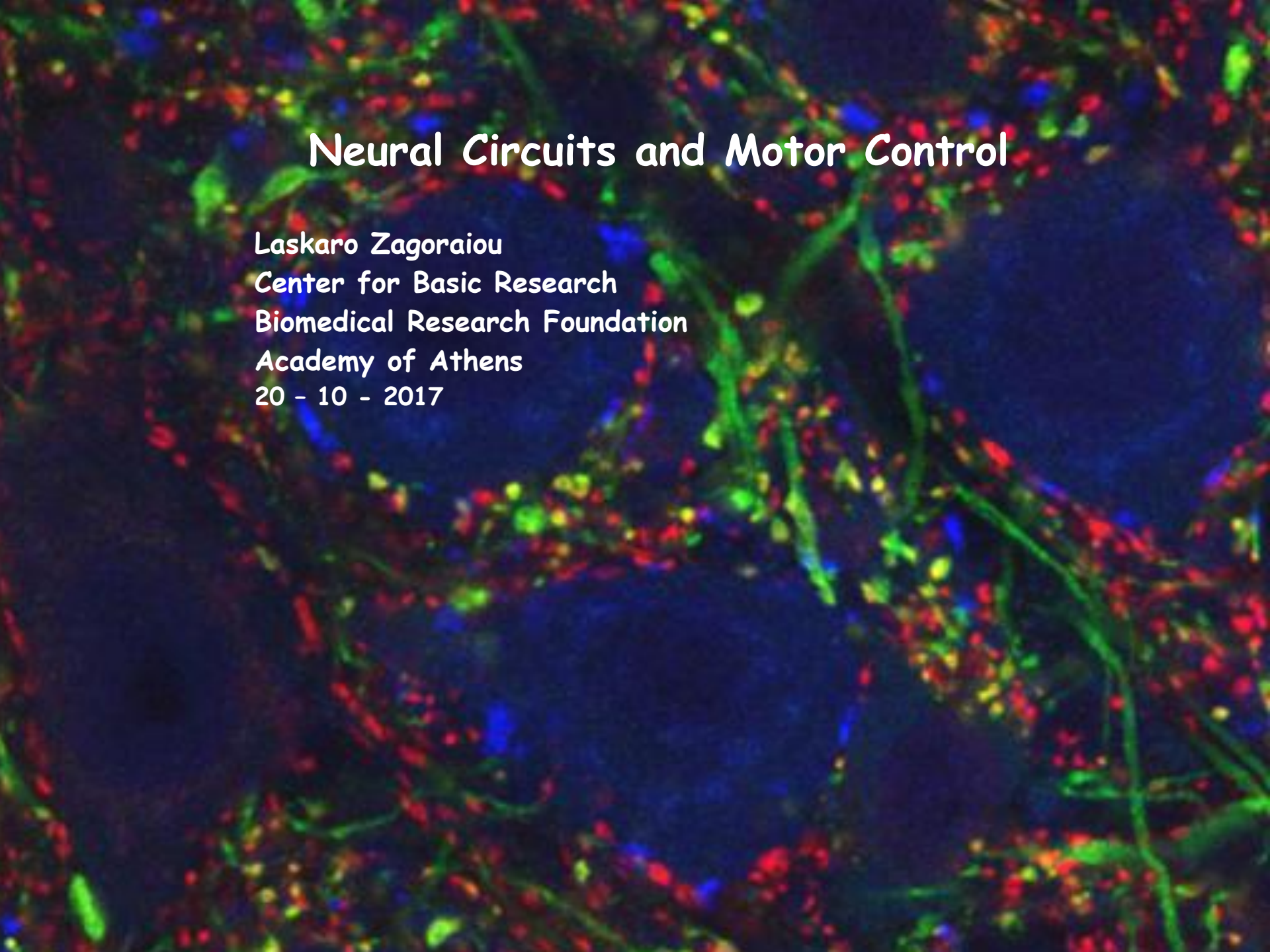
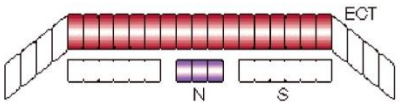


Neural Circuits and Motor Control

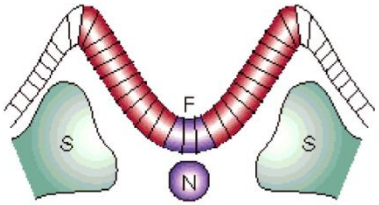
Laskaro Zagoraiou
Center for Basic Research
Biomedical Research Foundation
Academy of Athens
20 – 10 - 2017

Inductive signals generate the D/V cell type diversity in vertebrate neural tube

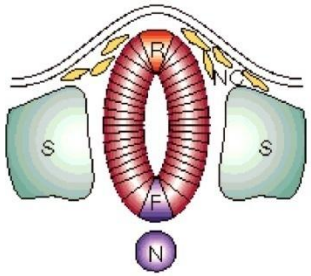
Neural Plate



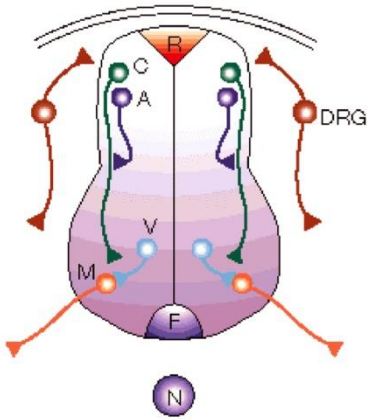
Neural Fold



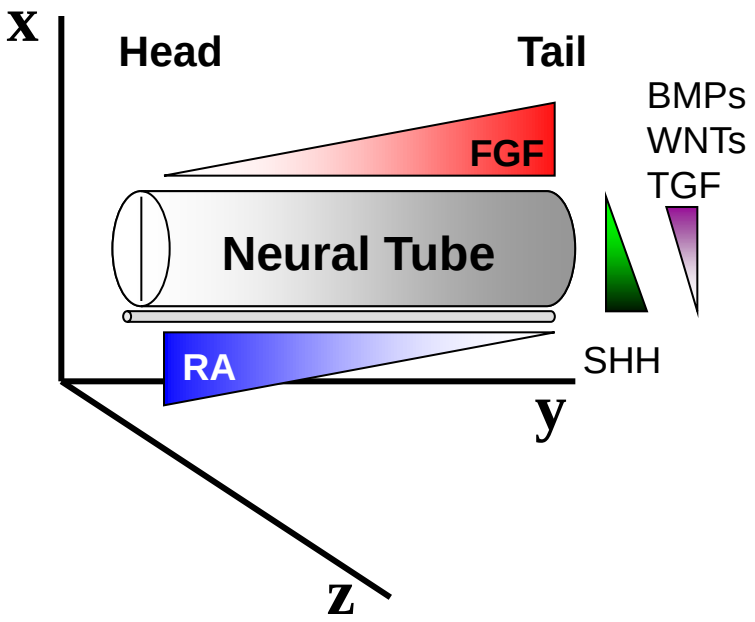
Neural Tube



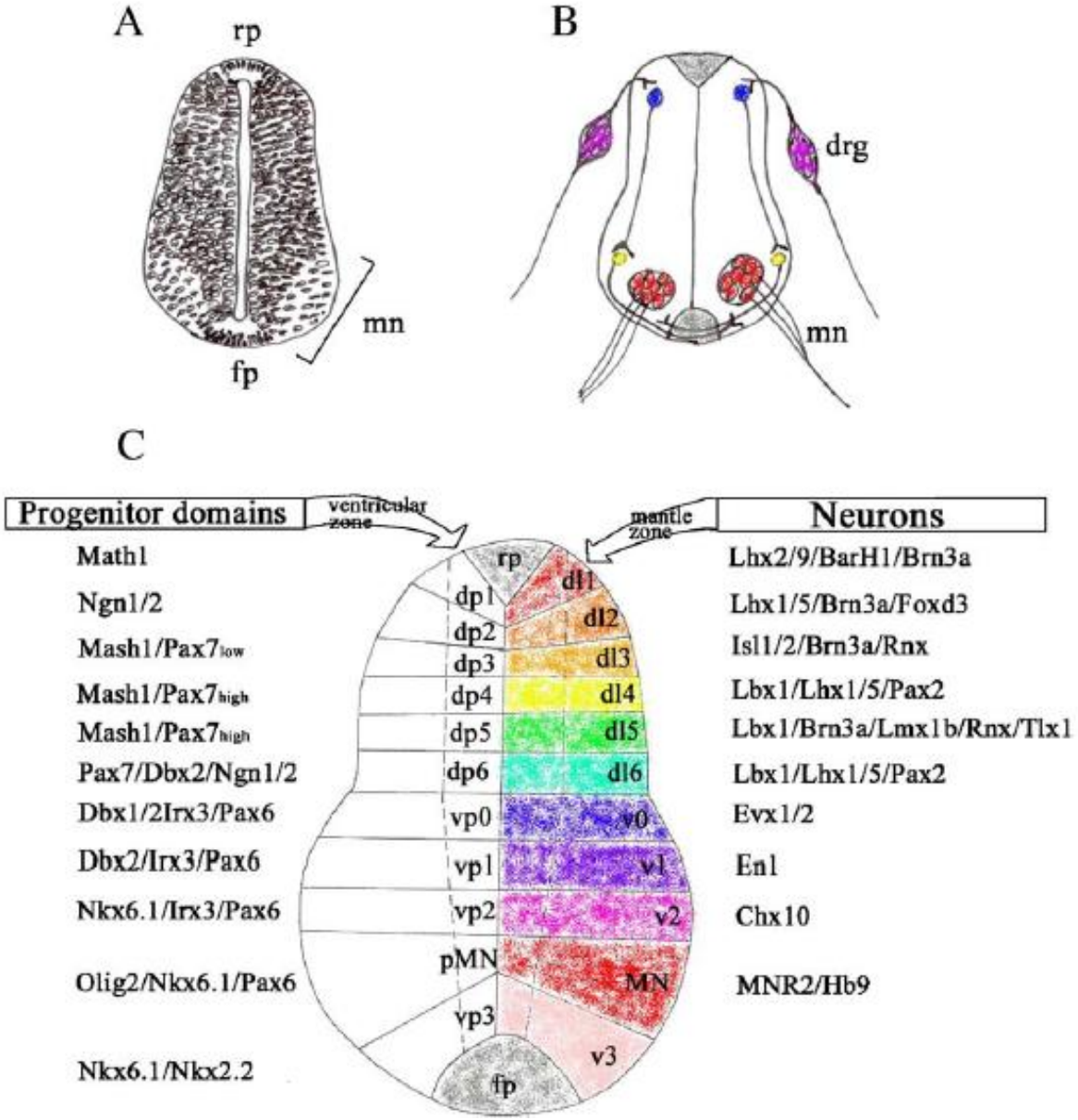
Spinal Cord



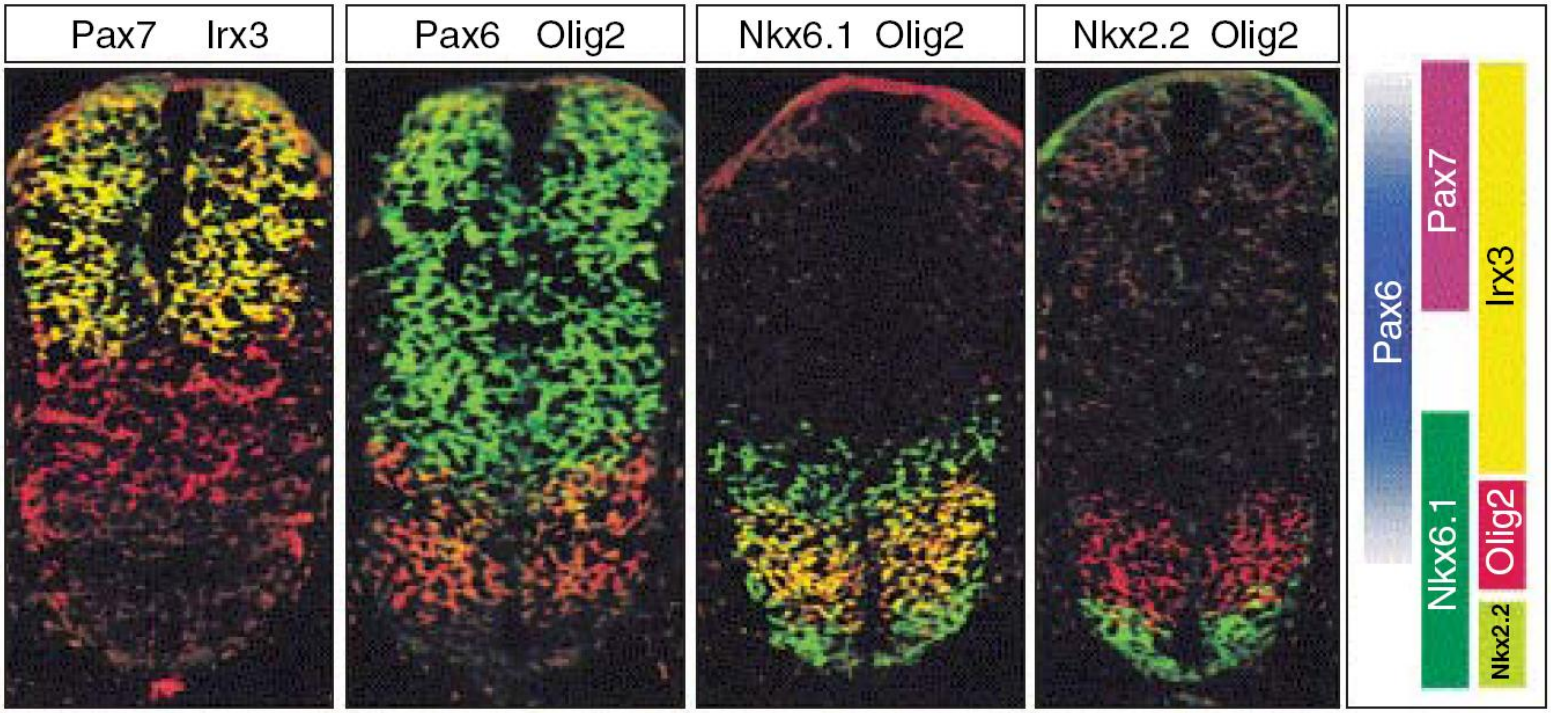
Graded signaling of extrinsic morphogenetic molecules defines the identity of each progenitor cell in D/V and rostro-caudal axes



Generation of Neuronal diversity in the vertebrate neural tube

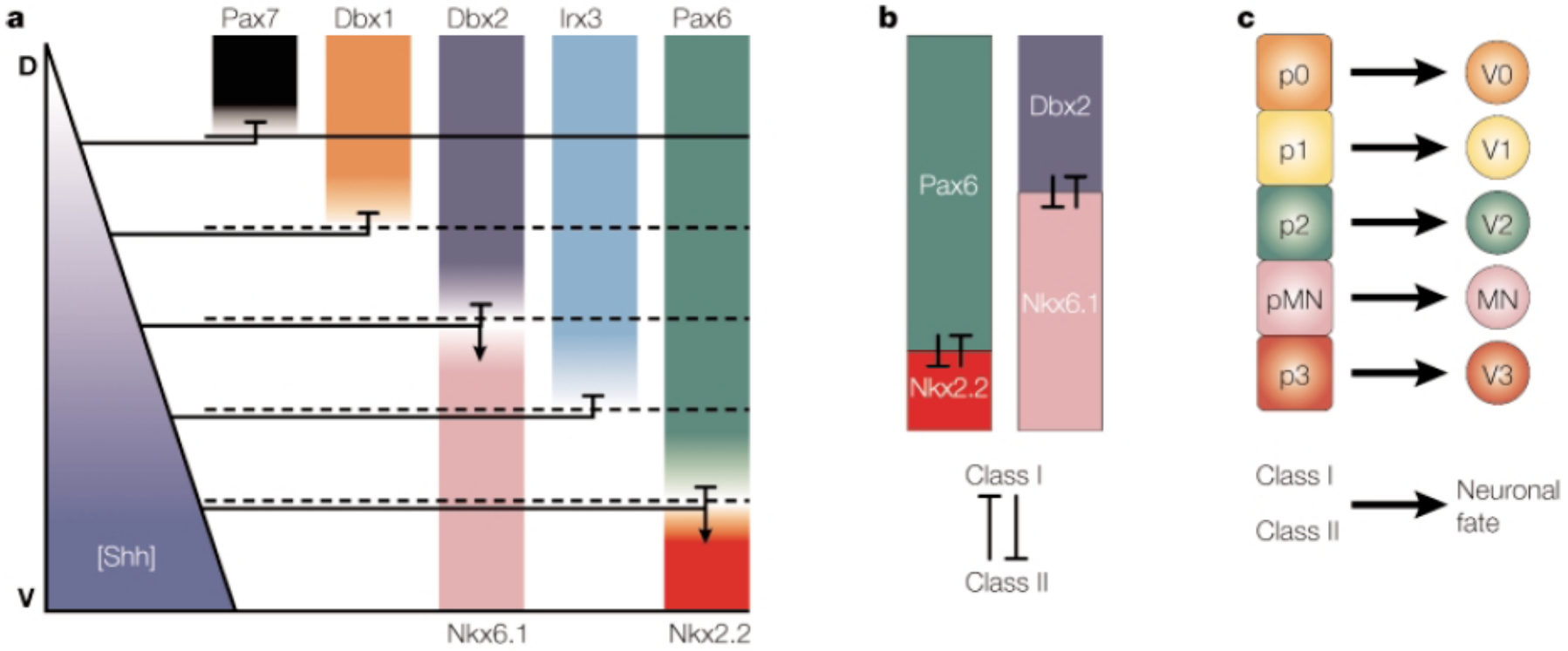


Each progenitor domain is defined by its position on the D/V axis and generates distinct cell types that express different HD factors

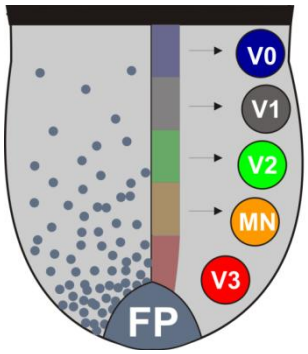


How neural progenitor cells interpret graded Shh signals?

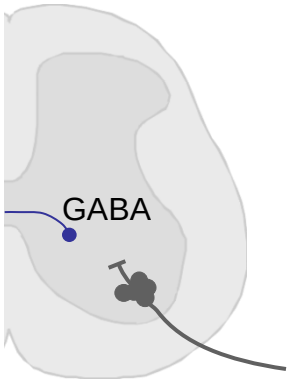
- A group of homeodomain proteins expressed by ventral progenitor cells act as intermediary factors in the interpretation of graded Shh signalling



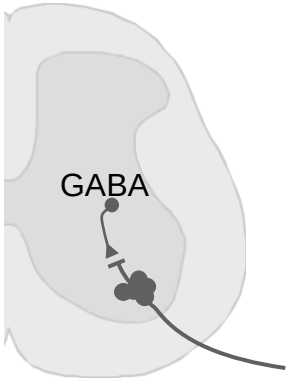
Marking subsets of interneurons by transcription factor expression



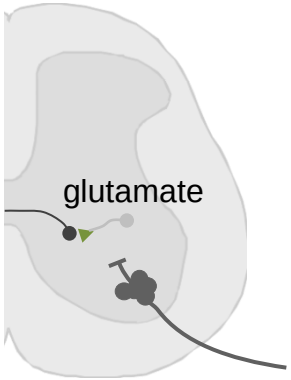
V0v: **Evx1**



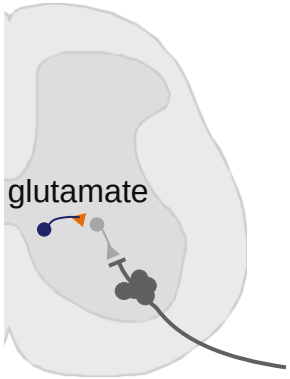
V1: **En1**

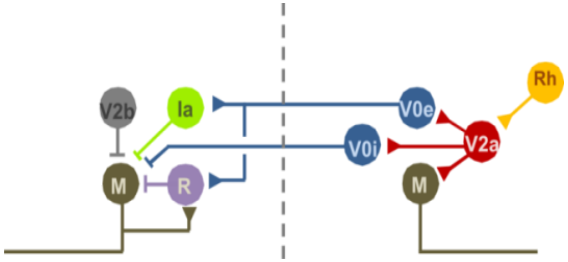


V2a: **Sox14**



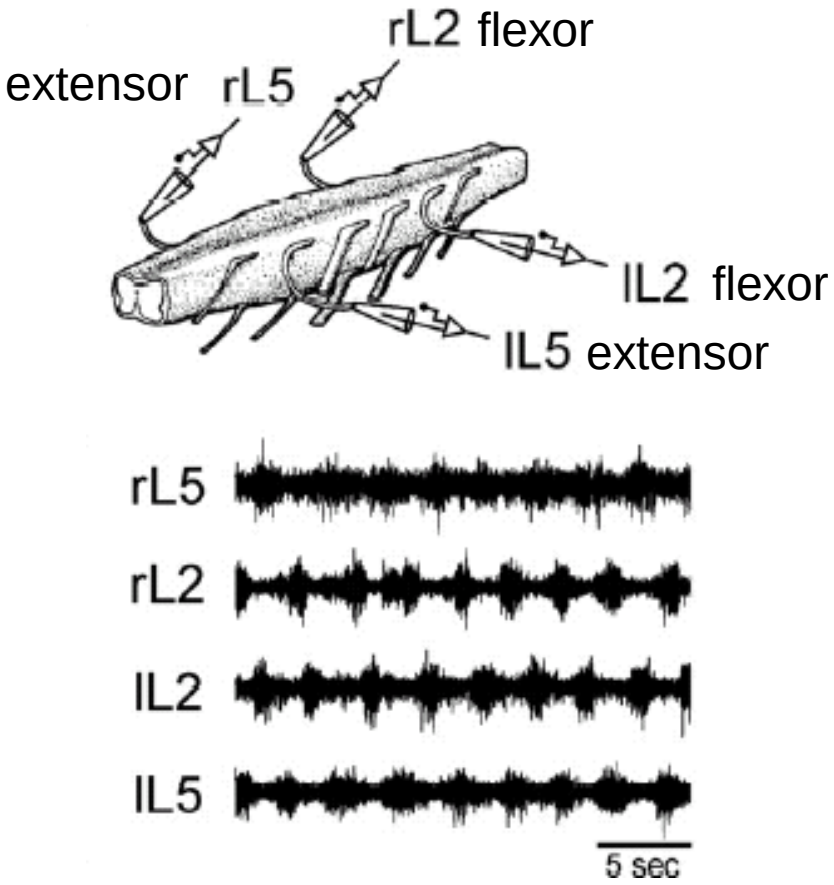
Vx: **Hb9**





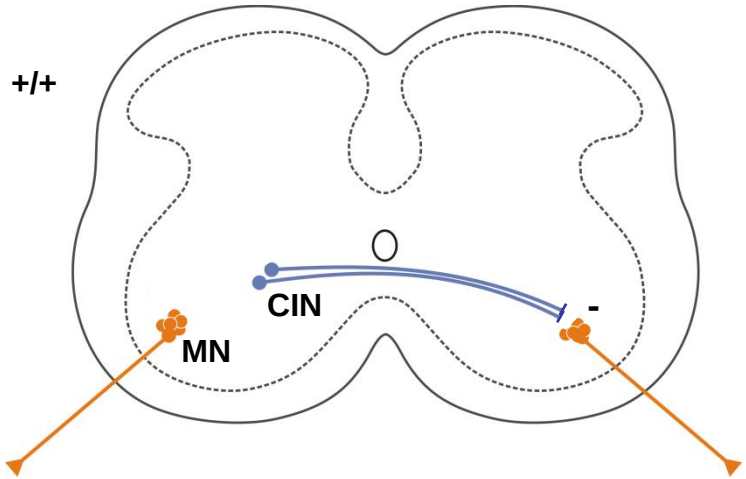
Zagoraiou, Pierani, Kiehn, Sharma,
Goulding, Brownstone, Harris-Warwick

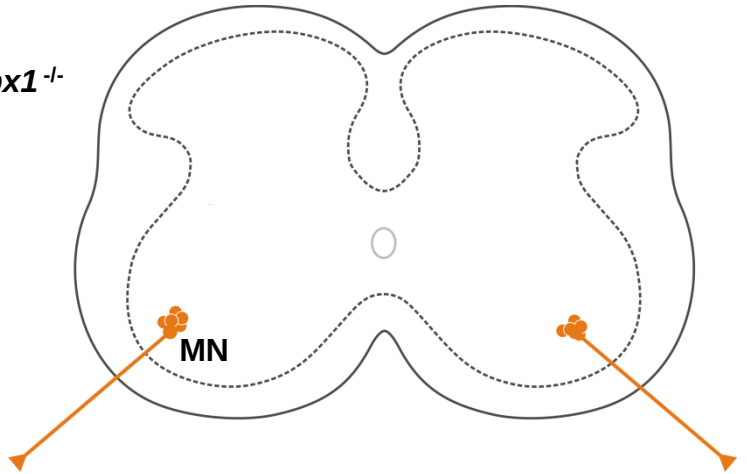
In vitro locomotion

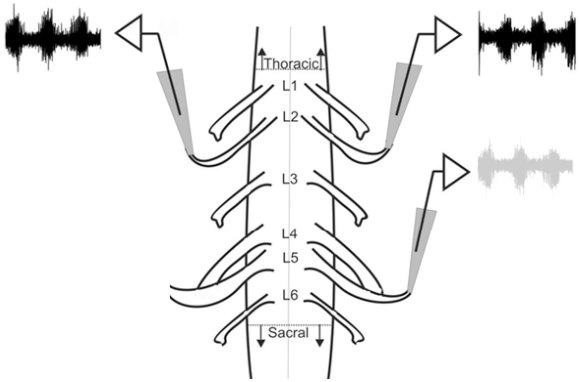


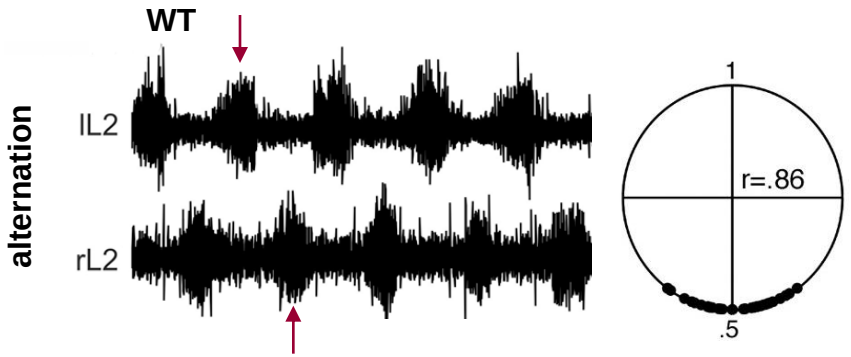
Kiehn lab

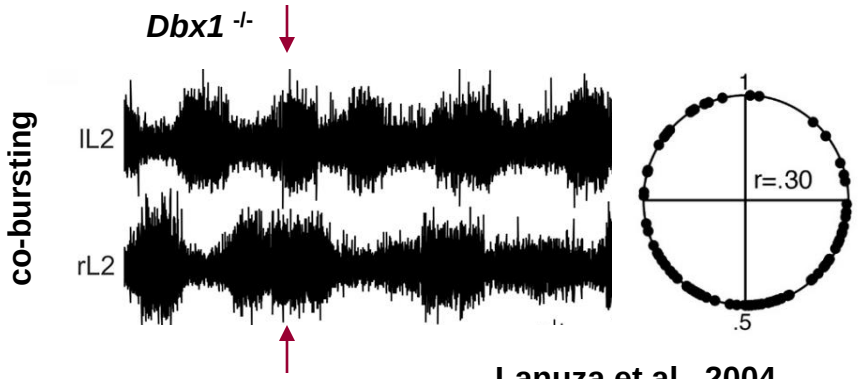
Loss of left-right alternation in *Dbx1*$^{-/-}$ mutant mice





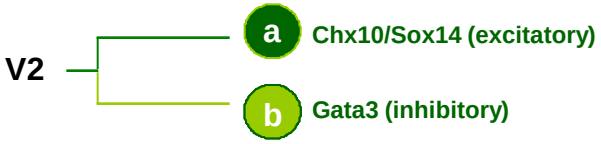


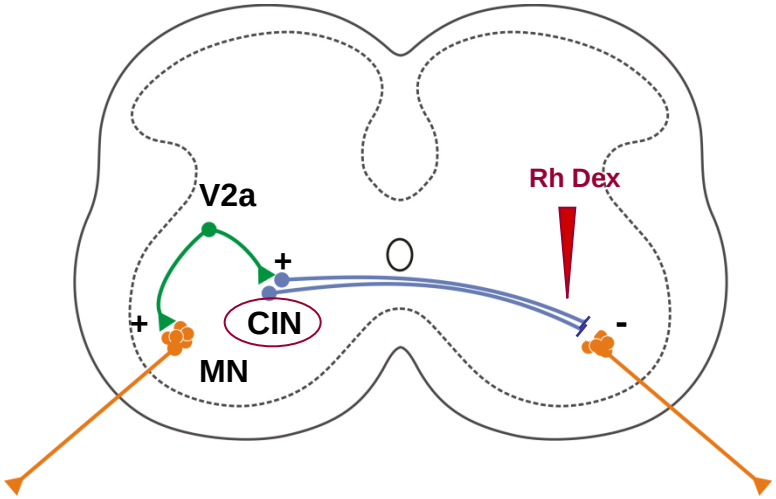


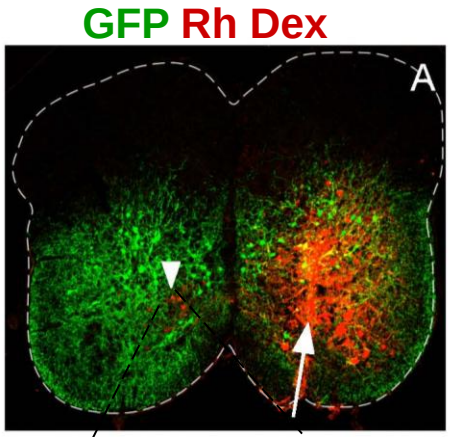


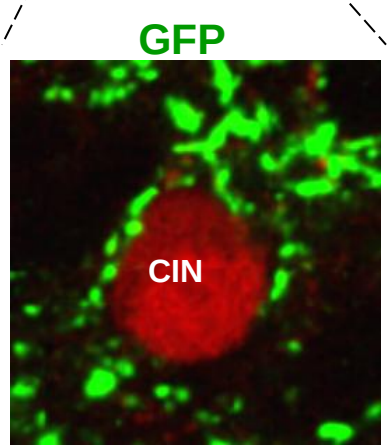
Lanuza et al, 2004

Mapping the projections of V2a interneurons



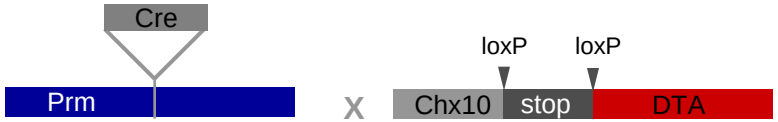


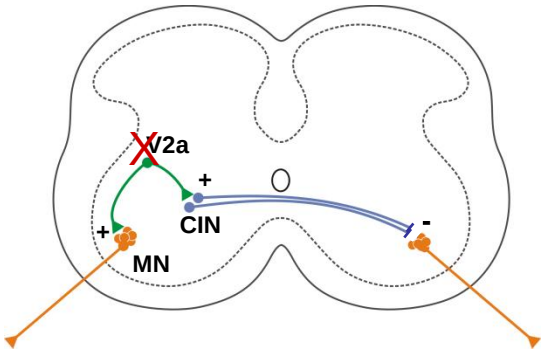


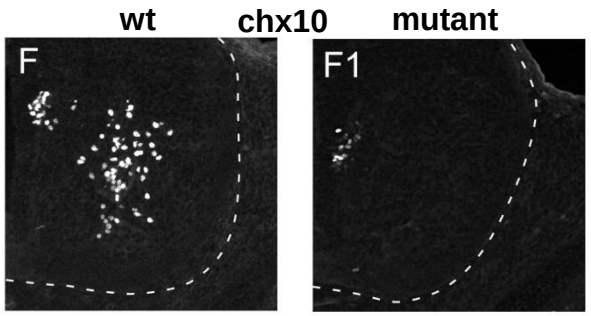


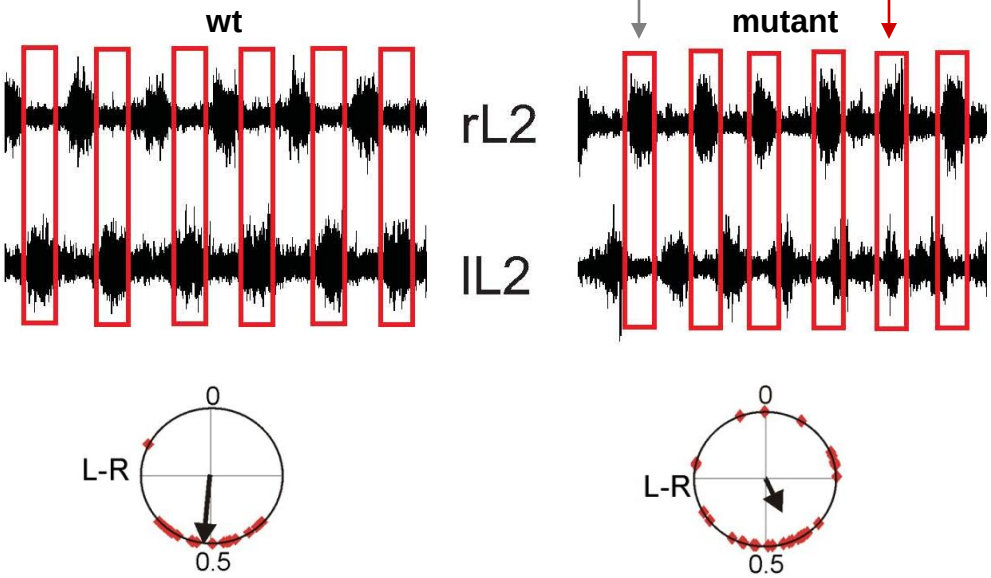
Crone et al., 2008

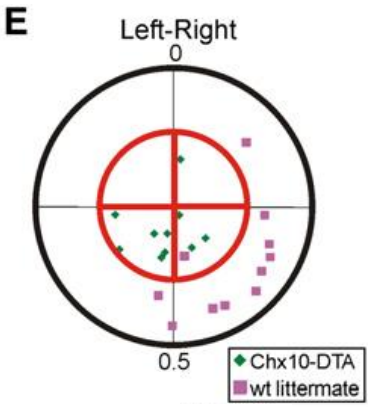
Genetic ablation of V2a INTs decouples left-right locomotor activity







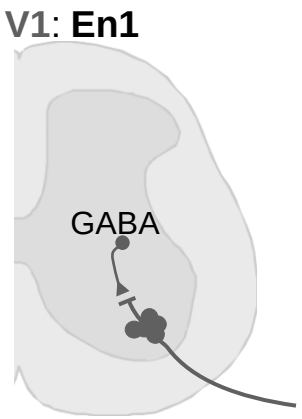


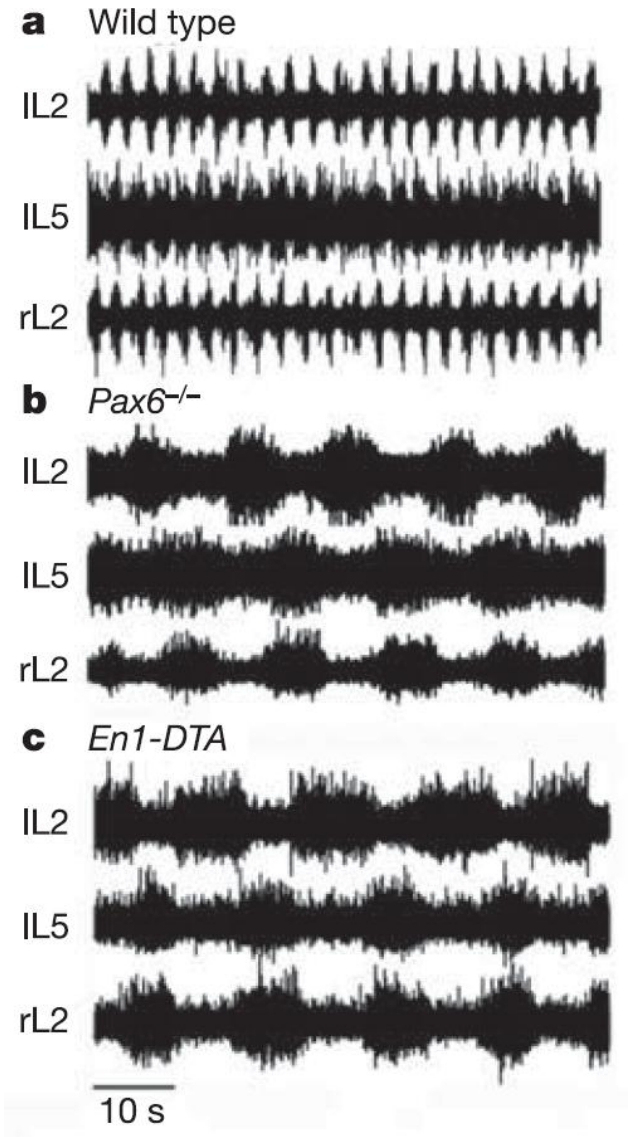


Crone et al., 2008

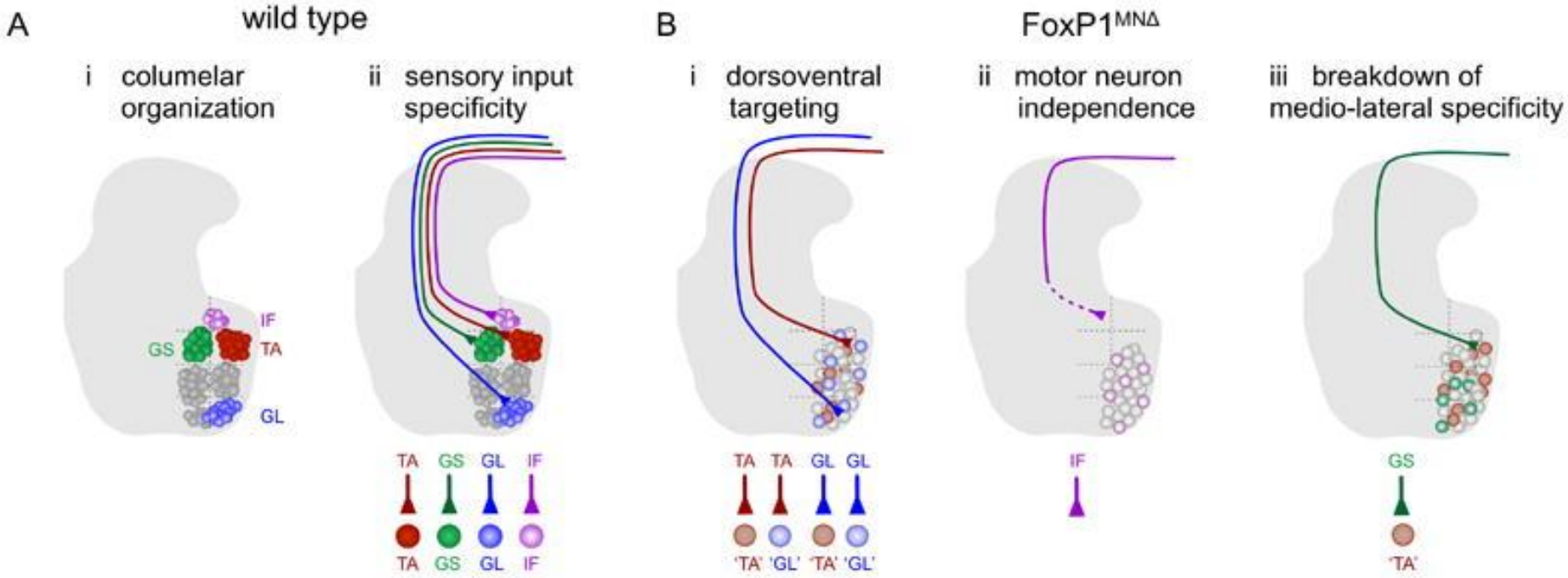
Ipsilateral inhibitory interneurons and locomotor speed:

- A population of ipsilaterally projecting inhibitory neurons (**V1 interneurons**) can be defined by the expression of the developmental genes Pax6 and En1.
- When V1 interneurons are deleted locomotor burst duration increases and the locomotor rhythm slows down.
- Thus, V1 interneurons appear to be involved in controlling the speed of locomotion.



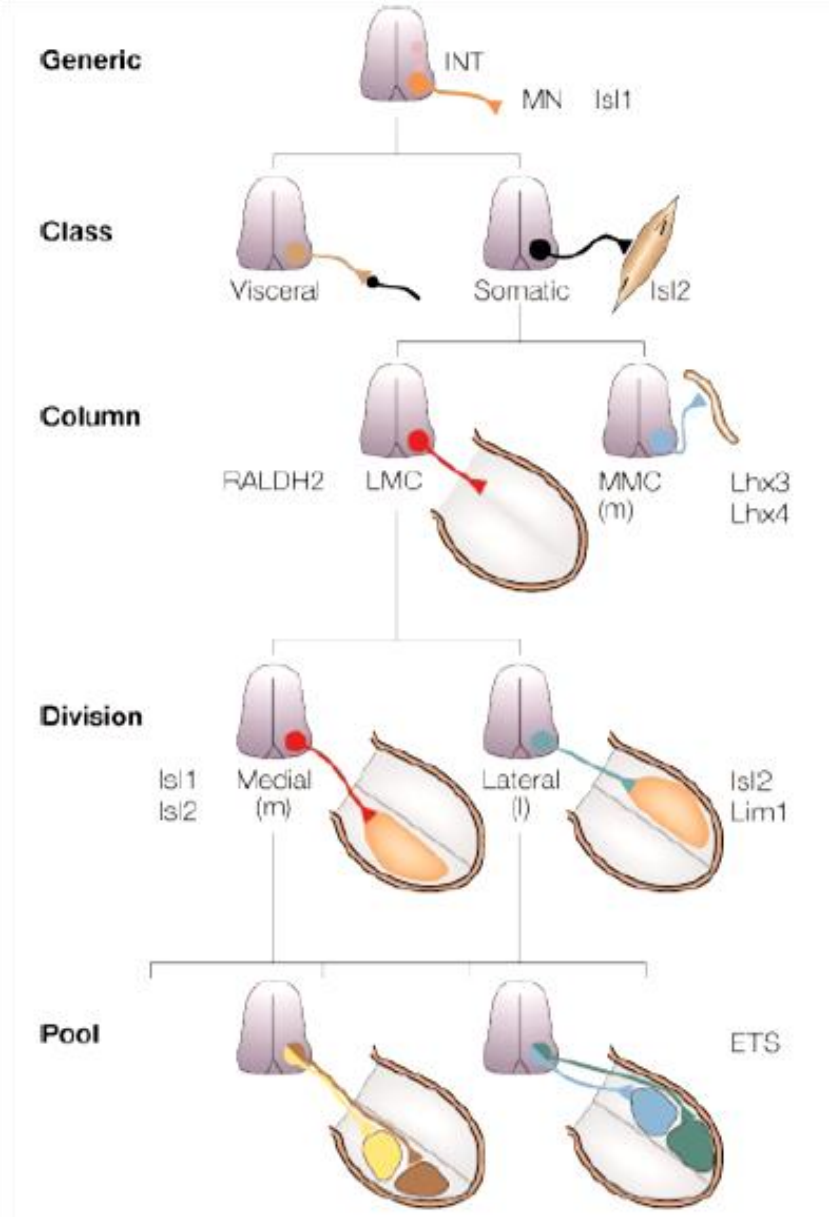


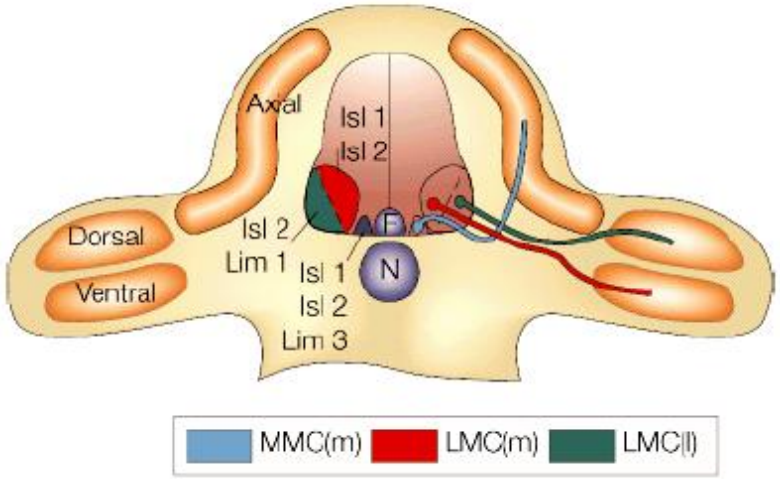
Patterns of spinal sensory-motor connectivity prescribed by a dorsoventral positional template



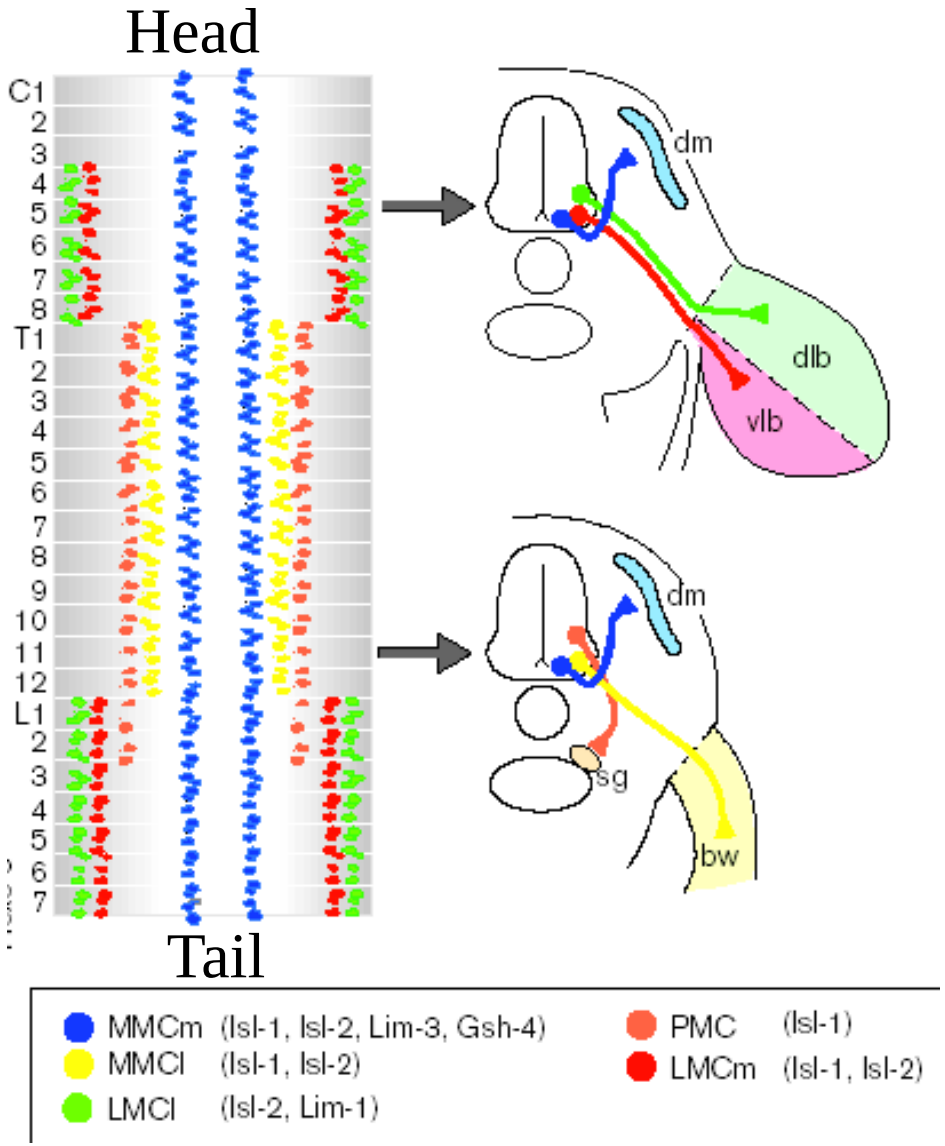
Surmeli et. al. 2011

Motor Neurons acquire subtype identities to generate Motor neuron Columns



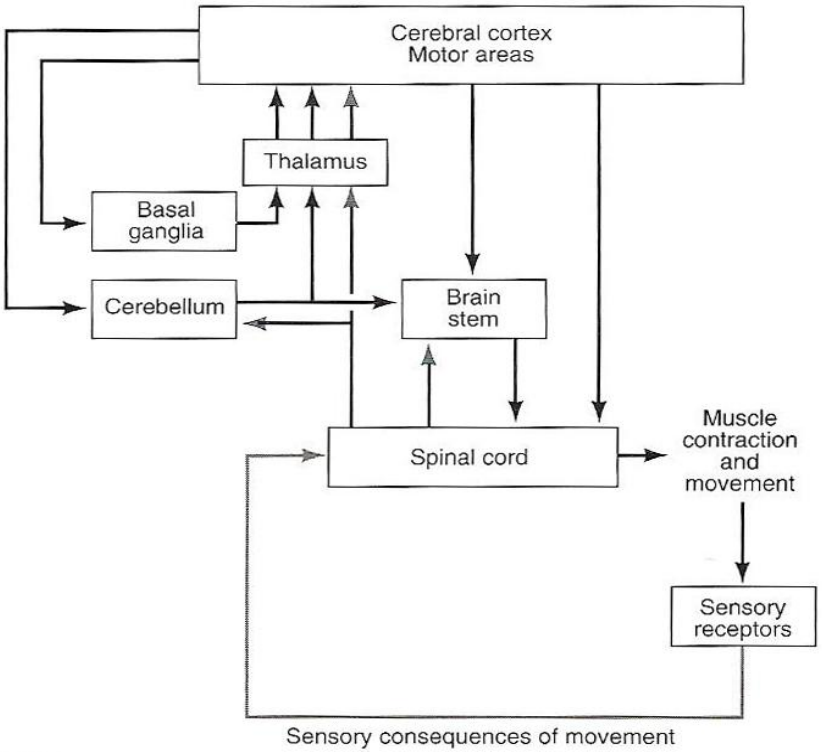


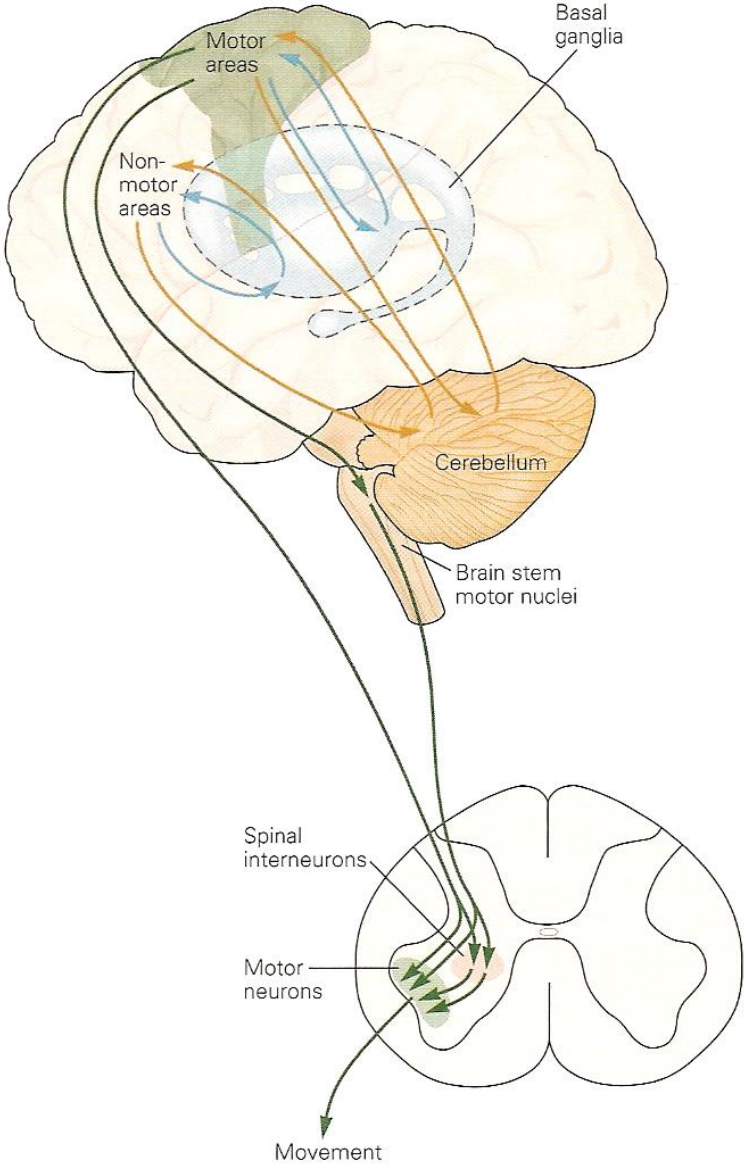
The rostrocaudal Columnar cell fate of Motor Neurons



Motor systems are organised hierarchically:

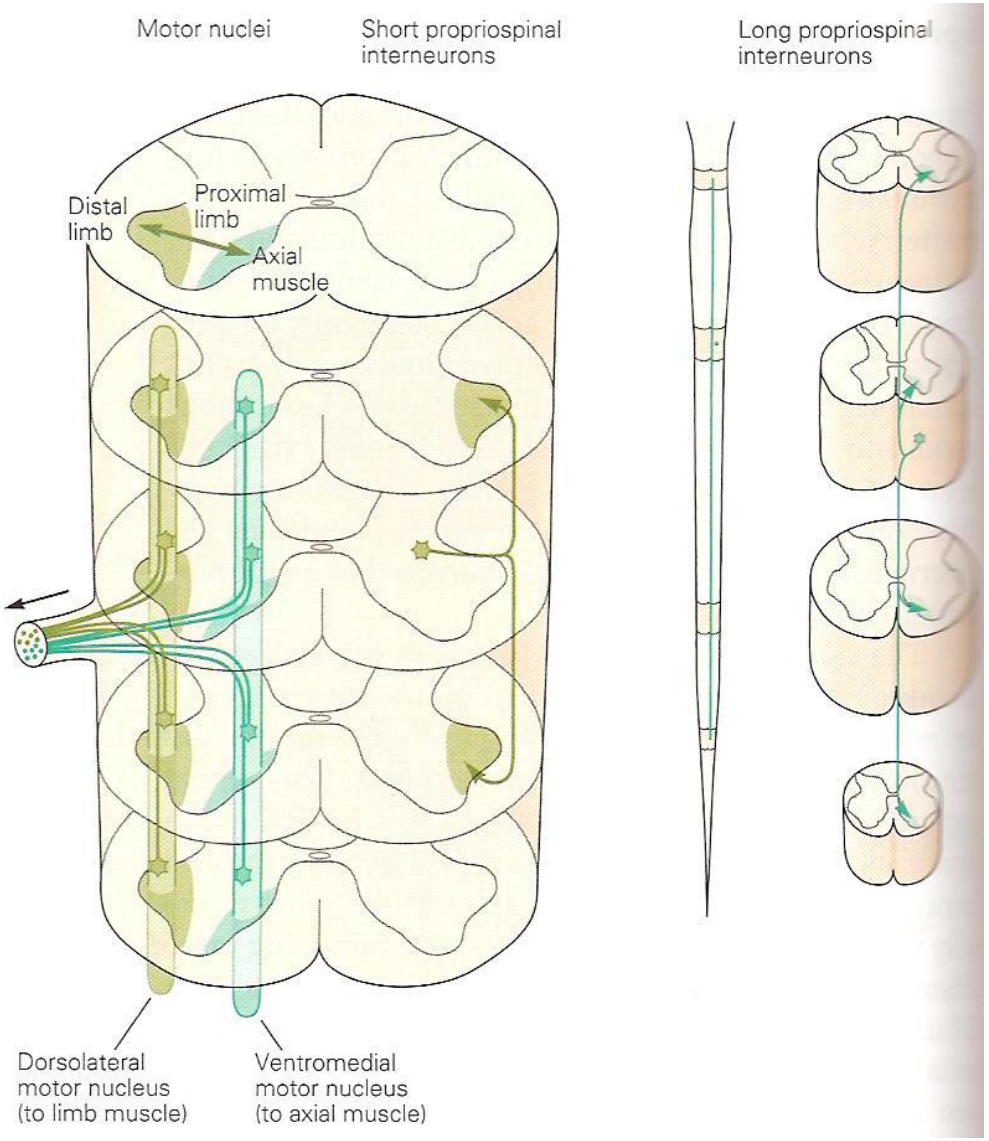
- **Spinal cord**, **Brain stem** and **Cortex** contain successively more complex motor circuits.
- **Cerebellum** and **Basal Ganglia** feedback to the cortex (via thalamus) and brainstem to regulate planning and execution. Necessary for smooth movement and posture (Parkinson, Huntington, Cerebellar ataxia).

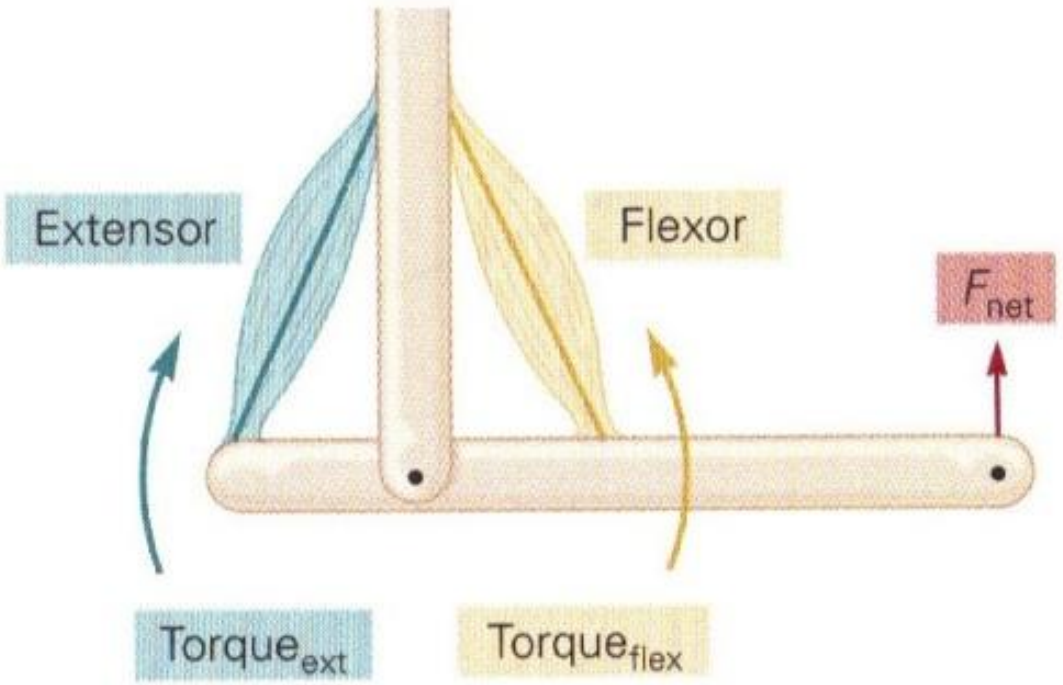




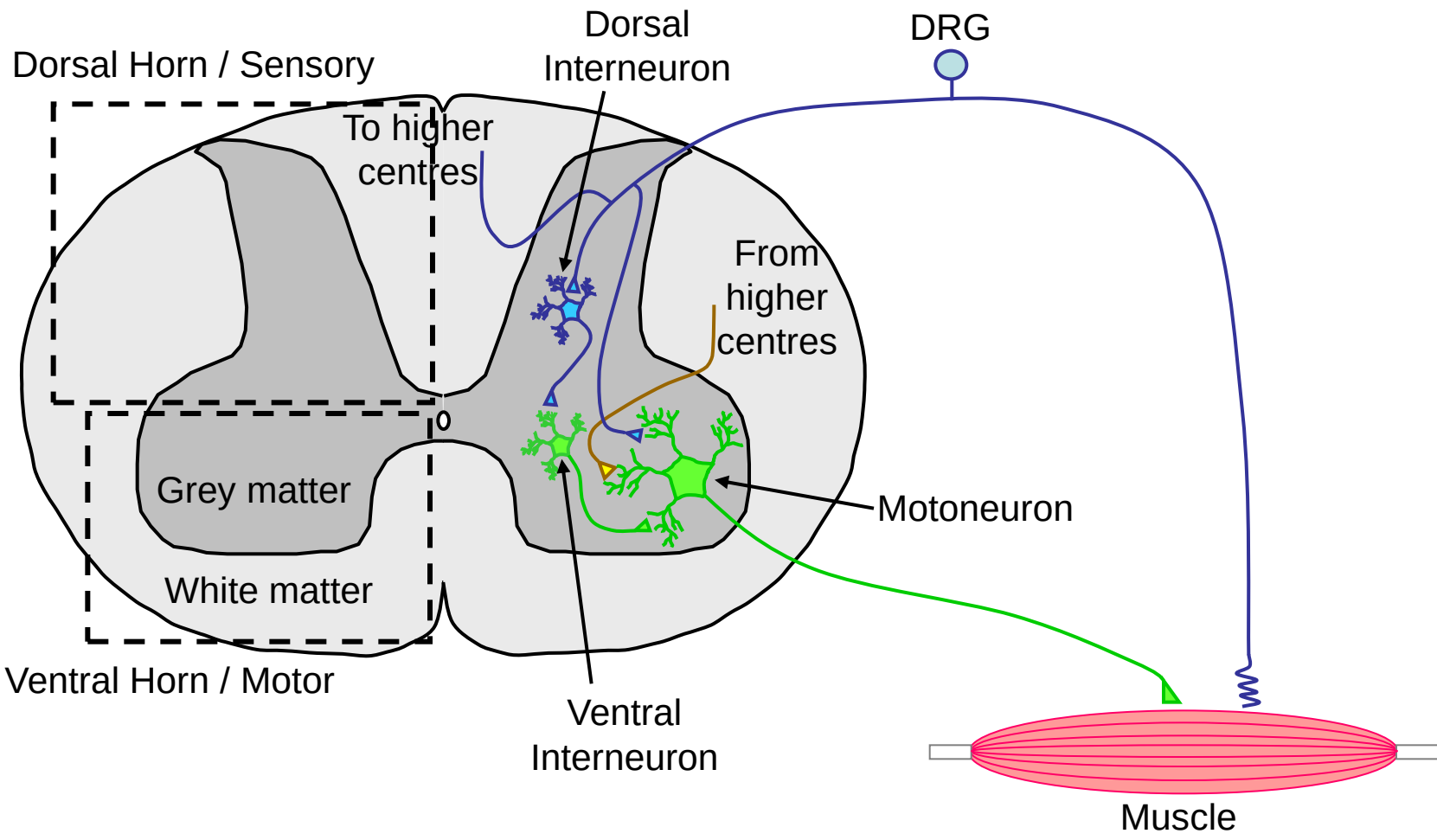
Organisation of the spinal cord:

- **Local interneurons:** axons are confined to the same or adjacent segments of the spinal cord – includes local networks giving rise to rhythmic movements.
- **Propriospinal neurons:** axons project to distant spinal segments.
- **Projection neurons:** axons ascend to higher brain centres.
- **Motoneurons:** innervate muscles, “final common pathway” for all motor action (Sherrington).

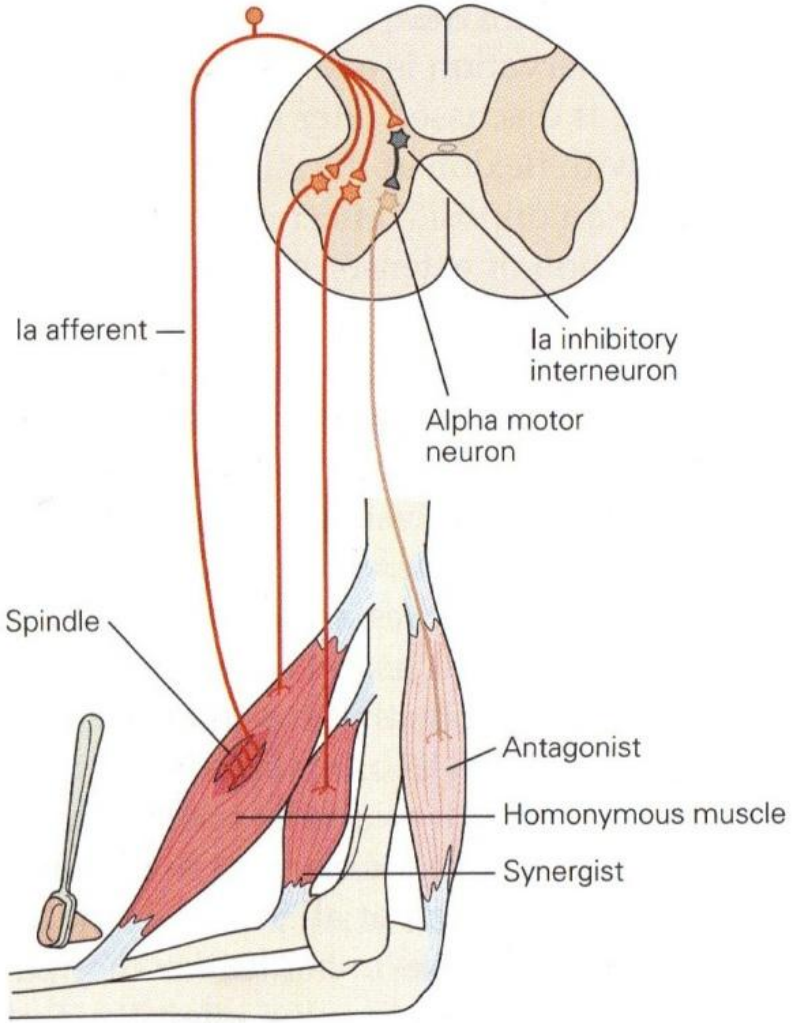



Extensor
Flexor
F_{net}
$Torque_{ext}$
$Torque_{flex}$

Organisation of the spinal cord:

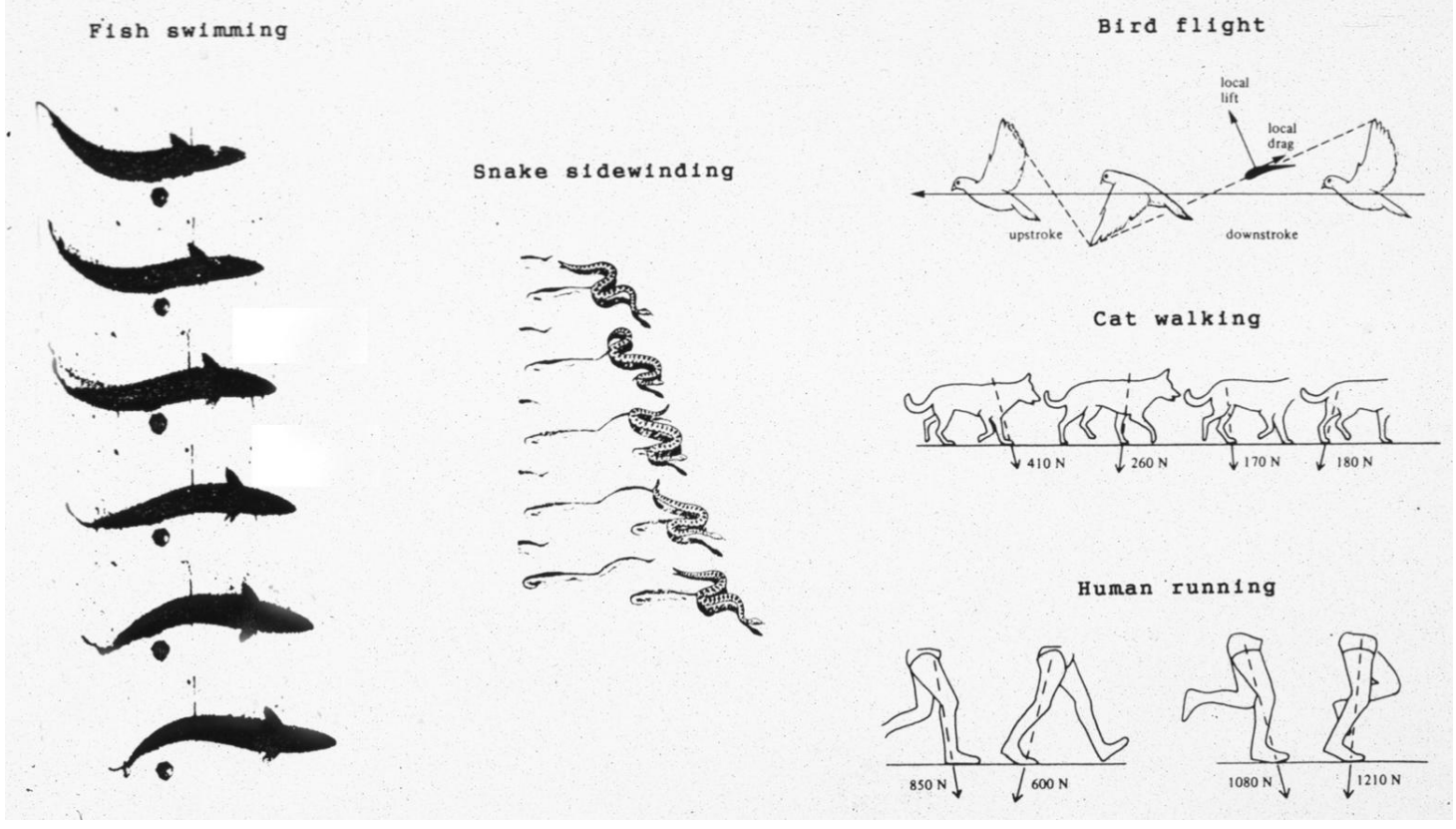


B_1 Stretch reflex



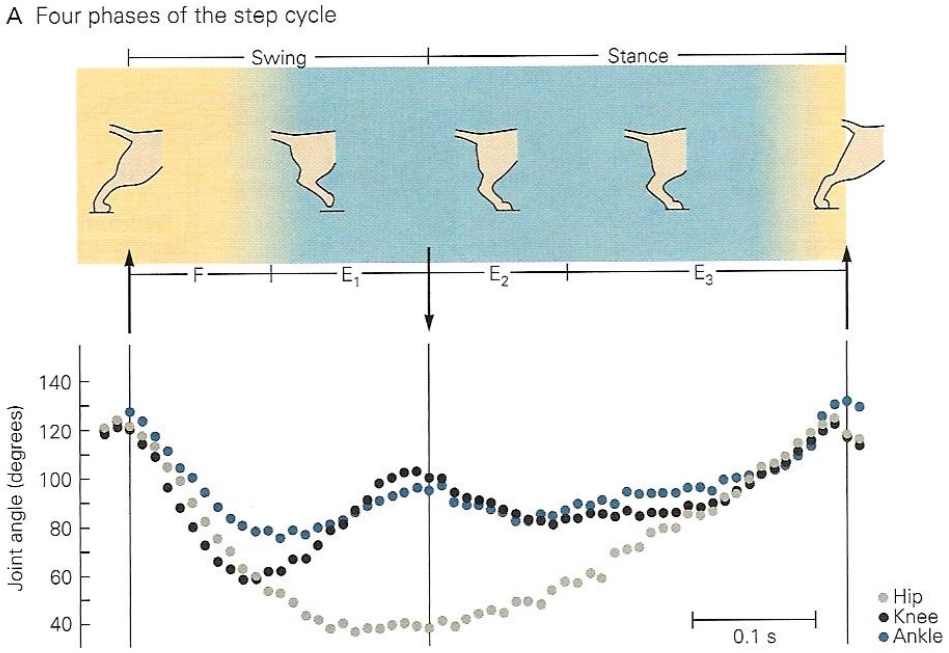
Rhythmic movement: locomotion

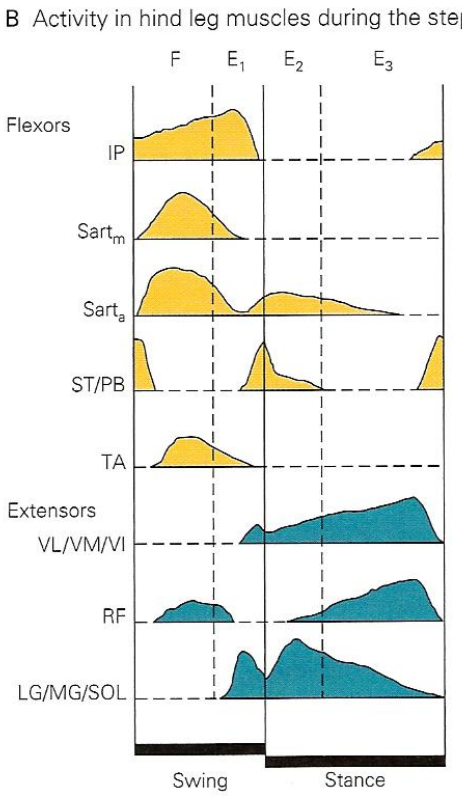
- Many different forms including: swimming, crawling, flying and walking.
- Rhythmic and alternating movements of the body or appendages generate propulsion.
- Skeletal muscles involved are arranged as functional antagonists.
- Each cycle comprises power & return stroke phases - in limbed animals power and return stroke phases are called stance and swing.

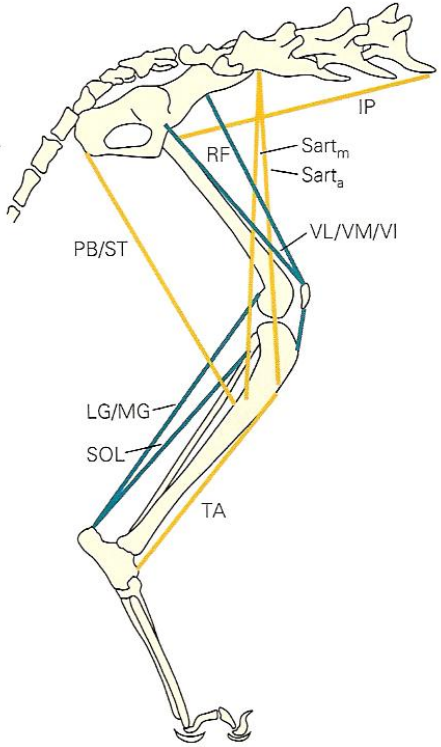


Complex sequence of muscle contractions produces stepping:

- A large number of muscles are involved. In general flexor muscles contract during F phase and extensor muscles contract during one or more of the E phases. However, timing and level of activity can vary widely in different muscles.

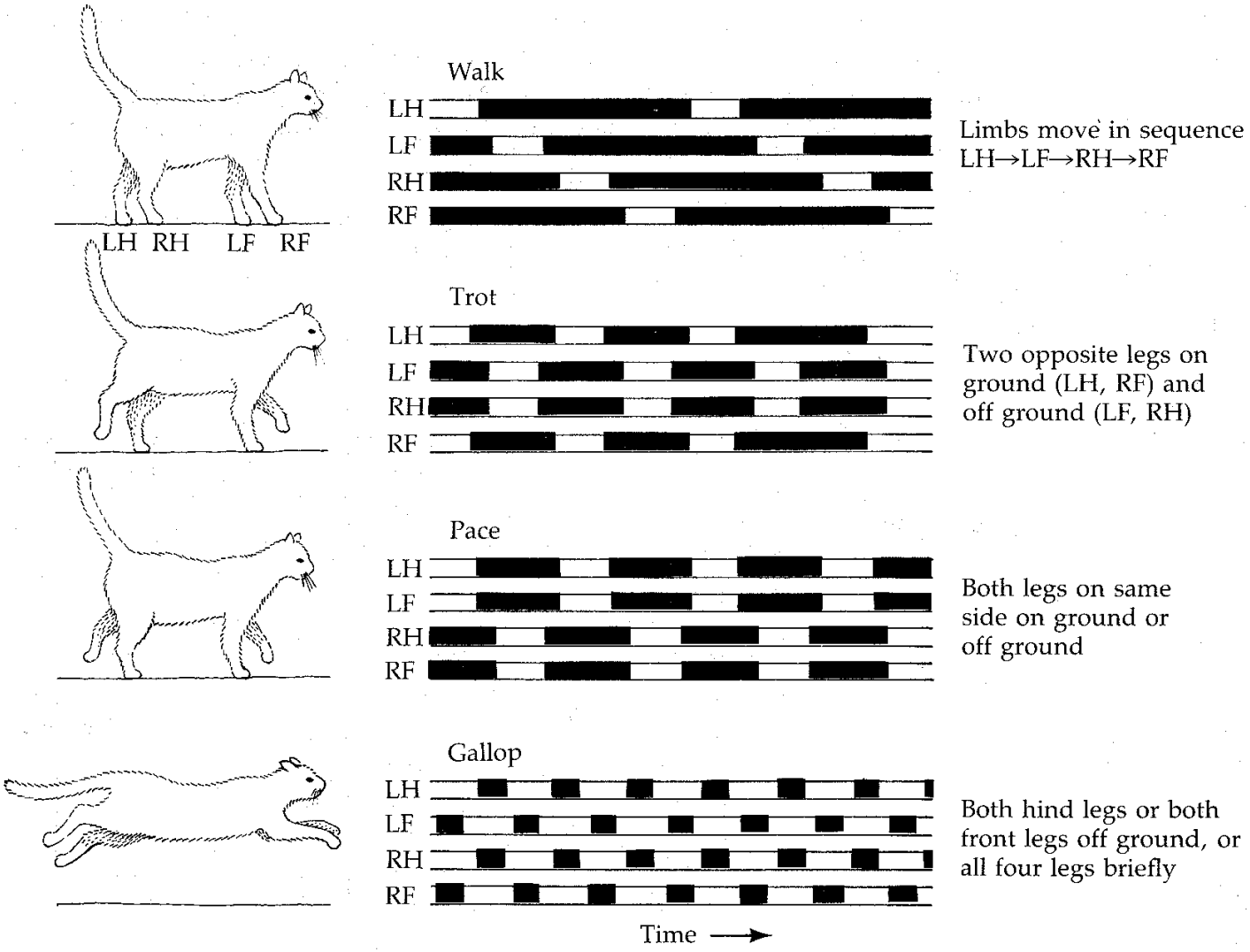




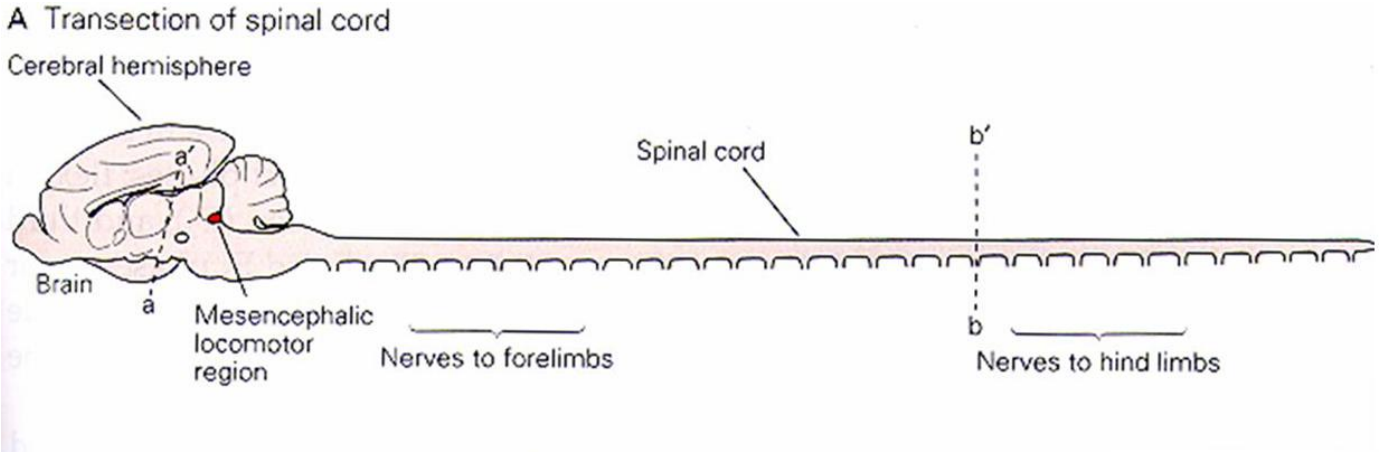


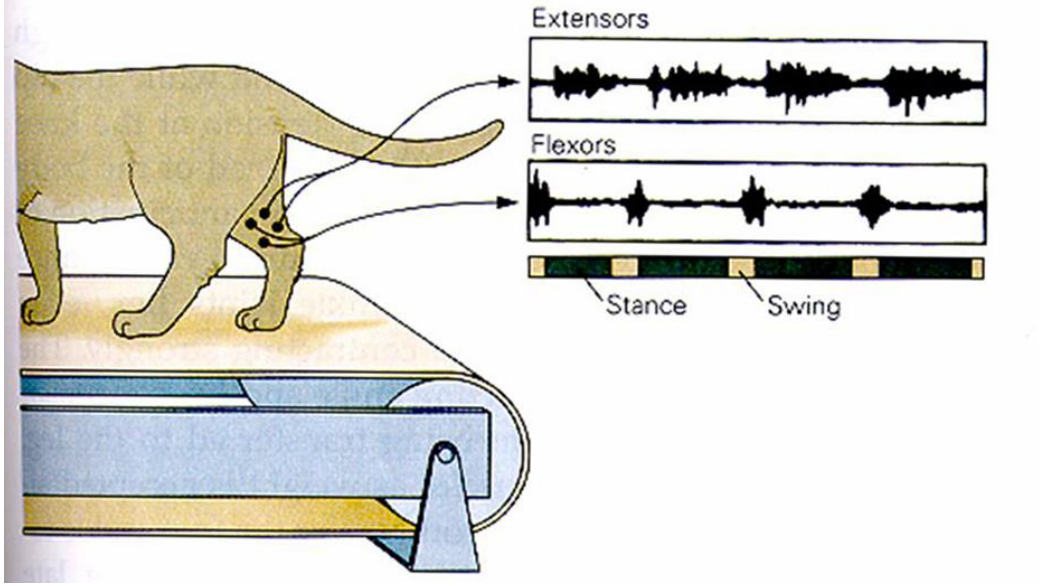
A variety of locomotor patterns can be generated:

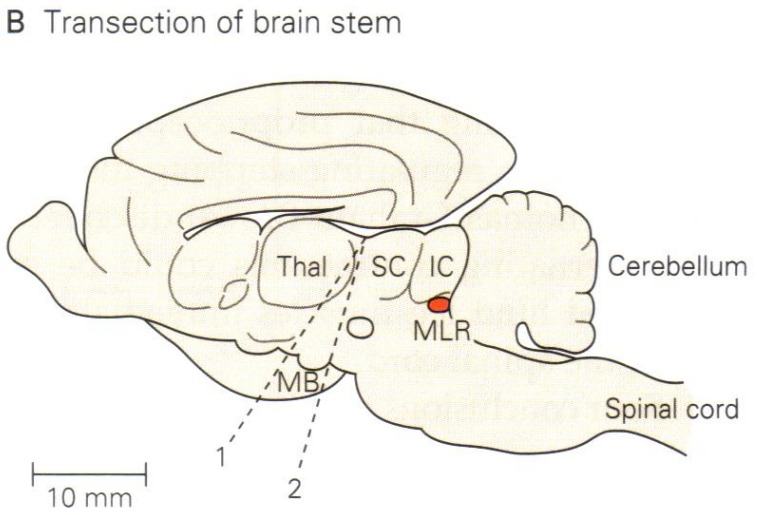
- There are many different gaits that can be employed during different speeds of locomotion



Brief history of locomotor research:

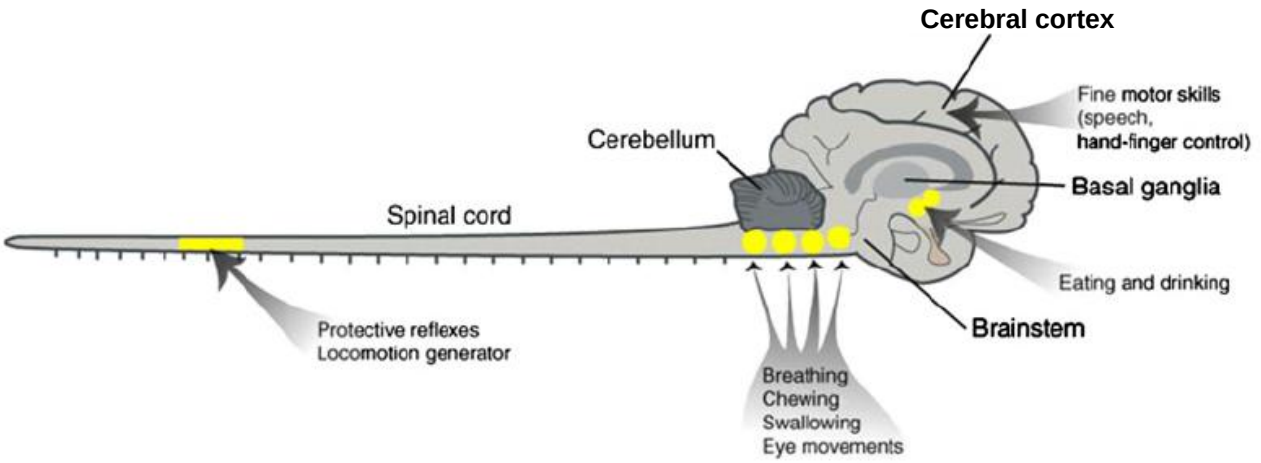


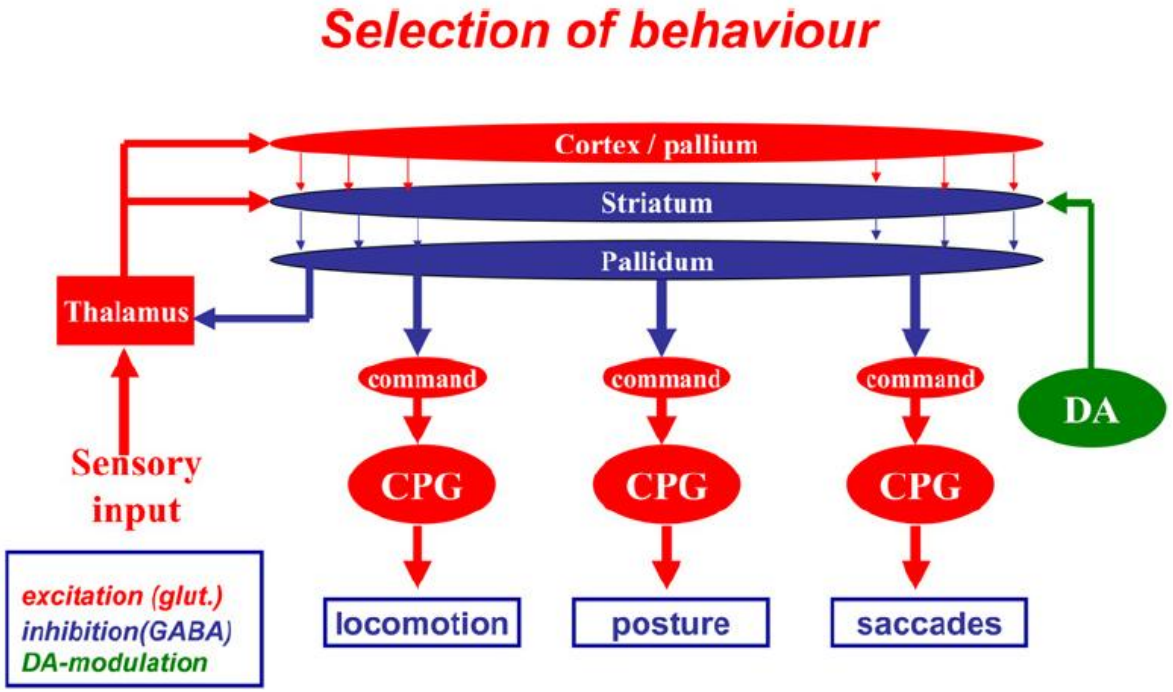




Central Pattern Generators:

- Central pattern generators (CPGs) are networks of neurons which generate a range of rhythmic movements including breathing, chewing, swallowing and locomotion.
- CPGs generate the timing and pattern of complex muscular activities.
- Different CPGs are distributed throughout the central nervous system.
- For a behaviour to be elicited the particular CPG or the input that drives it needs to be disinhibited.





Central pattern generators:

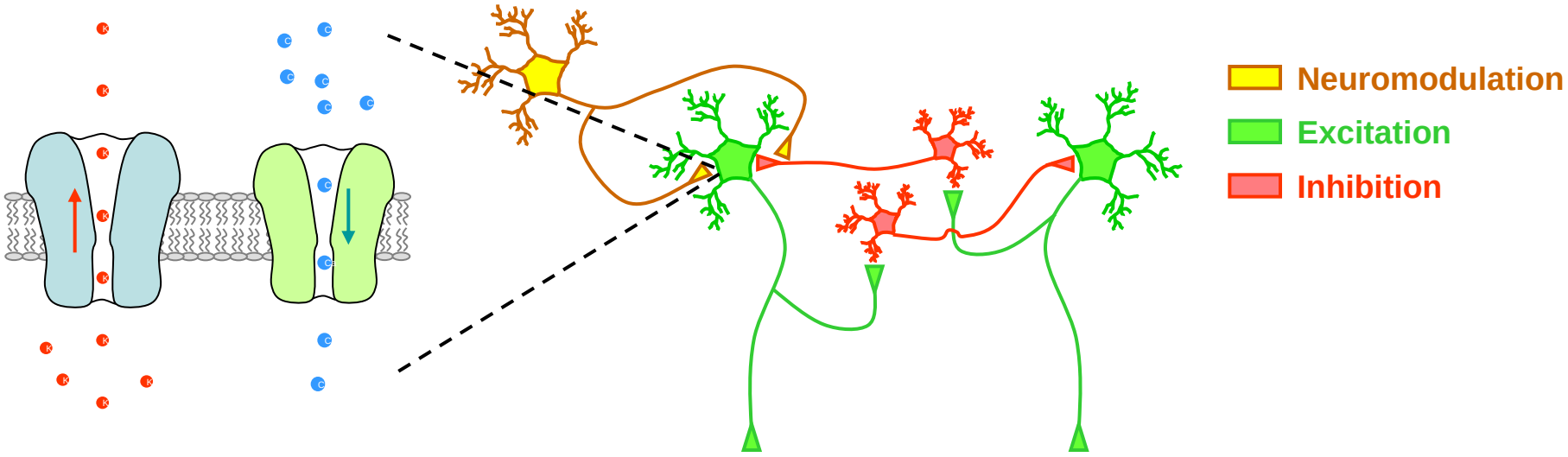
- The output of a CPG is determined by:

– The **intrinsic properties** of individual neurons within the network (e.g. ion channel and neurotransmitter receptor expression) which determine their output (pattern of action potential firing).

– The **synaptic connectivity** between neurons which make up the network.

– The **properties of the synapses** between neurons within the network.

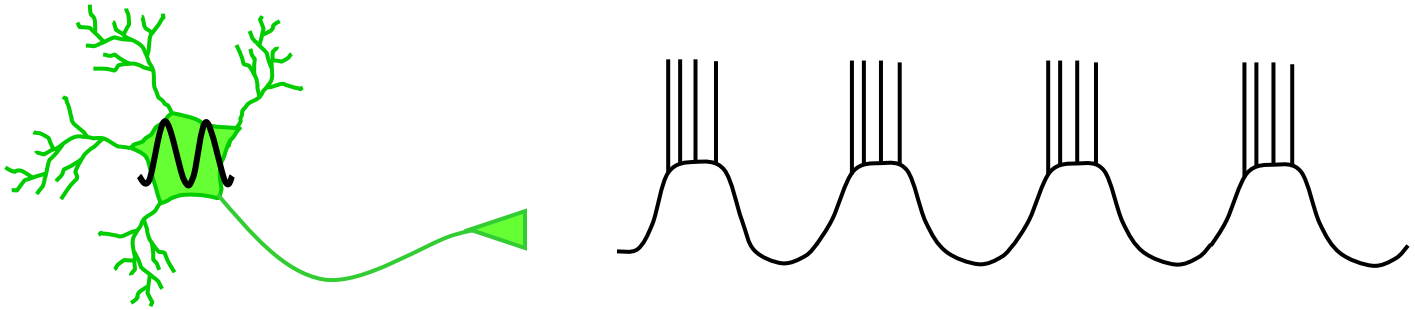
– **Neuromodulation** of the intrinsic properties of neurons within the network and of the synaptic connections present.



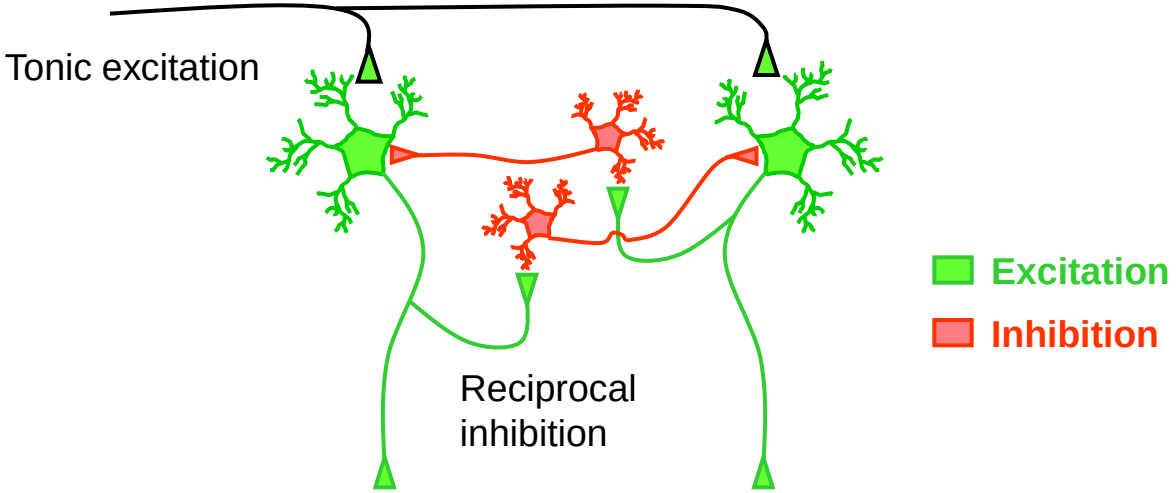
Rhythm generation in CPGs:

- The rhythmicity of a CPG can reflect one or both of the following:

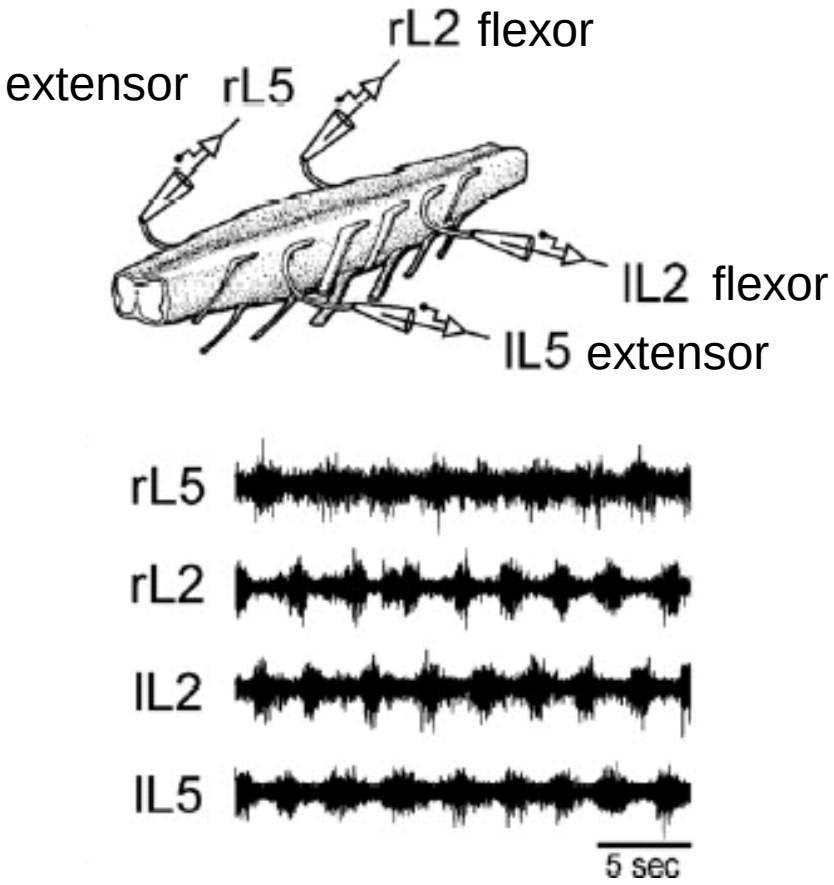
– **Endogenous bursters:** (pacemaker cells) neurons with the capacity to burst spontaneously.



– **Oscillating networks**: simple networks of neurons can generate rhythmic activity if time dependent processes exist to enhance or reduce activity within some of the neurons – (e.g. fatigue in the inhibitory connections in the half-centre model).



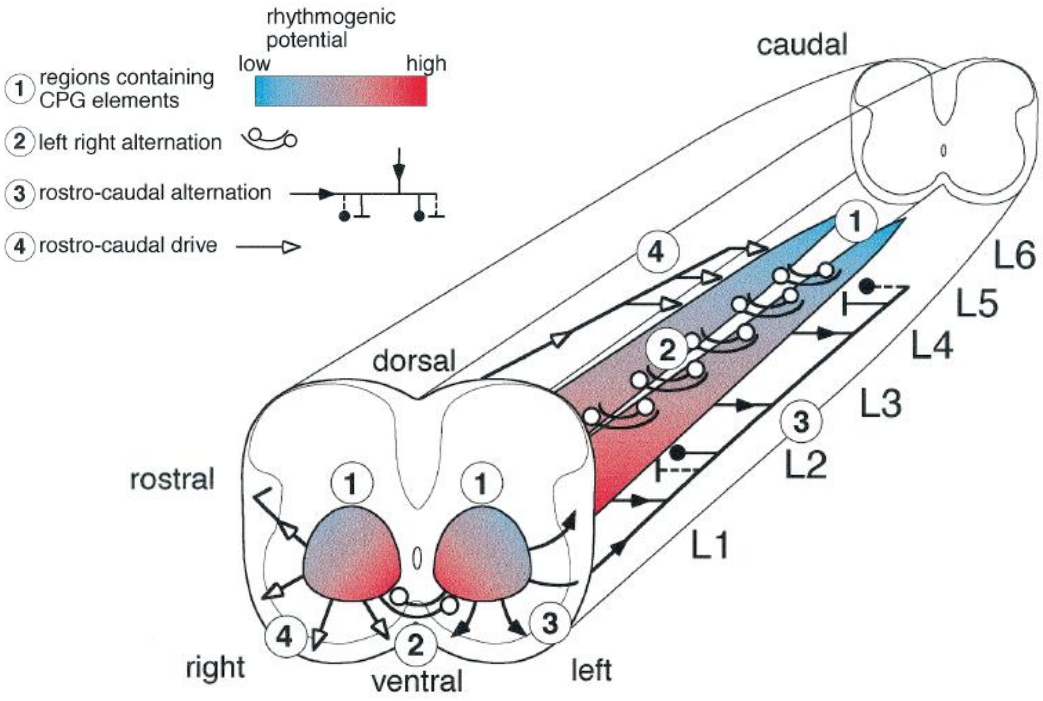
In vitro locomotion

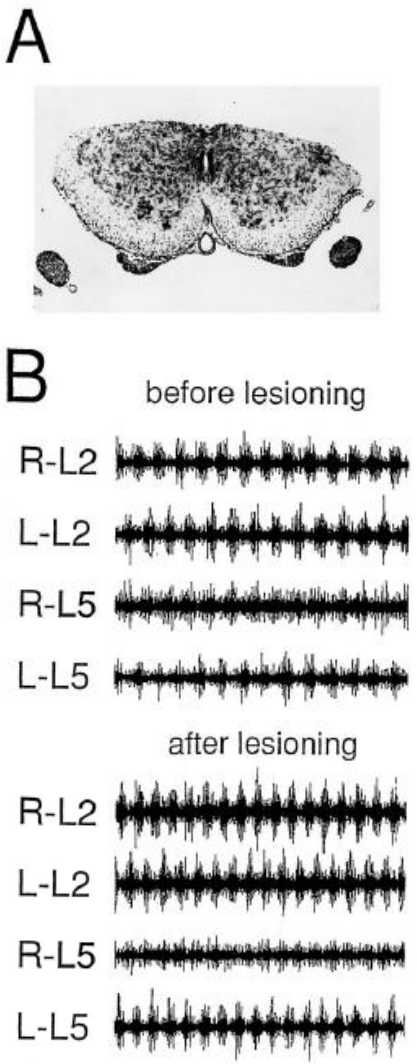


Kiehn lab

Localising the locomotor CPG in mammals

- The rhythmogenic capacity of the mammalian hind limb locomotor CPG is distributed along the lumbar cord but with a rostrocaudal excitability gradient.
- The CPG is located in the ventral horn of the spinal cord.



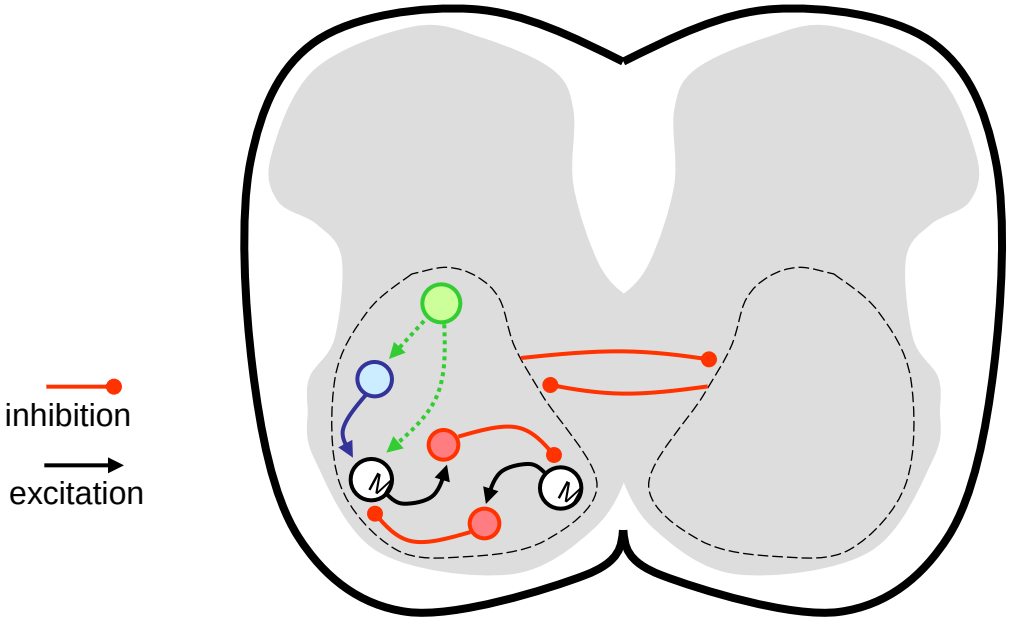


General features of vertebrate locomotor CPGs:

- Locomotor rhythm is most likely to be generated by glutamatergic excitatory interneurons which have ipsilateral projections either directly onto motoneurons or onto other excitatory interneurons.

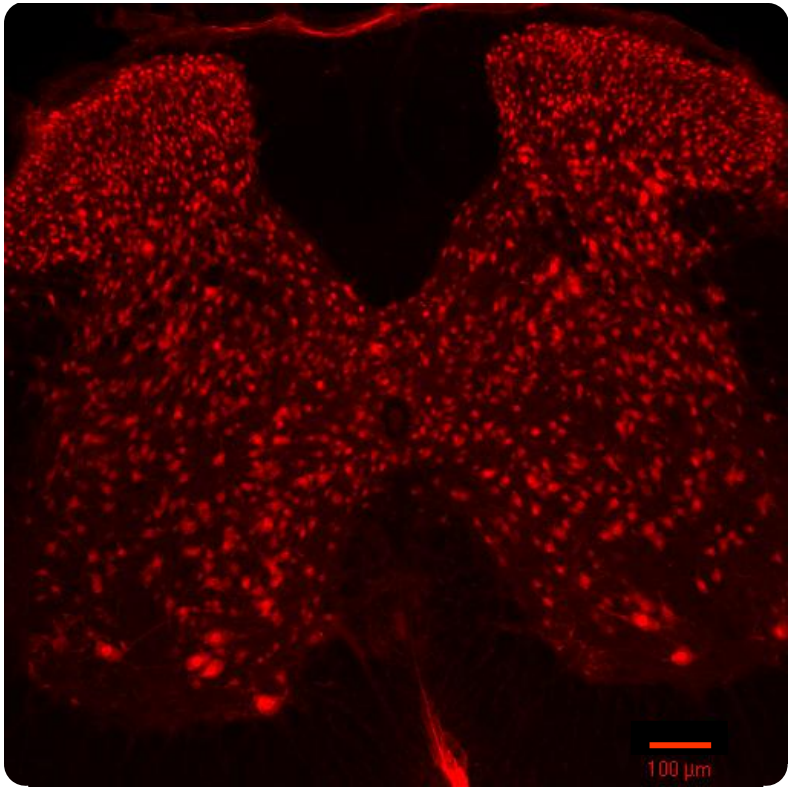
- Reciprocal inhibition contributes to alternating activity between different groups of motoneurons (ipsilateral and contralateral).

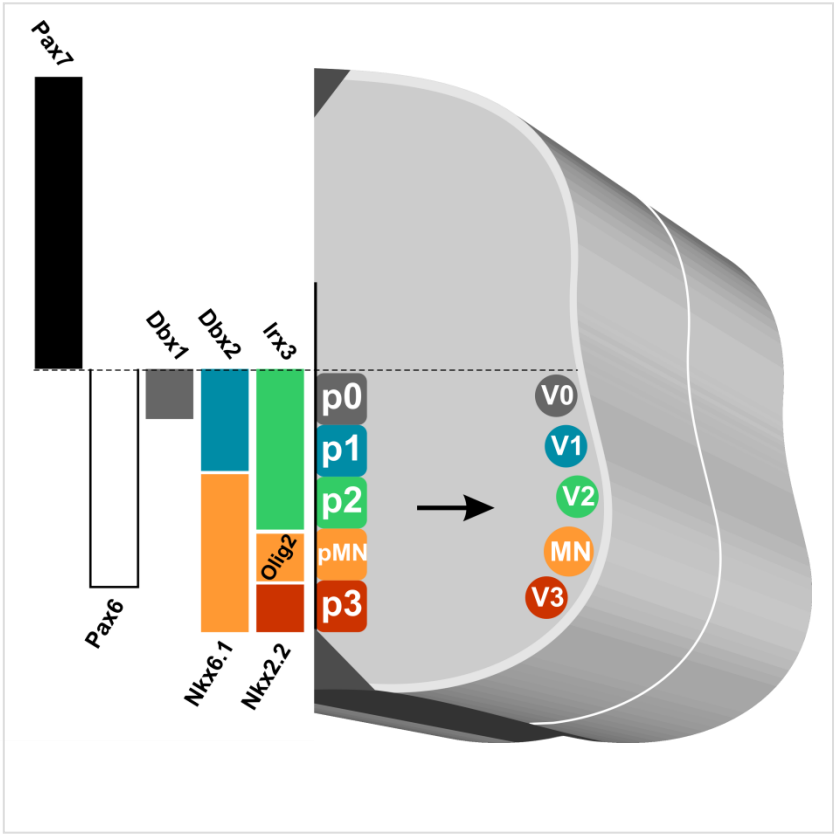
- Reciprocal inhibition controlling coordination between left and right sides of the spinal cord involves commissural inhibitory interneurons.



Identifying interneurons in the mammalian spinal cord:

- It is difficult to identify discrete populations of interneurons in the mammalian spinal cord due to the number of cells and the lack of clear structure.
- Recently, our knowledge of transcription factors which are involved in the differentiation of specific types of spinal neurons has been utilised to label specific populations of interneurons.

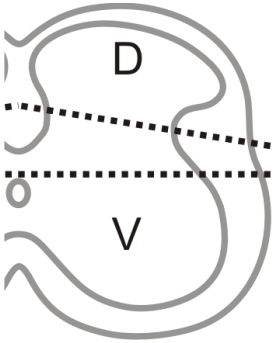


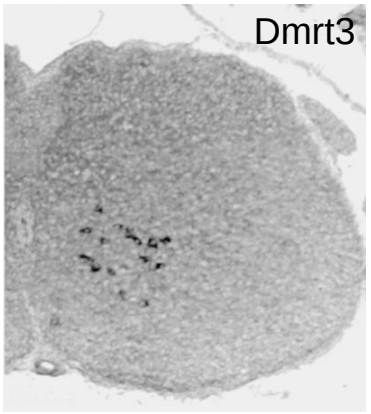


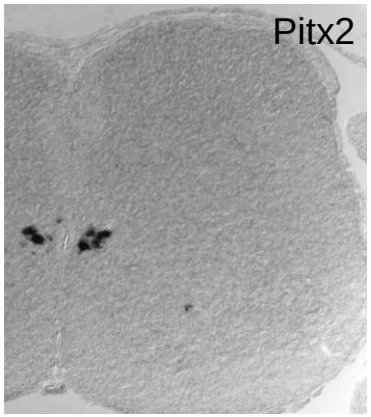
Identifying interneurons in the mammalian spinal cord:

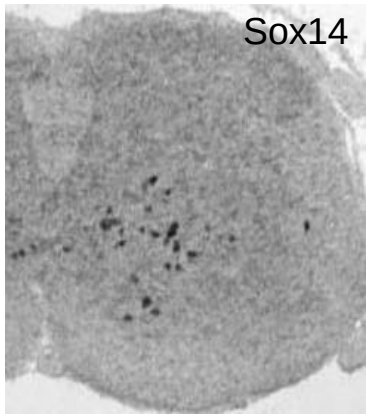
- Candidate screen (all Hox genes)

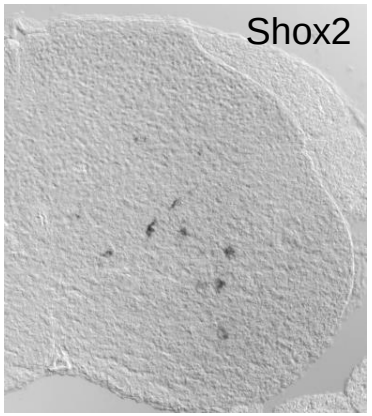
- A screen for interneuron subpopulations

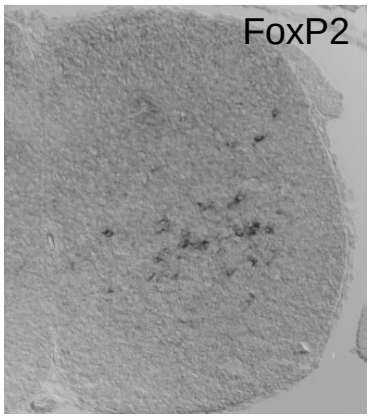


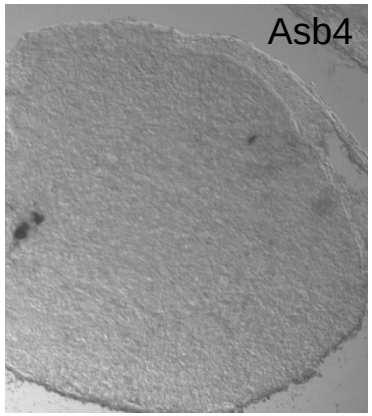






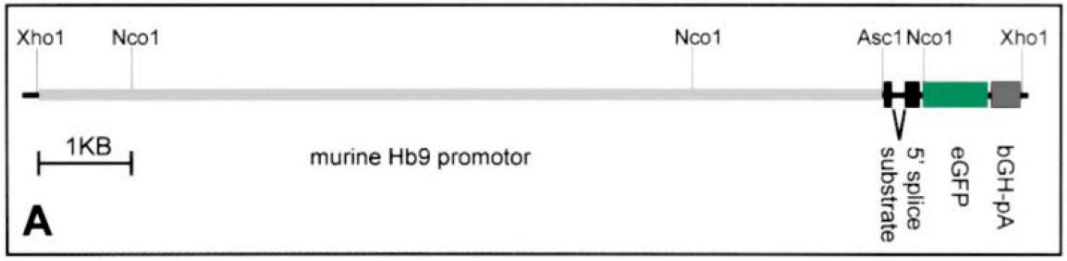




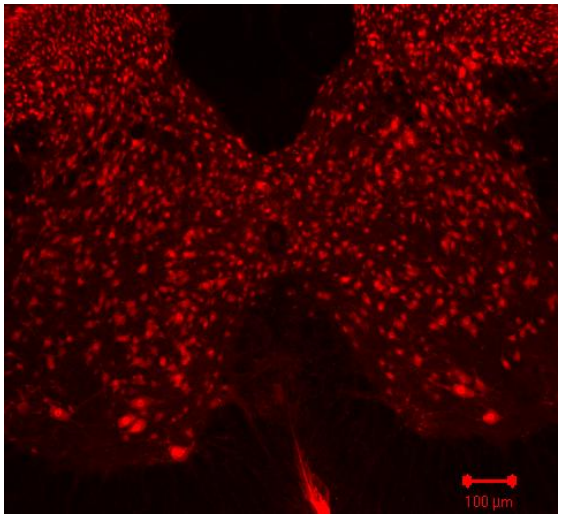


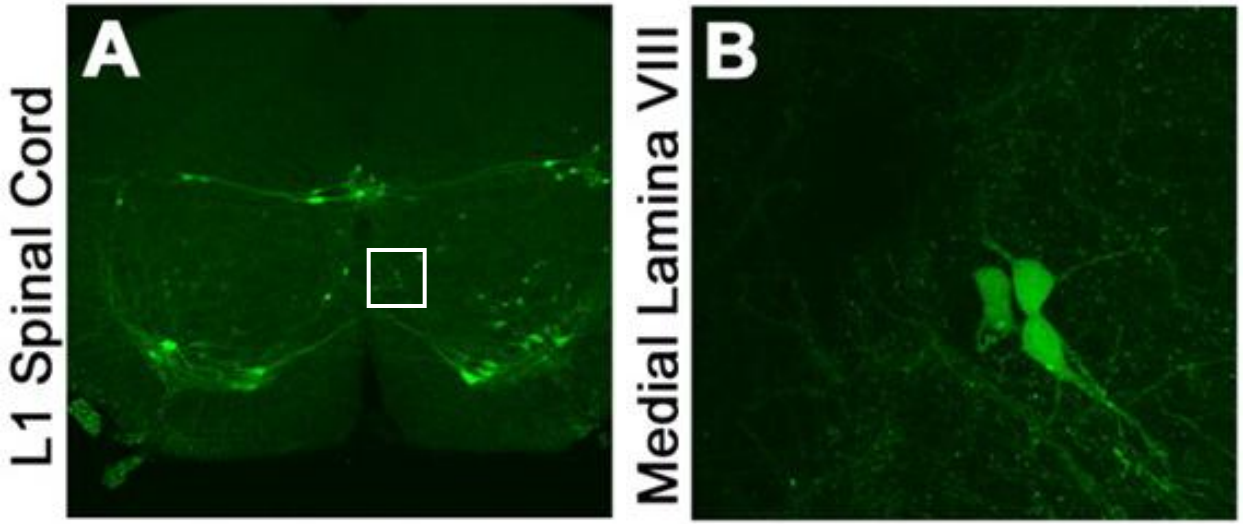
Identifying interneurons in the mammalian spinal cord:

- Genetically encoded fluorescent reporters, most commonly Green Fluorescent Protein (GFP), can be expressed under the control of neuron-specific promotor genes.



- Specific interneurons can then be visualised and their physiology with respect to locomotion studied.





Genetically Engineered Mice (GEM)

Transgenic Mouse: an engineered mouse that has its complete genome plus extra DNA sequence of any origin integrated into some position in its genome.

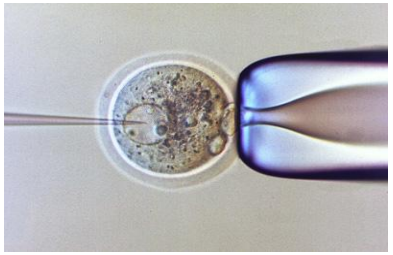
Knockout Mouse: A GEM in which the normal gene is missing or engineered so that is not transcribed or translated (non functional). "Knocks out" that gene.

Knockin Mouse: A GEM in which the engineered "transgene" is subtly manipulated to: (A) alter the function of the gene (e.g., replace one amino acid with another in a site to determine if that site is essential for the protein's function); (B) change transcription rate to overproduce or underproduce the gene product; or (C) create a fluorescent gene product to map its distribution in tissue.

Conditional Knockout (Knockin) Mouse: A GEM in which the transgene is knocked out (or in) in specific tissues, at a specific developmental stage, or in response to an exogenous substance (e.g., an antibiotic).

Transgenics

Pronuclear microinjection



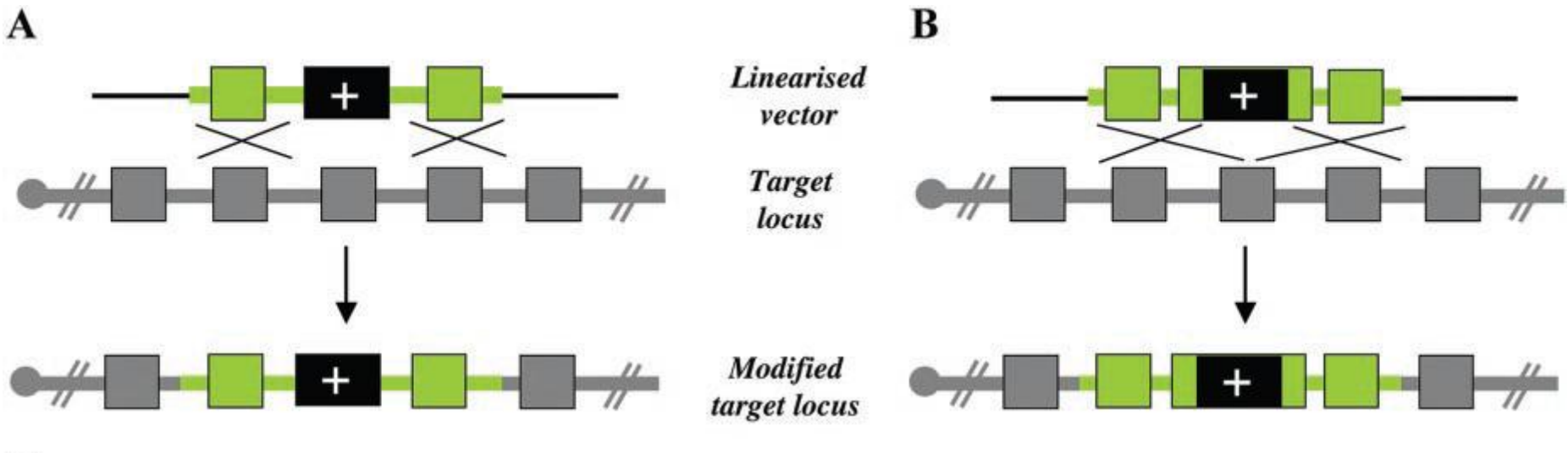
pros

Relatively simple
Long transgenes possible

cons

Inneficient
Very high embryo mortality
Multicopy insertions
Only random integration
Species / Strain limitations

Knockin mice



Conditional schemes