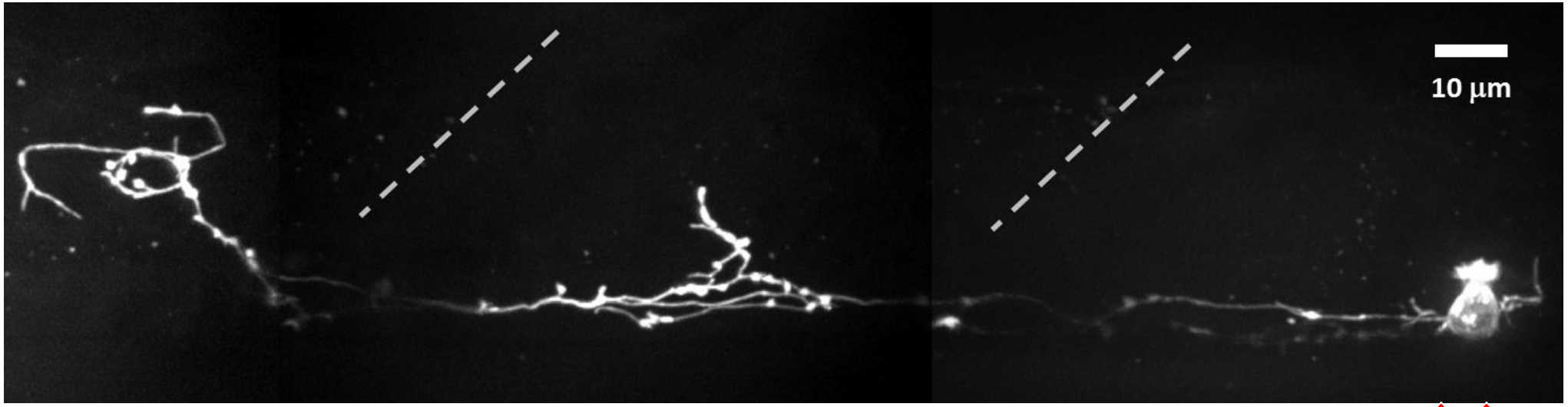
2D light patterning to activate single cells

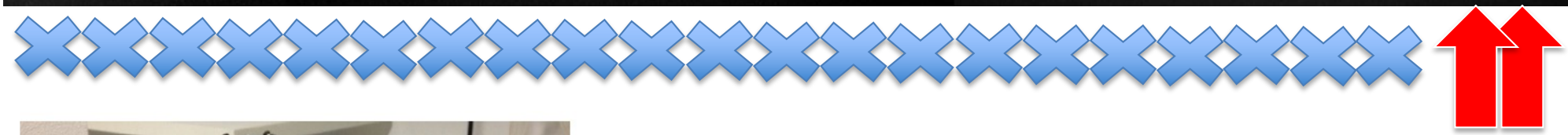


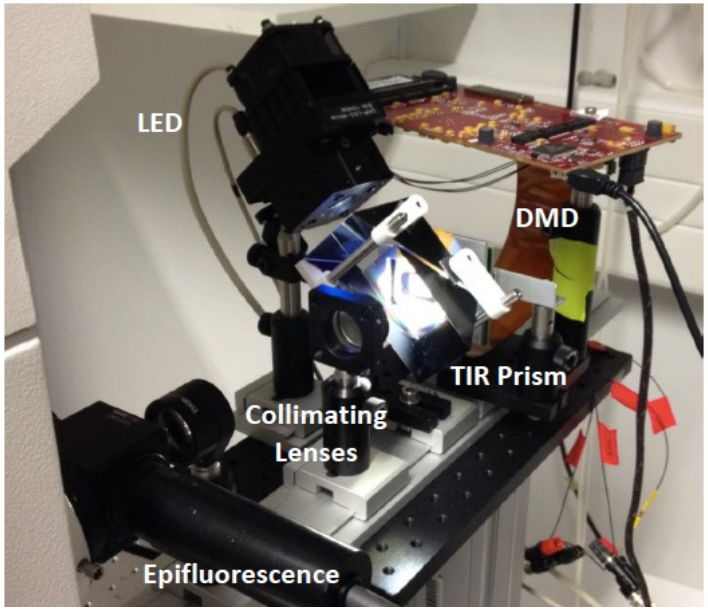
Wyart, 2009
Warp, 2012

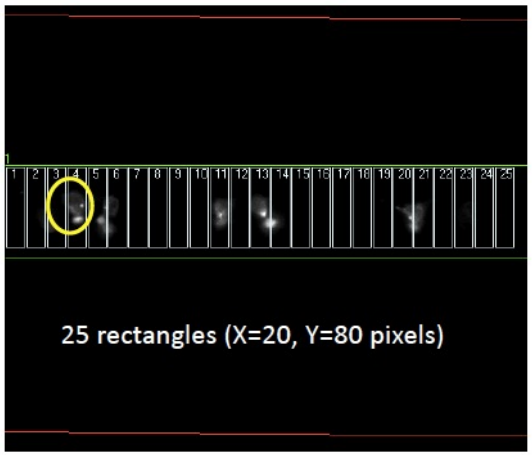
DMD Setup
Urs Böhm

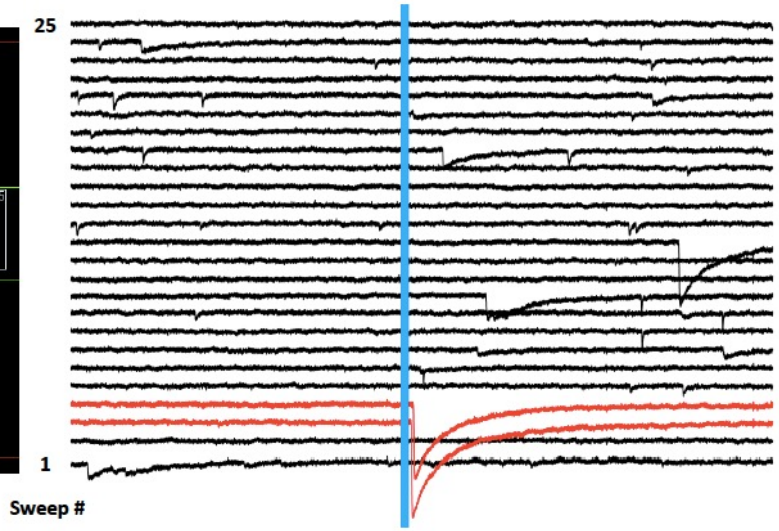
CSF-cN spiking requires illumination of soma or initial segment



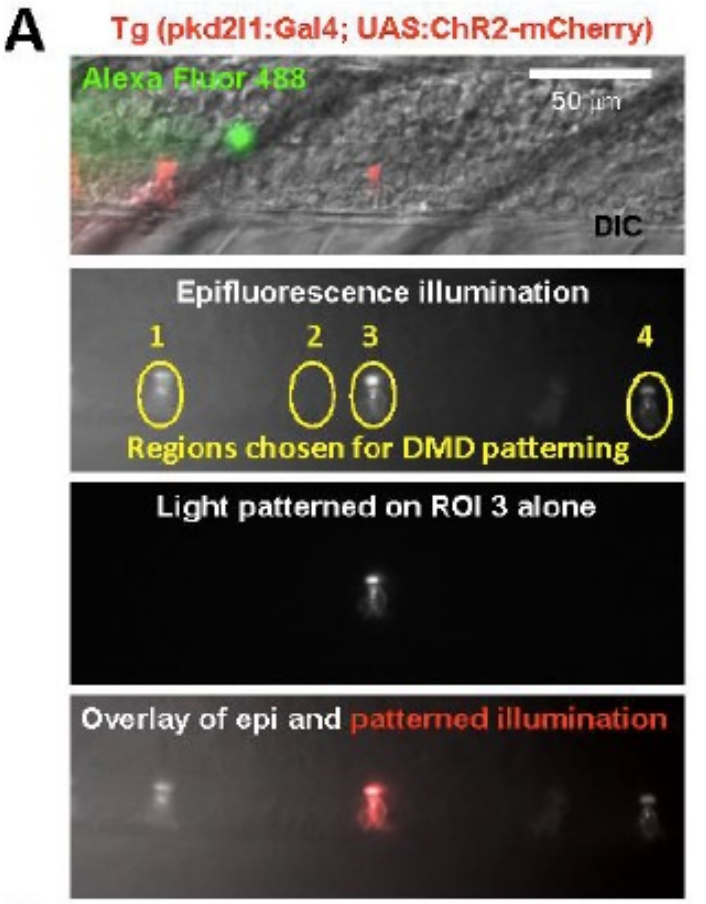


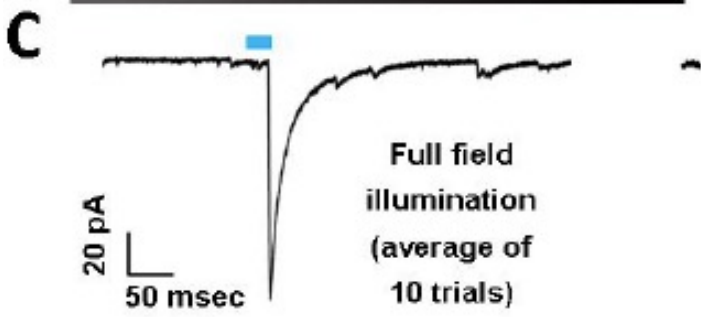






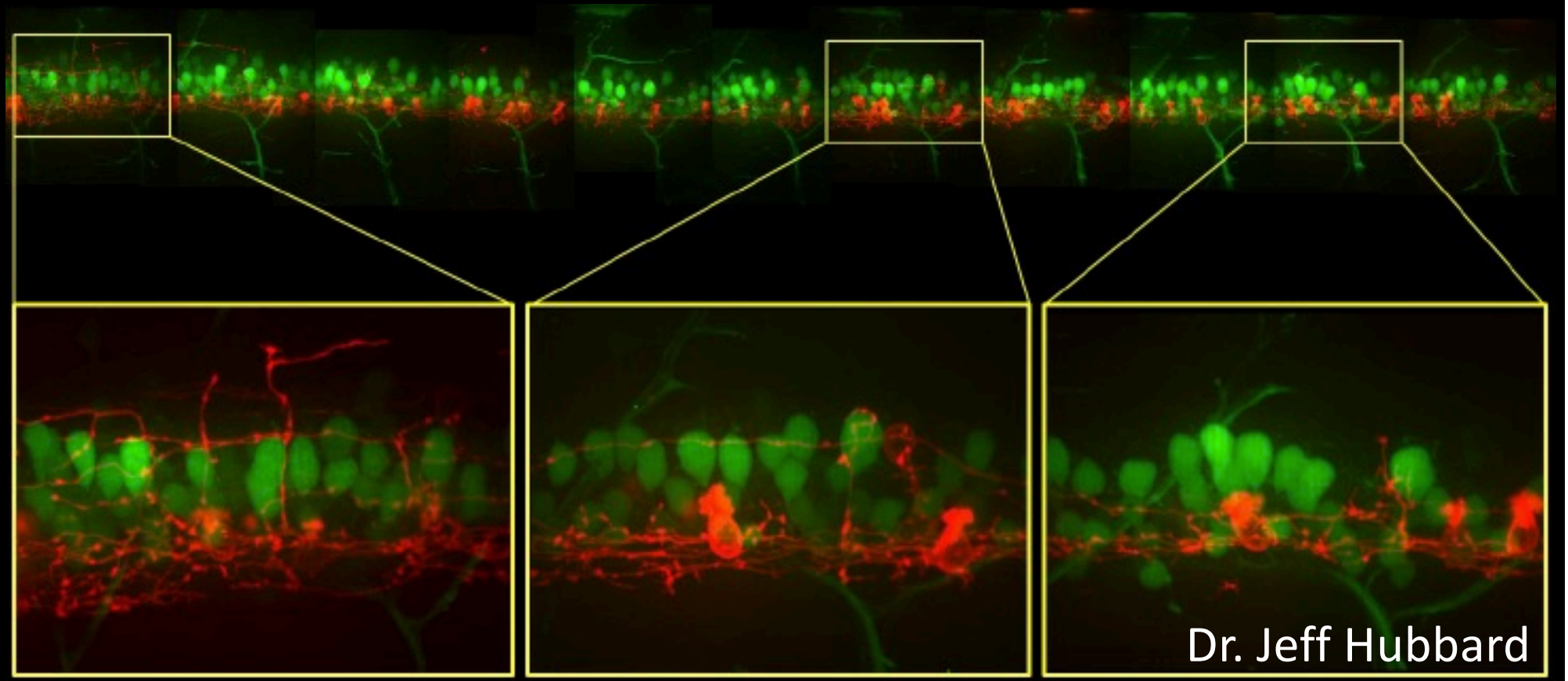
Convergence of multiple inputs onto one target



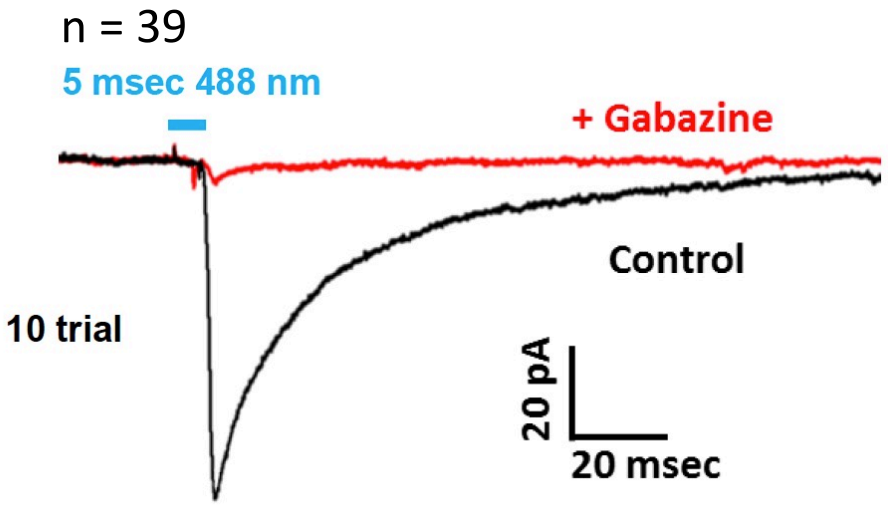


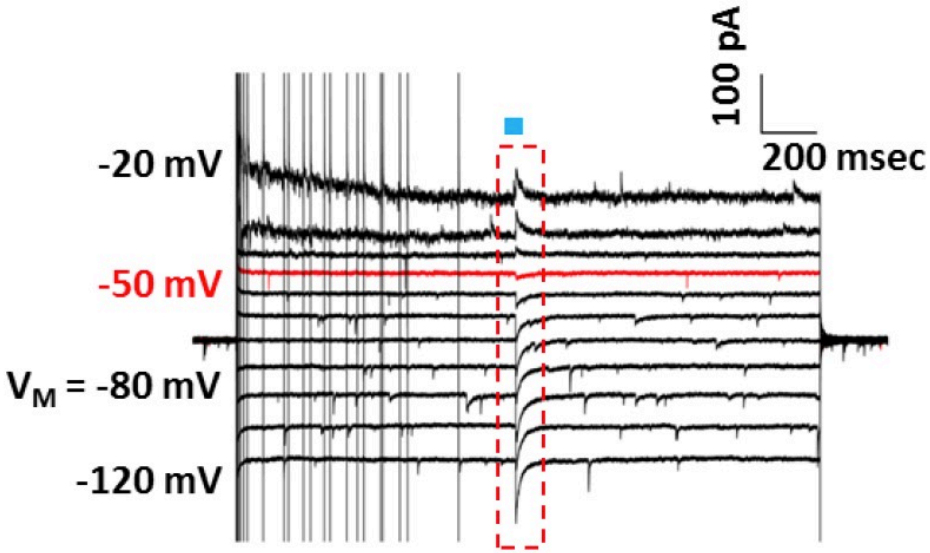
Projection onto motor neurons controlling posture

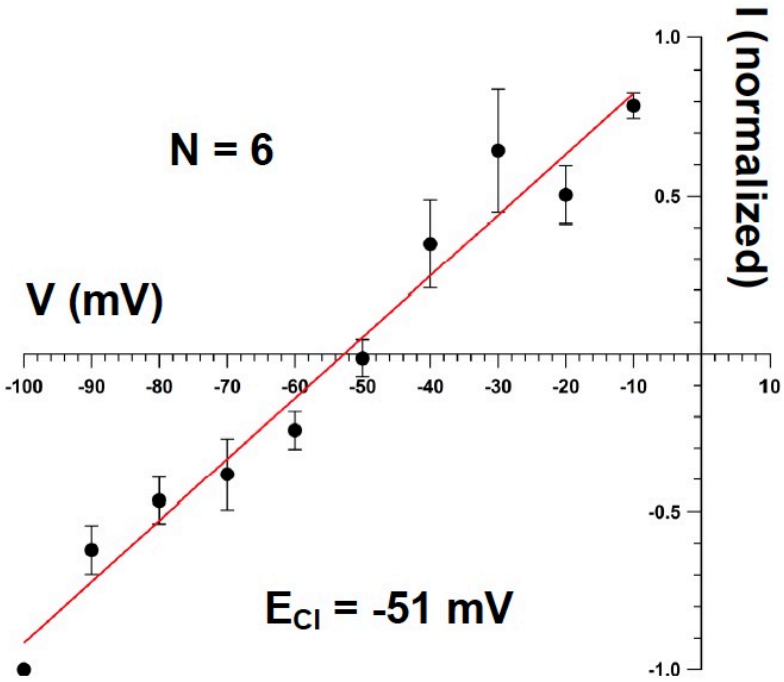
Motor neurons, CSF-cNs

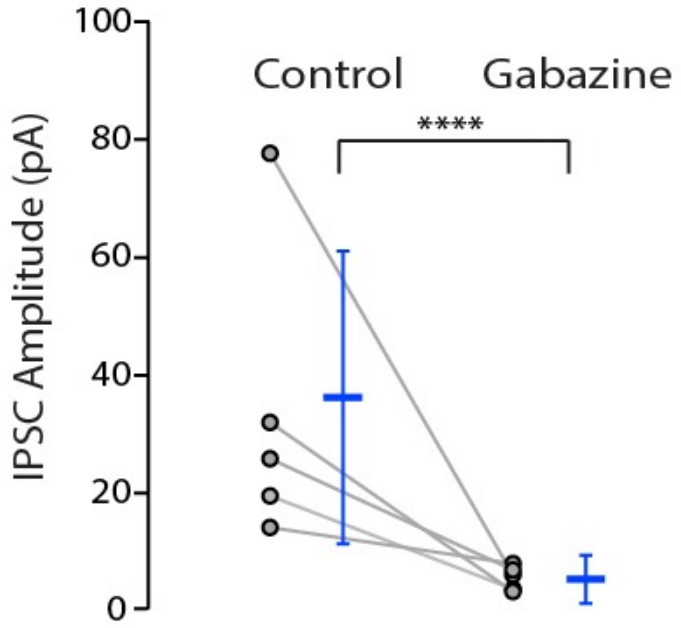


CSF neurons form synapses onto primary motoneurons





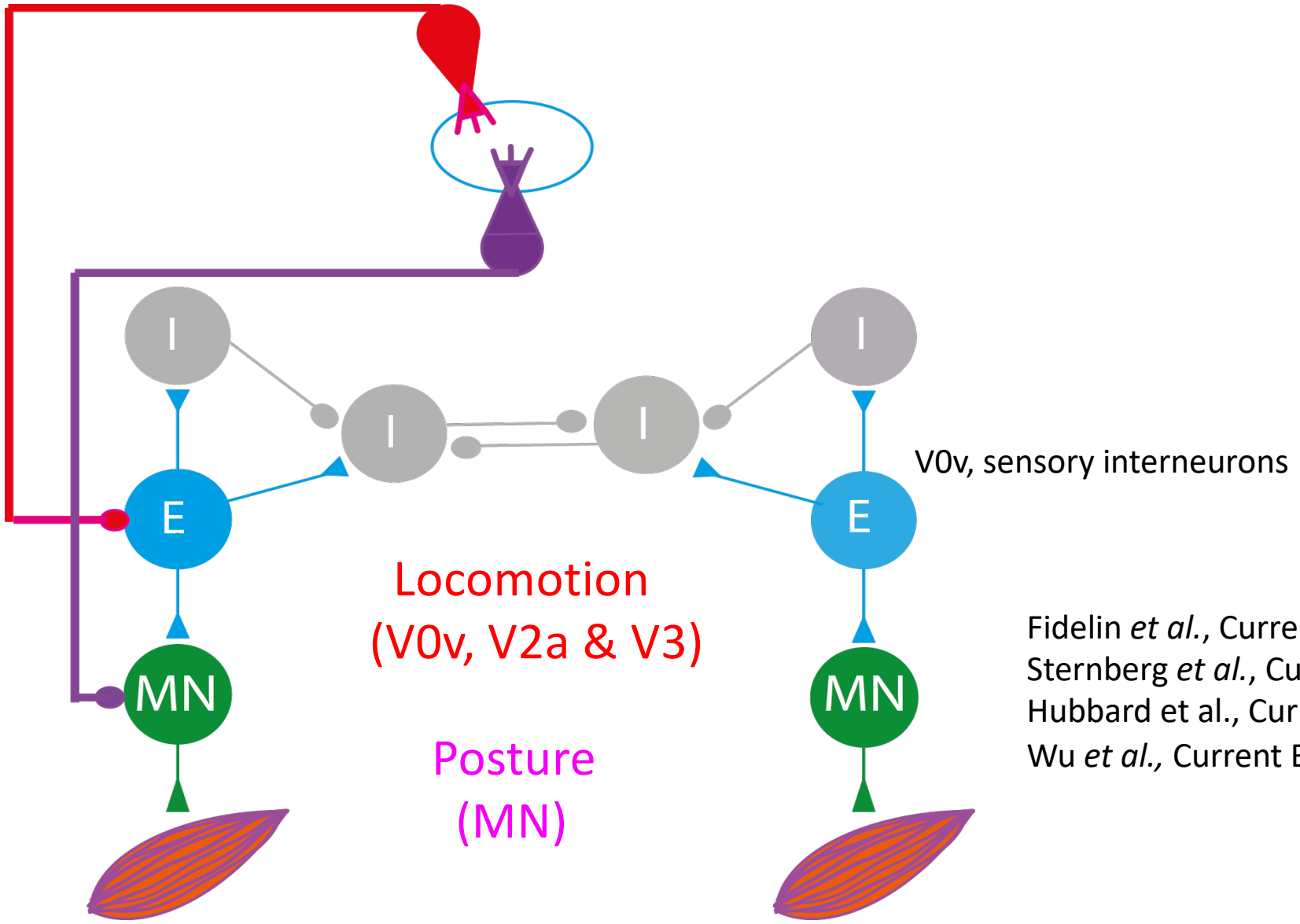




Hubbard *et al., 2016*

Sternberg *et al.,* Current Biology *2016*

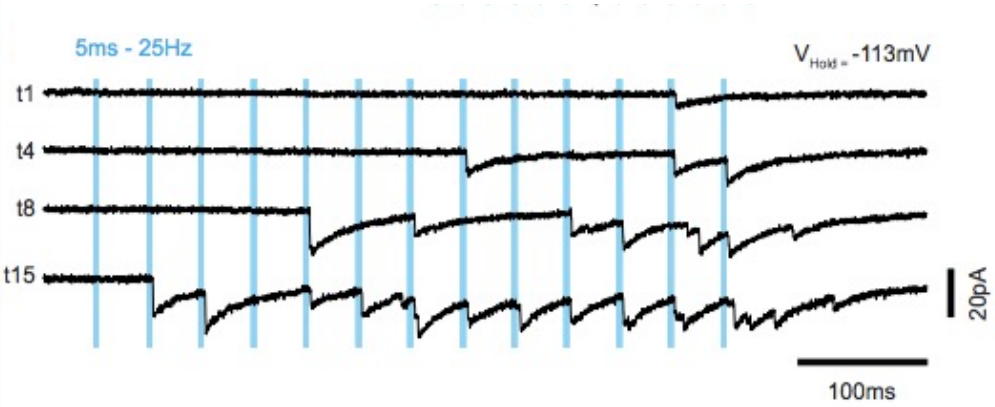
Multiple targets in hindbrain and spinal cord controling locomotion, speed & posture

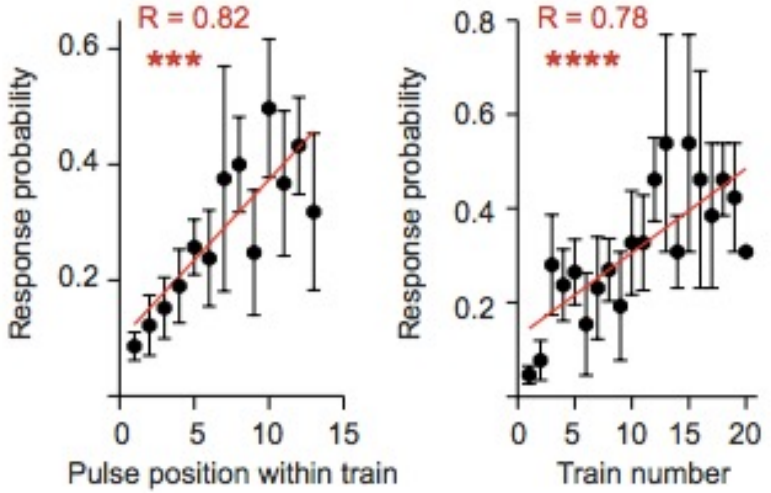


Fidelin *et al.*, Current Biology 2015
Sternberg *et al.*, Current Biology 2016
Hubbard et al., Current Biology 2016
Wu *et al.*, Current Biology 2021

Short term plasticity differs between synapses formed onto slow and fast circuits

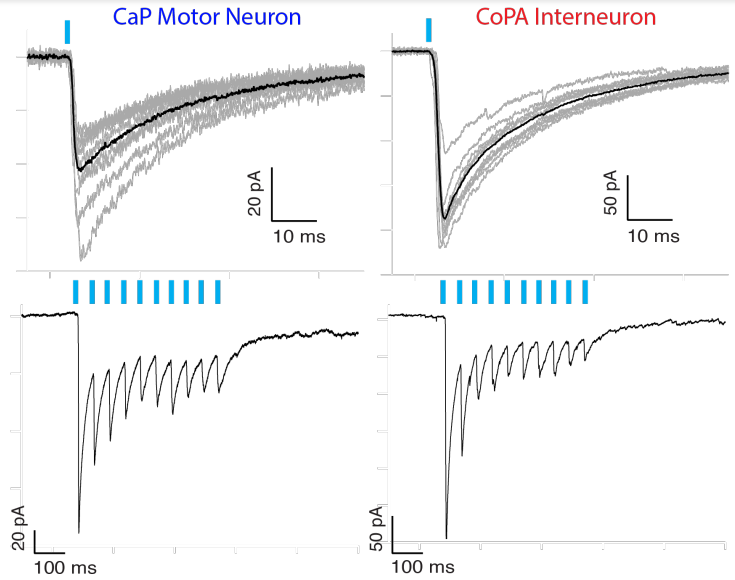
SLOW

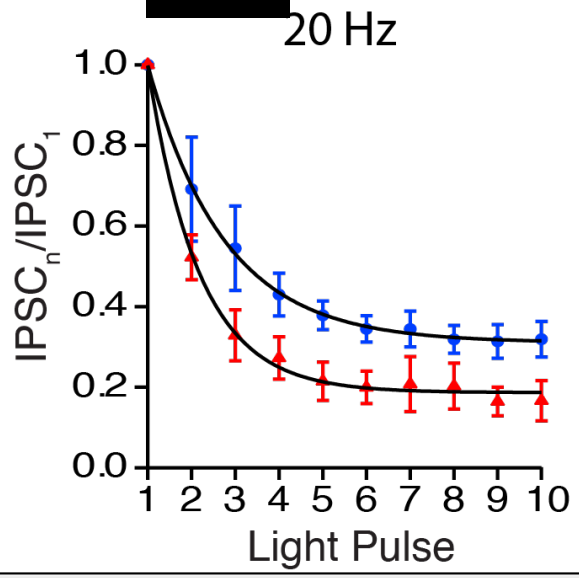




Fidelin *et al.*, Current Biology 2015

FAST





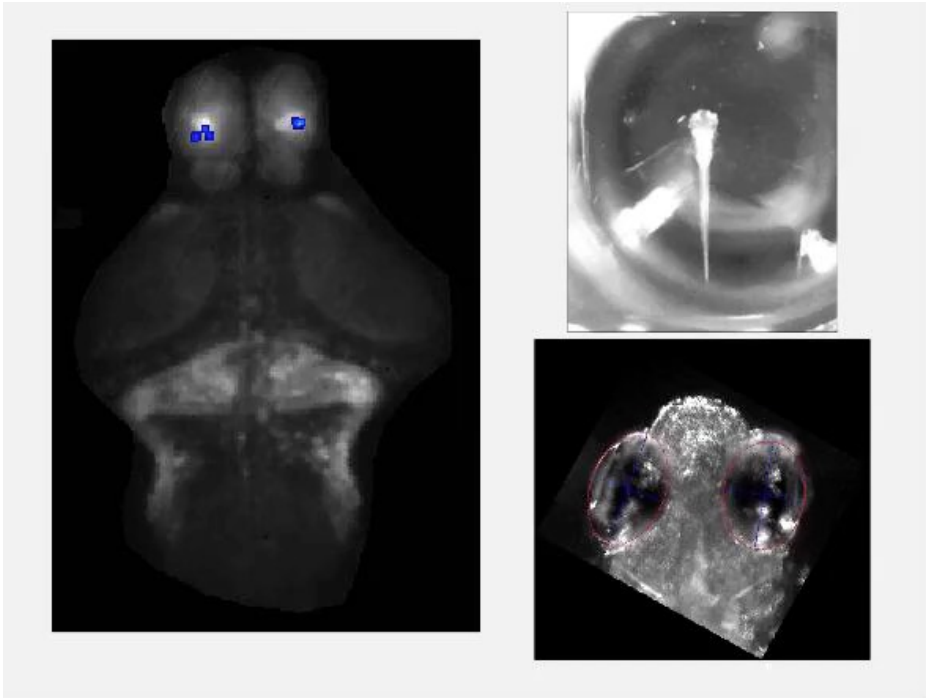
Hubbard *et al.*, Current Biology 2016

A combination of tools for recording & manipulation of neuronal activity in vivo

Perspectives: Control of an integrated behavior via a minimal circuit

Exploration

Prey Capture



Oldfield *et al.,* eLife 2020

Acknowledgements

Urs Böhm
Lydia Djenoune
Kevin Fidelin
Jeff Hubbard
Jenna Sternberg
Olivier Thouvenin
Steven Knafo

Andy Prendergast
Yasmine Cantaut-Belarif
Adeline Orts Del'Imagine
Feng Quan
Julian Roussel
Olivier Mirat
Martin Carbo-Tano
Mathilde Lapoix

Apply we are hiring !

Optics & hardware engineer
Postdoc in electrophysiology

Contact: claire.wyart@icm-institute.org

Spinal Sensory Signalling