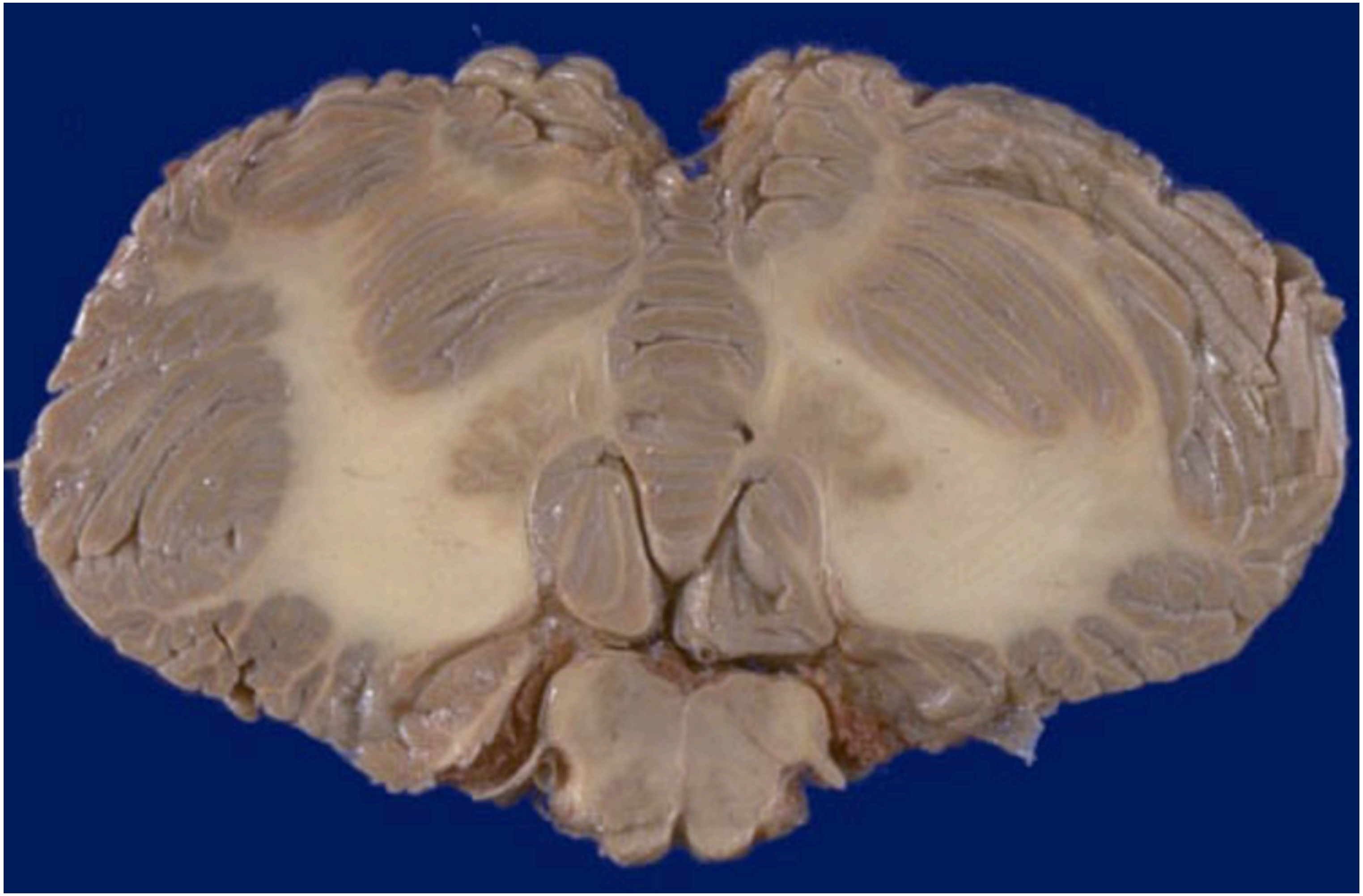
The Cerebellum

Neuroanatomy 2015

Daniel Scoles, Ph.D.
daniel.scoles@hsc.utah.edu



Gray matter of the cerebrum not cross the midline (PREVIOUS SLIDE).
Gray matter of the cerebellum spans the midline (this slide).

Overview

Part I

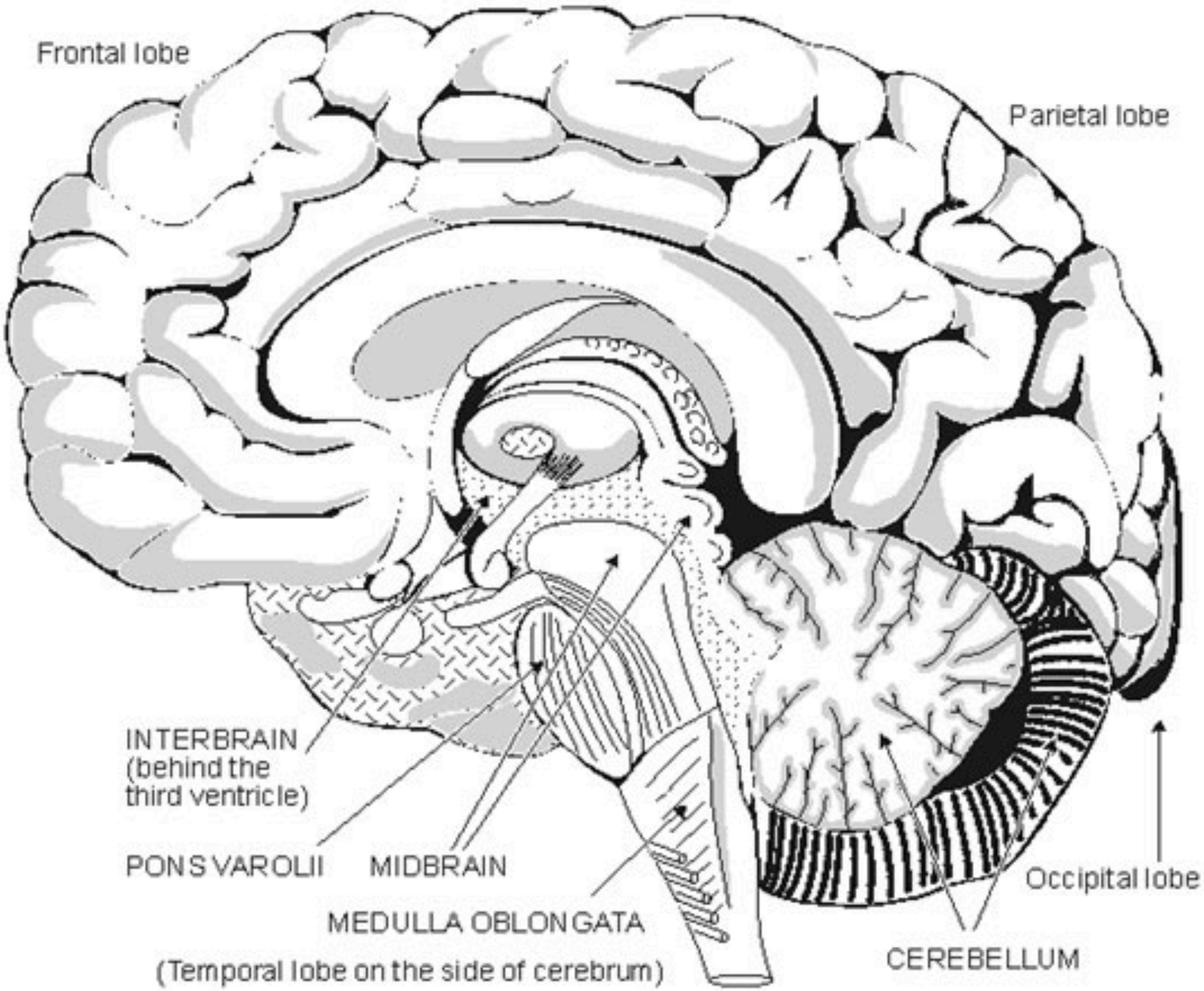
Gross anatomy
&
Cerebellar Circuitry

Part II

Cerebellar Diseases
&
Therapeutics

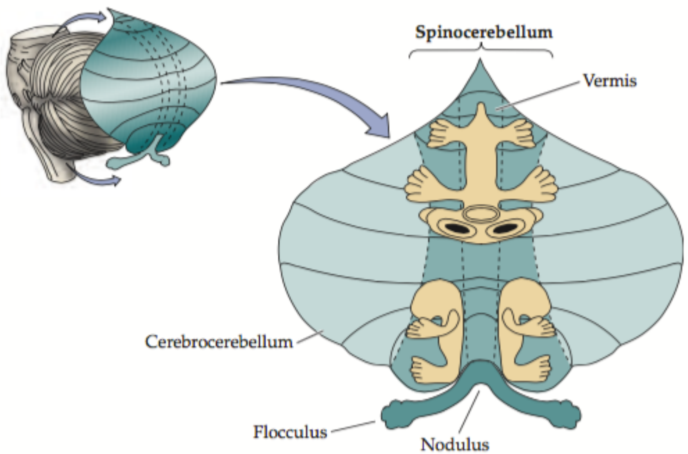
PART I

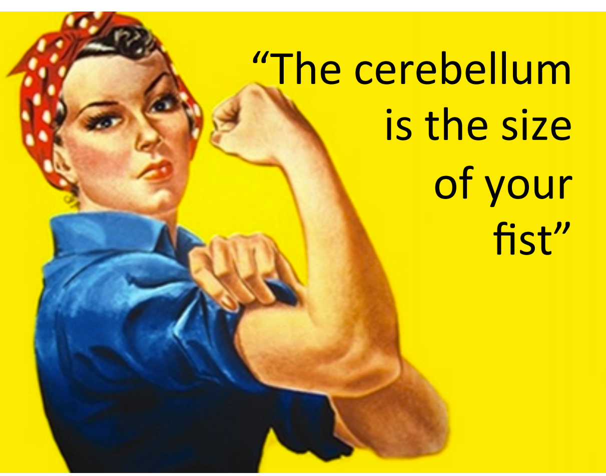
The Cerebellum
Latin for "The little brain"

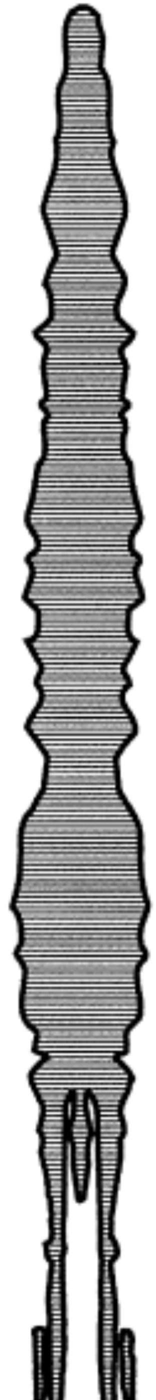


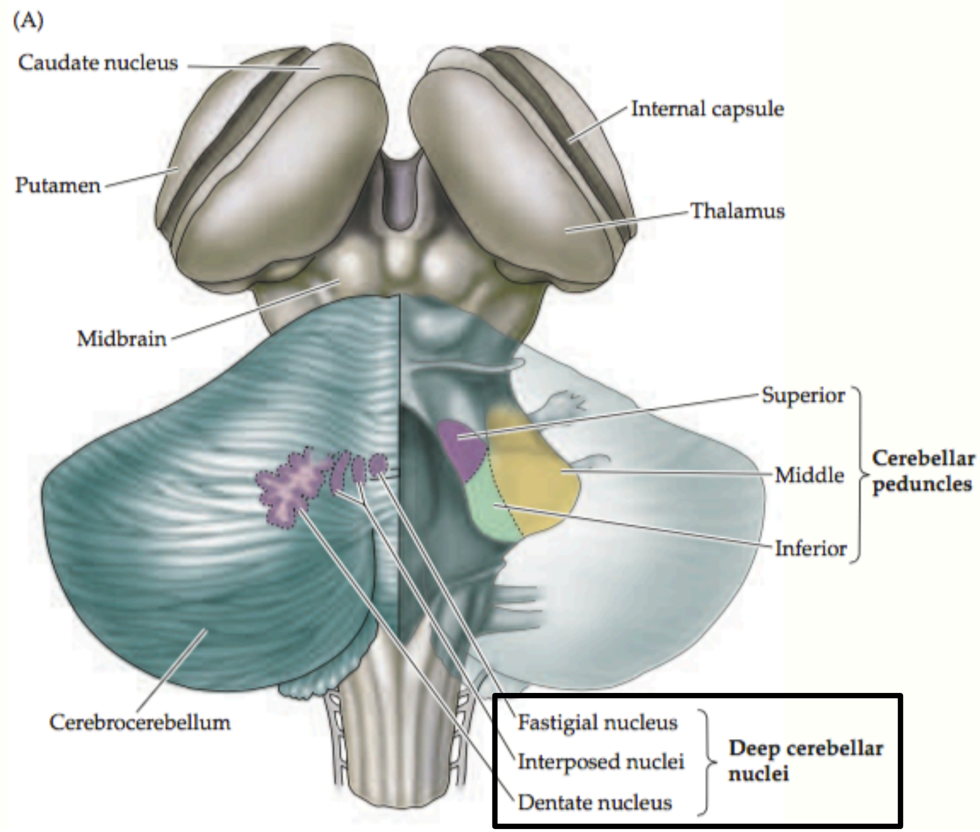
The unfolded cerebellum
Is 2.3 meters long
(Heck & Sultan 2002):

The spinocerebellum contains two maps of the body









- The cerebellum connects to the pons and midbrain.
- There are three major components of the Deep Cerebellar Nuclei

Pruves 5th Ed. 2012.

10 Folia

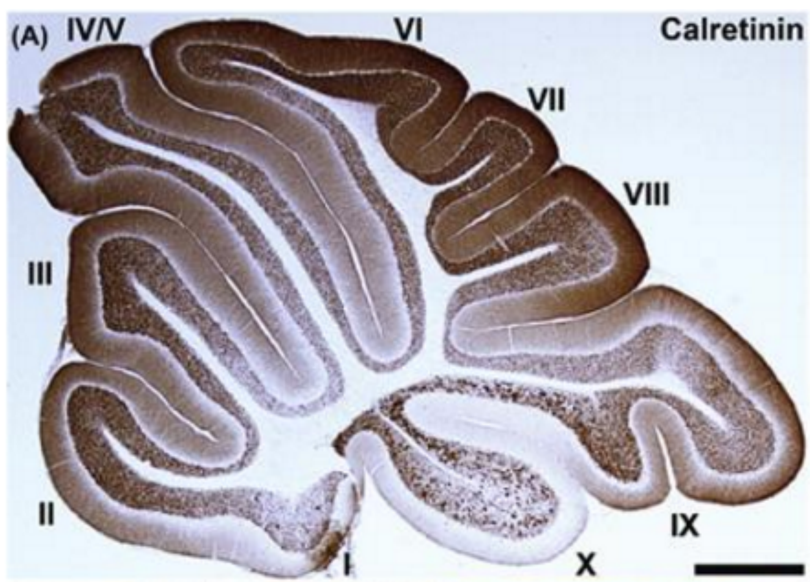
One lobe is called a folium

Folds are called folia or lobes. There are 10:

Human

http://www.edoctoronline.com/medical-atlas.asp?c=4&id=21803

Mouse

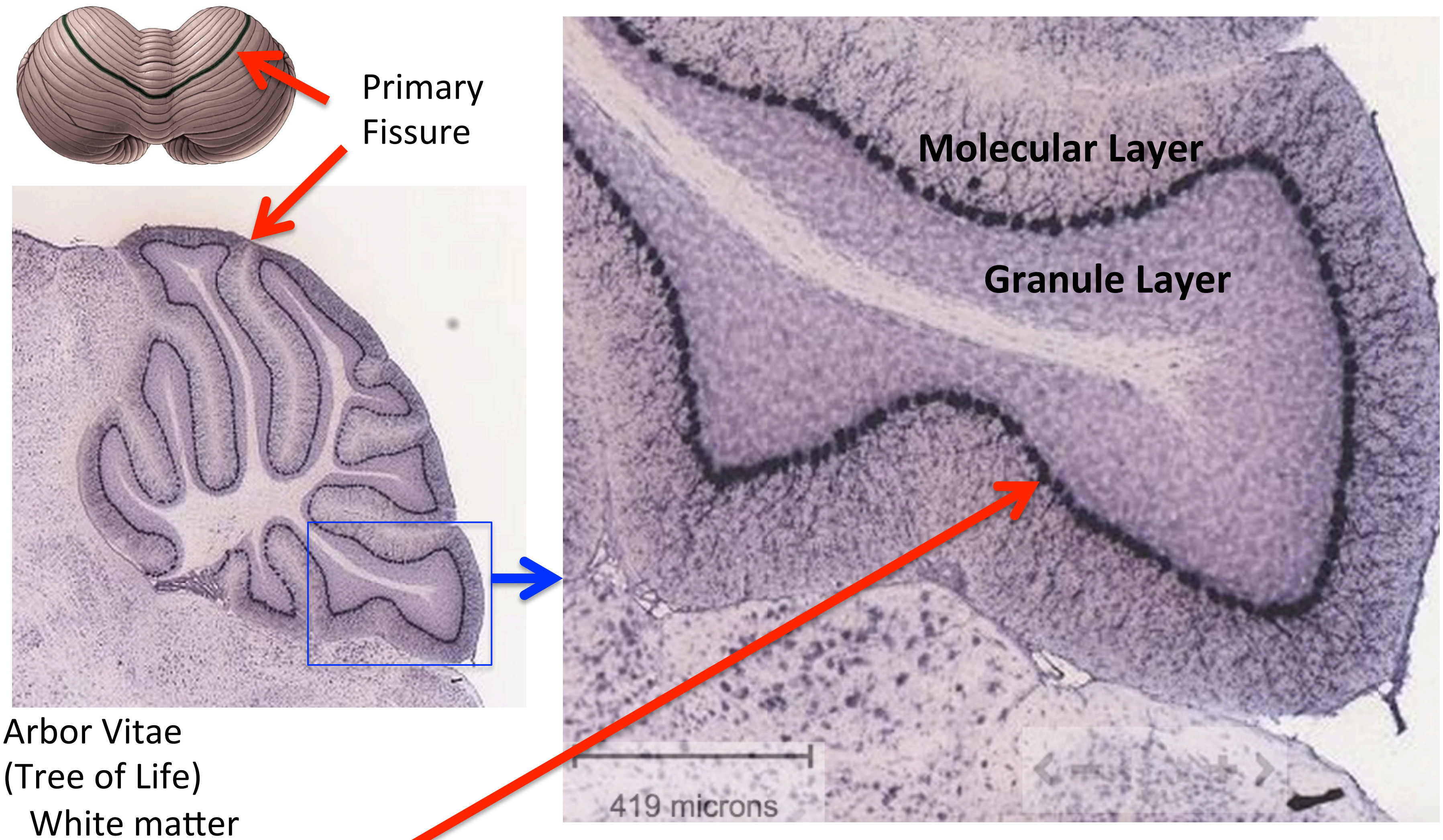


Primary fissure is between the 5th and 6th lobes.

From:
The Mouse Nervous System
By Charles Watson & George Paxinos
2011

Itpr1 from Allen Brain Atlas

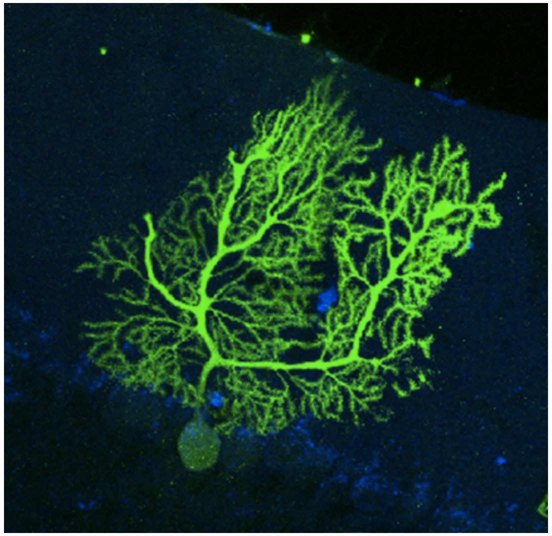
Mouse cerebellum

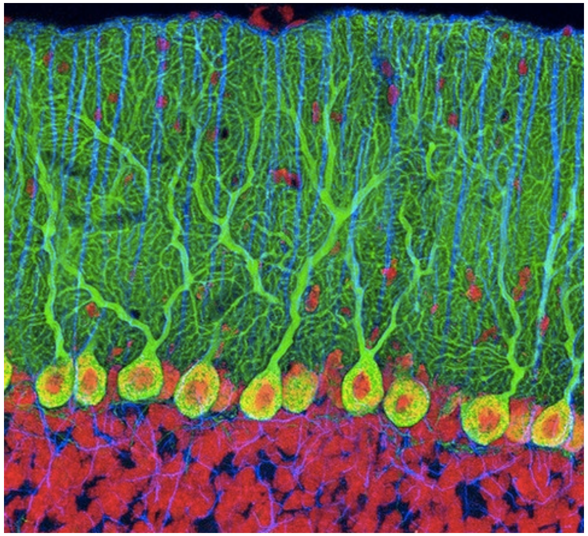


ISH for *Itpr1*

The cerebellum by the numbers

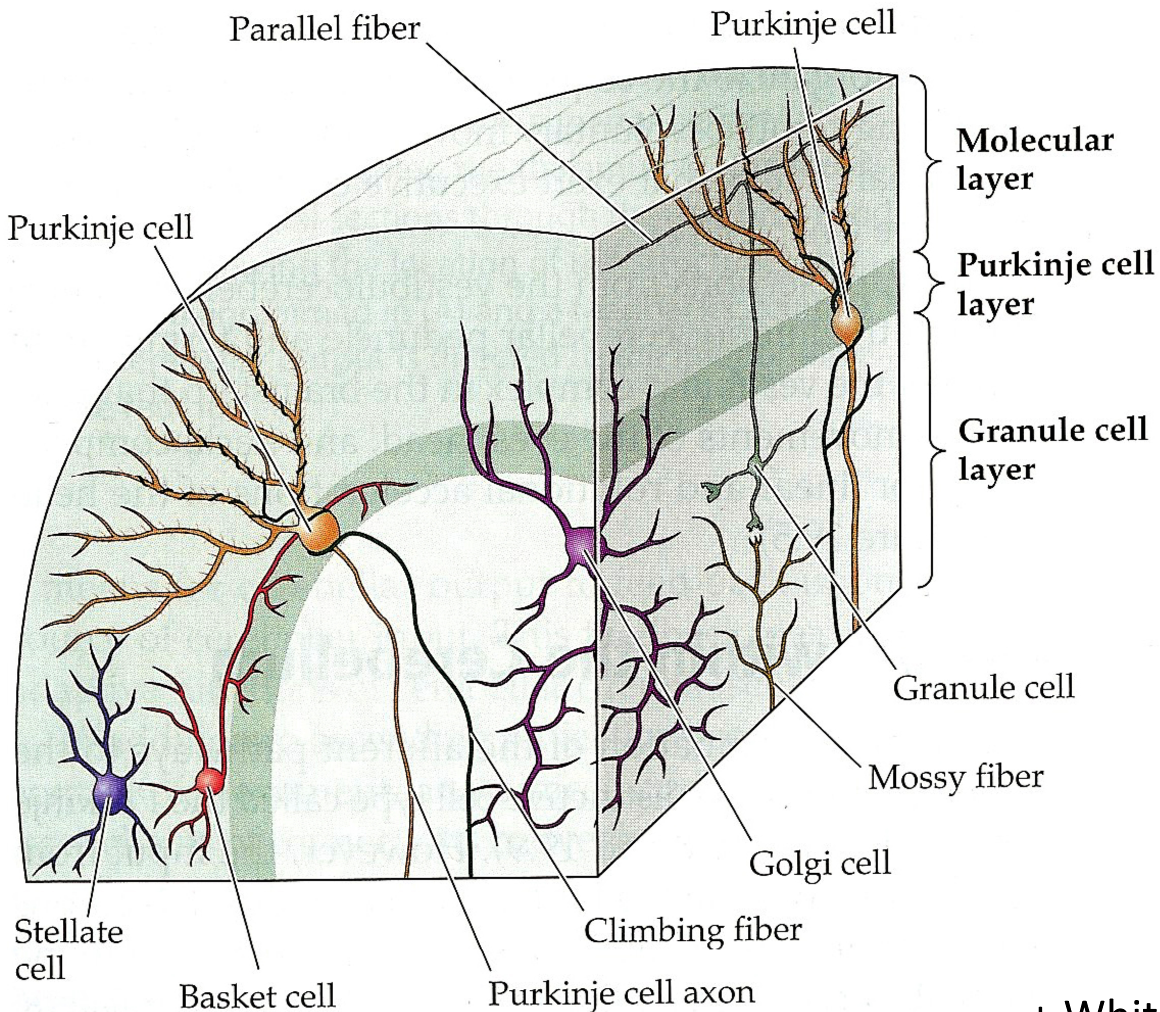
- Cerebellum is 11% of the brain by weight
- Each granule cell makes 200 synapses
- There are 10^{10} to 10^{11} granule cells in the cerebellum, which is more than all other cells in the brain. There are 9×10^{10} neurons in the brain*.
- Each Purkinje cell receives 500 climbing fiber synapses (Wadiche & Jahr, 2001).
- Each Purkinje cell receives 175,000 parallel fiber synapses (Napper & Harvey, 1998).
- There are 40-50 thousand beams in the cerebellum (Heck & Sultan 2002).
- Output is to the Deep Cerebellar Nucleus (DCN) and is inhibitory.





* en.wikipedia.org/wiki/List_of_animals_by_number_of_neurons

Cellular Organization of the Cerebellum



Sagittal Plane

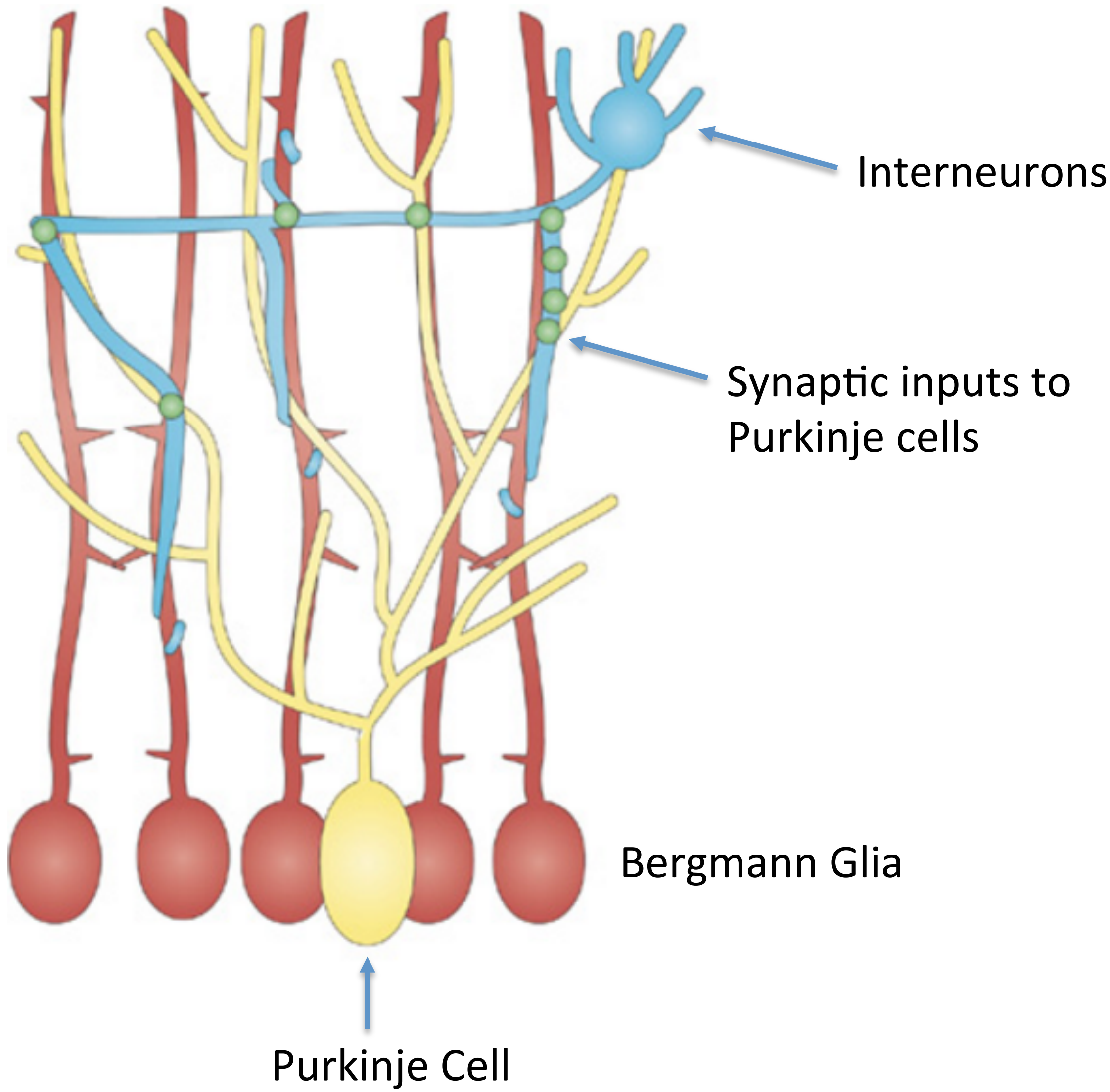
+ White matter

Bergmann Glia

Bergmann glia

- are a type of radial glia.
- guide interneuron arborization onto Purkinje cells.
- serve as guideposts for Purkinje cell and granule cell migration during development, but unlike other radial glia in the brain, Bergmann glia persist in adults and function in synaptic pruning.

Other cerebellar glia: GFAP positive glia & Aif1/Iba1 positive microglia.

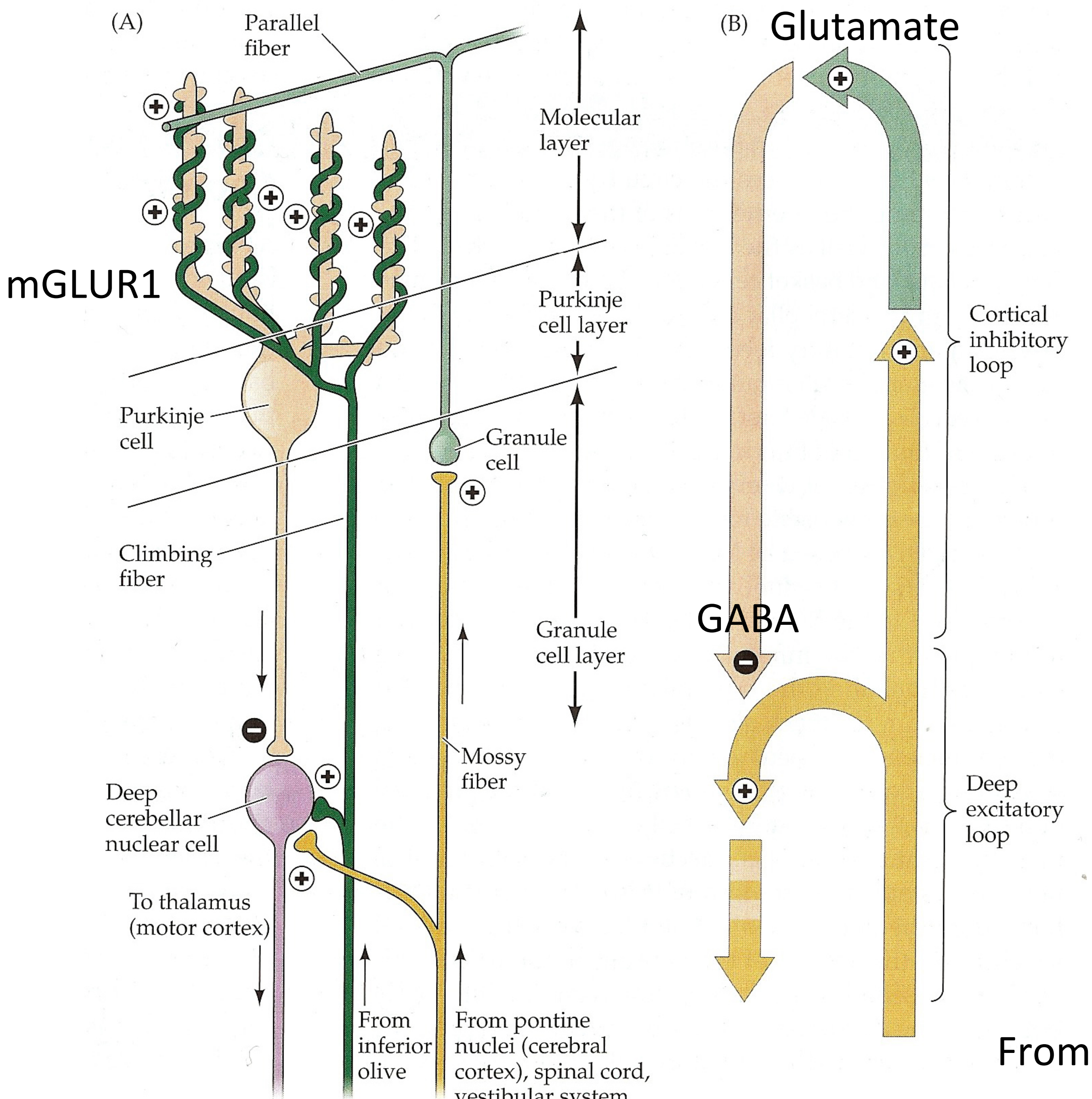


Chao et al., 2009.

The principal cerebellum neurotransmitters

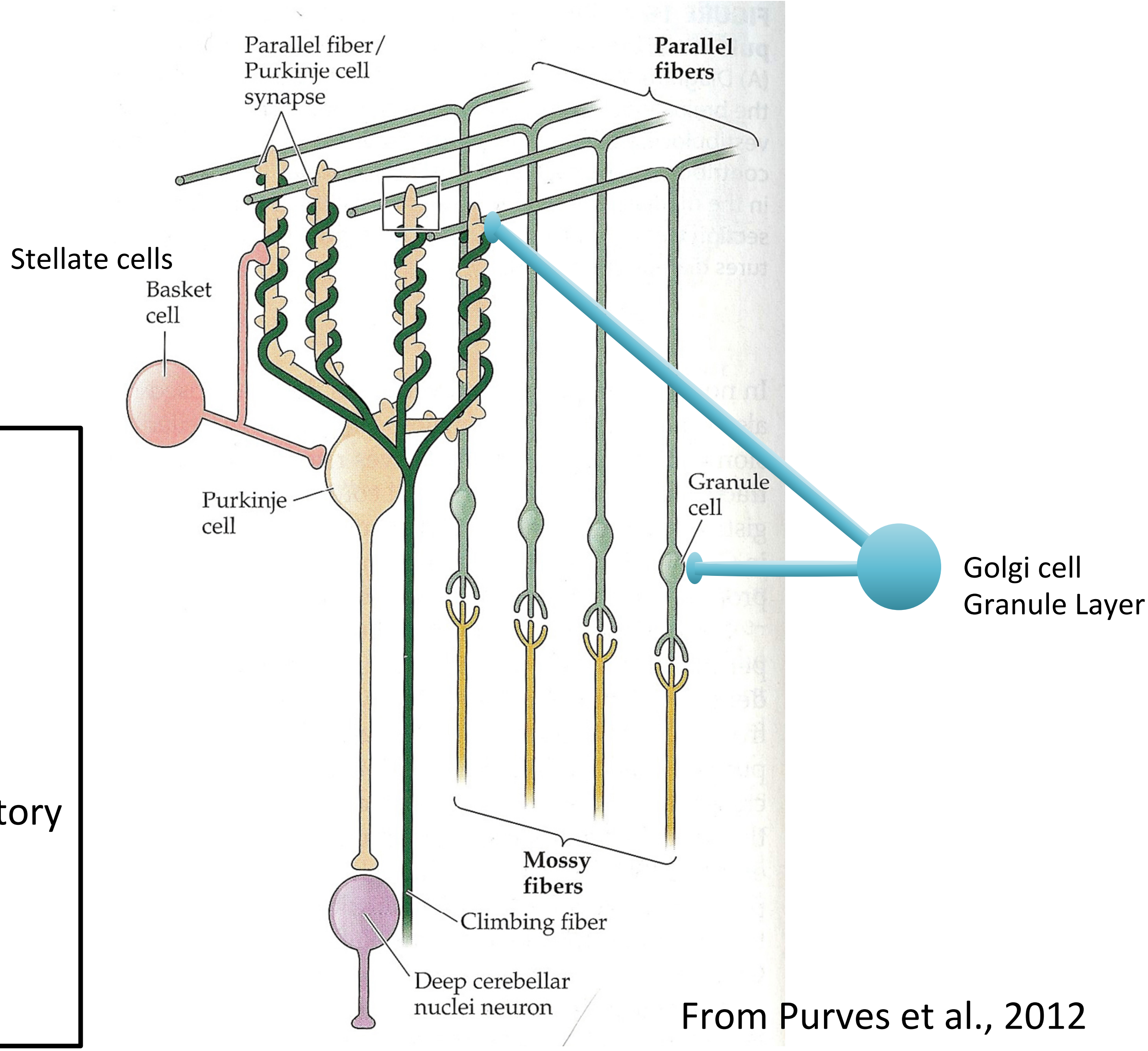
- Glutamate is the principal neurotransmitter in excitatory granule cell-Purkinje cell synapse.
- Purkinje cells release GABA and is inhibitory.

Cellular Connections in the Cerebellum



From Purves et al., 2012

Cellular Connections in the Cerebellum



From Purves et al., 2012

<u>Cerebellar Interneurons</u>

Molecular Layer

Gabaergic, inhibitory

- Basket cells
- Stellate cells

Granule Layer

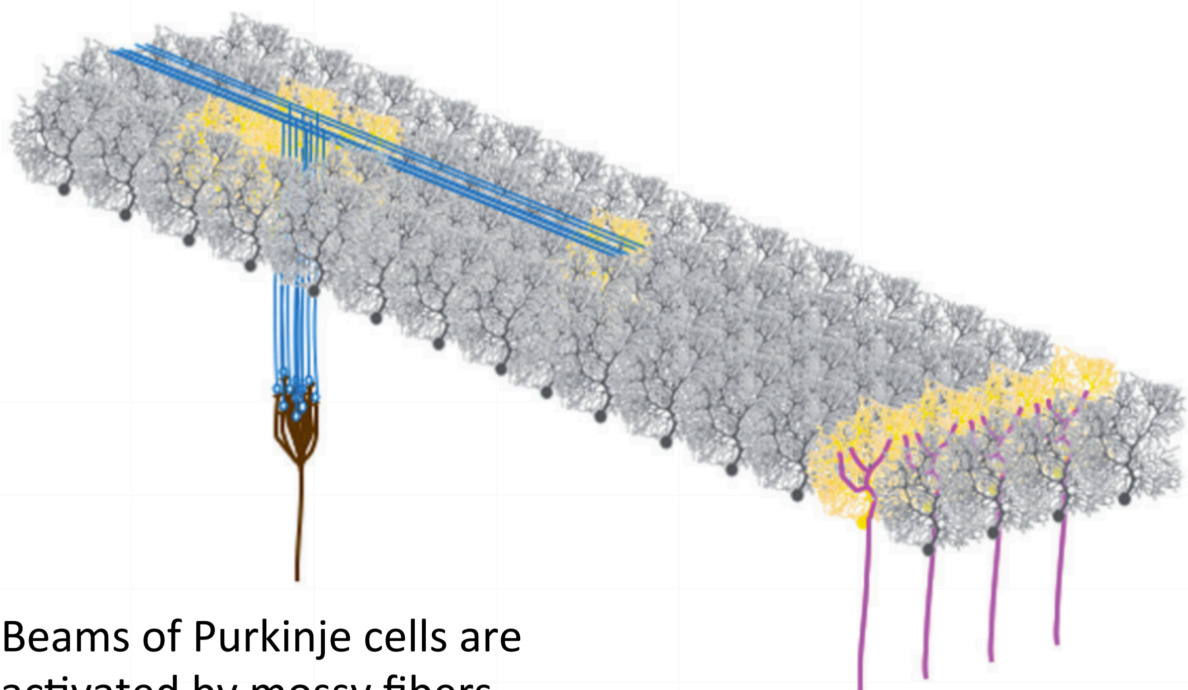
Glutamatergic, excitatory

- Brush cells

Gabaergic, inhibitory

- Golgi cells
- Lugaro cells

Beams and Parasagittal Bands of Purkinje Cells



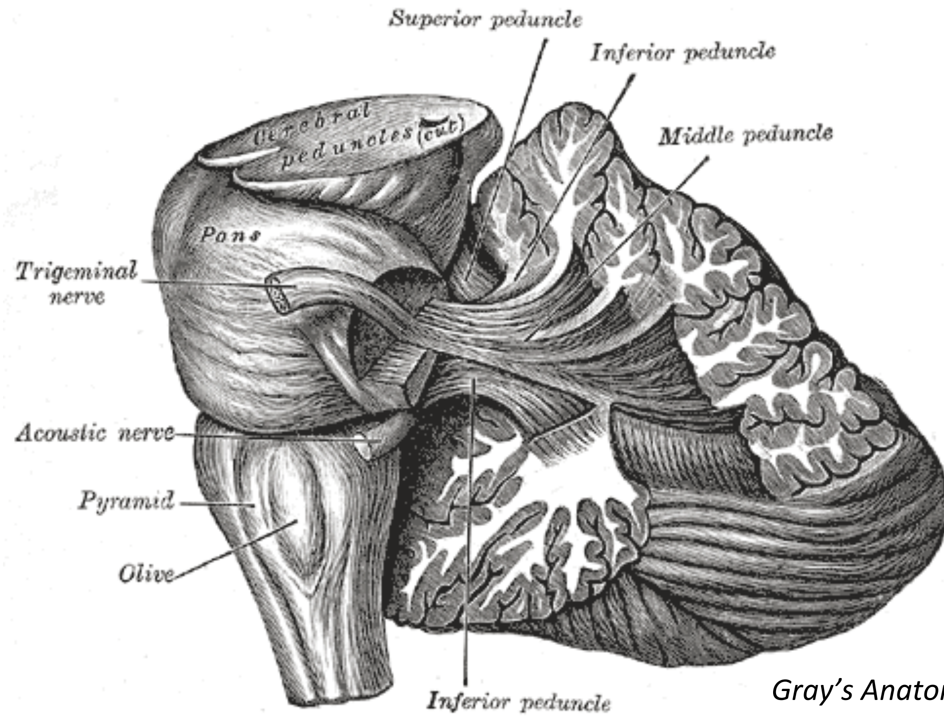
Beams of Purkinje cells are activated by mossy fibers

Parasagittal bands of Purkinje cells are activated by climbing fibers

Rokni D, Llinas R & Yarom Y. The morpho/functional discrepancy in the cerebellar cortex: looks alone are deceptive. Frontiers in Neuroscience 2(2):192-8 (2008).

Cerebellar Peduncles

Peduncle means stalk.

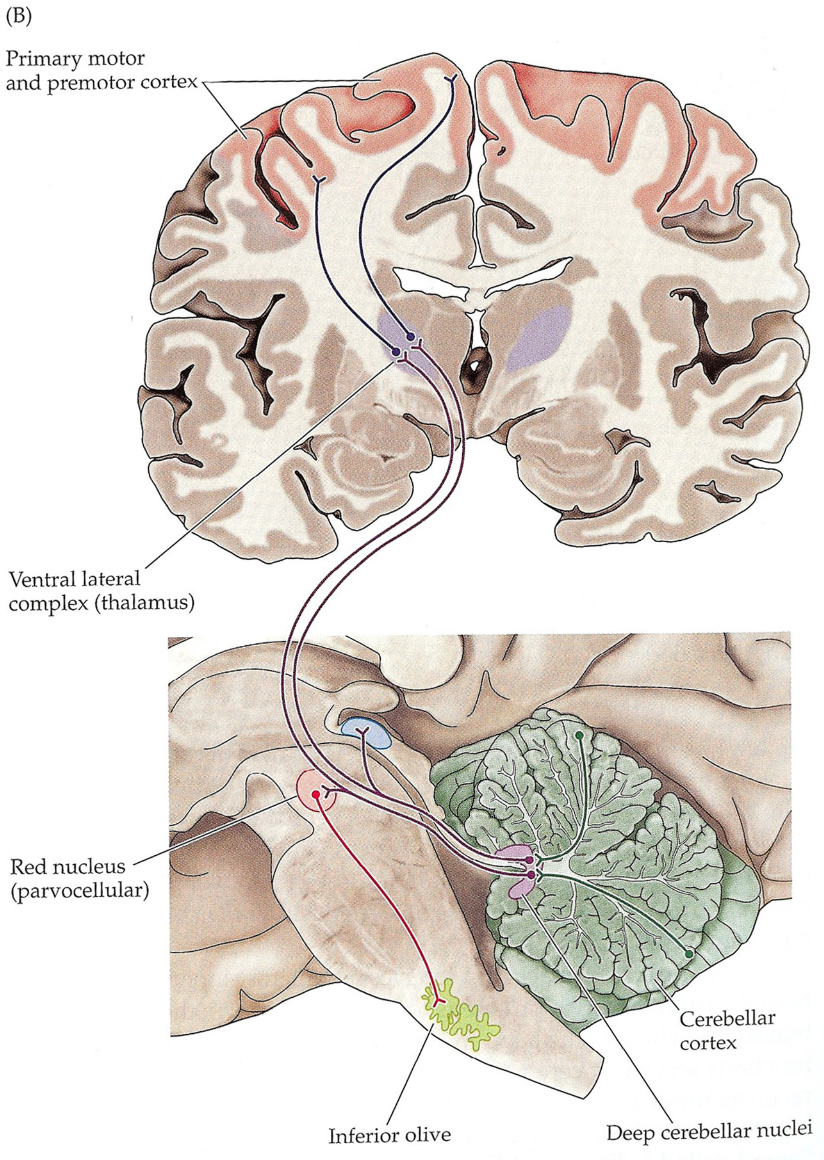


Gray's Anatomy

The cerebellar peduncles are white matter tracts connecting the cerebellum and the remaining CNS (5). They occur in 3 pairs:

- Inferior peduncles communicate sensory information about limb positions.
- Middle peduncles communicate information on the desired limb positions.
- Superior peduncles communicates information to the midbrain (pons, medulla oblongata) and spinal cord to stimulate or inhibit muscles into the desired positions.

Main Cerebellar Outputs (efferents)



Inputs synapse in Deep Cerebellar Nuclei

Red Nucleus

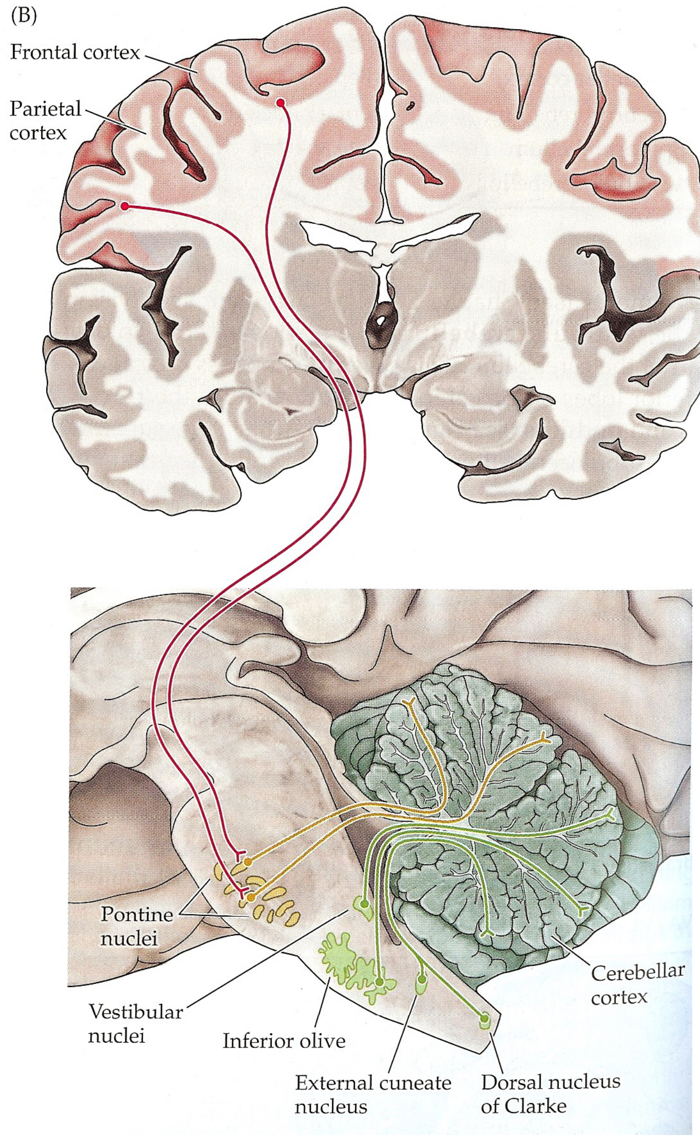
Output from DCN to Inferior Olive to SC

Thalamus

Output from DCN to Motor Cortex

From Purves et al., 2012

Main Cerebellar Inputs (afferents)



Inputs do not synapse in Deep Cerebellar Nuclei

Pontine Nuclei

Mossy Fiber input from the Motor Cotex to the Cerebellar Cortex

Inferior Olive

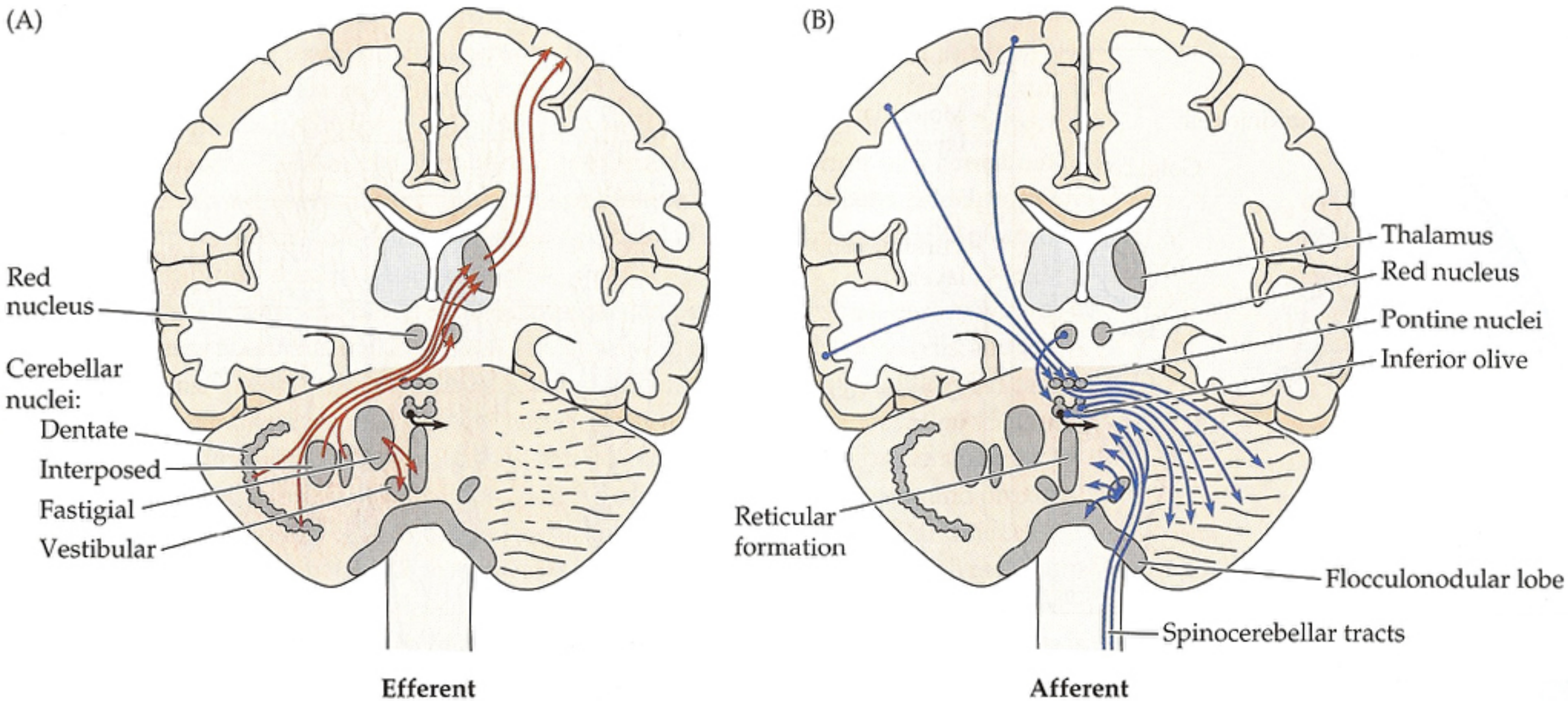
Climbing fiber input from the Spinal Cord to the Cerebellar Cortex

Spinocerebellar Tract

Mossy fiber input from the spinal cord

From Purves et al., 2012

Cerebellar afferents & efferents cross, but not the spinocerebellar tracts.



Nicholls et al., 5th Ed. 2012
From Neuron to Brain

Spinocerebellar tracts travel the same side of the body. This is a reason for a key concept in clinical neurology:

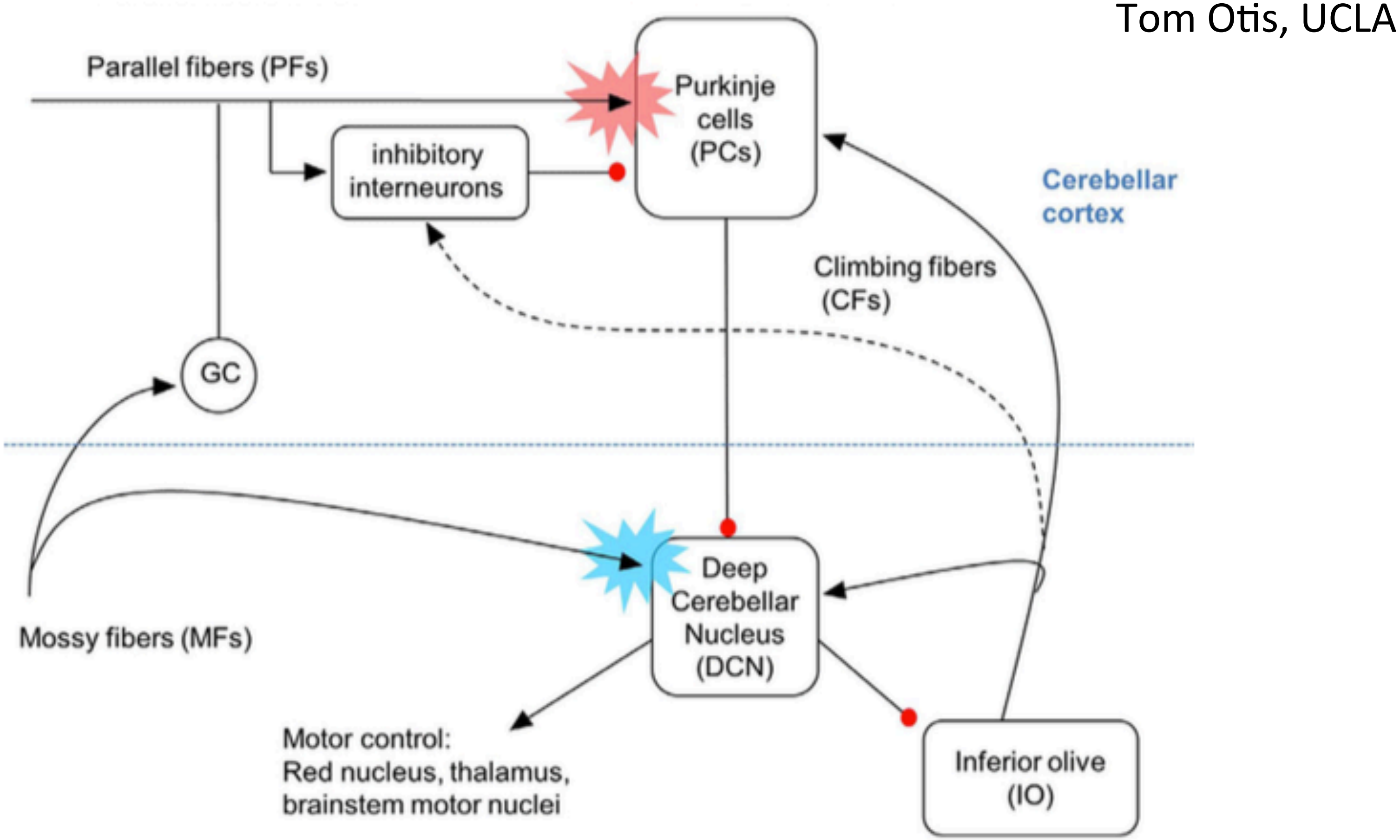
Cerebellar signs are ipsilateral.

Some key points:

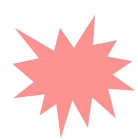
- All outputs originate from PCs and connect in the DCN.
- Mossy Fiber and Climbing Fiber inputs activate the cortical inhibitory loop at Granule Cells and Purkinje cells.
- Mossy Fibers and Climbing Fibers activate the deep excitatory loop at the DCN.
- Mossy fiber inputs to DCN and Parallel Fiber inputs to PCs are hypothesized points of synaptic plasticity in motor learning:

The Cerebellar Circuit (Meera et al., submitted)

Tom Otis, UCLA



Hypothesized sites of CF-instructed, associative forms of synaptic plasticity required for associative motor learning:

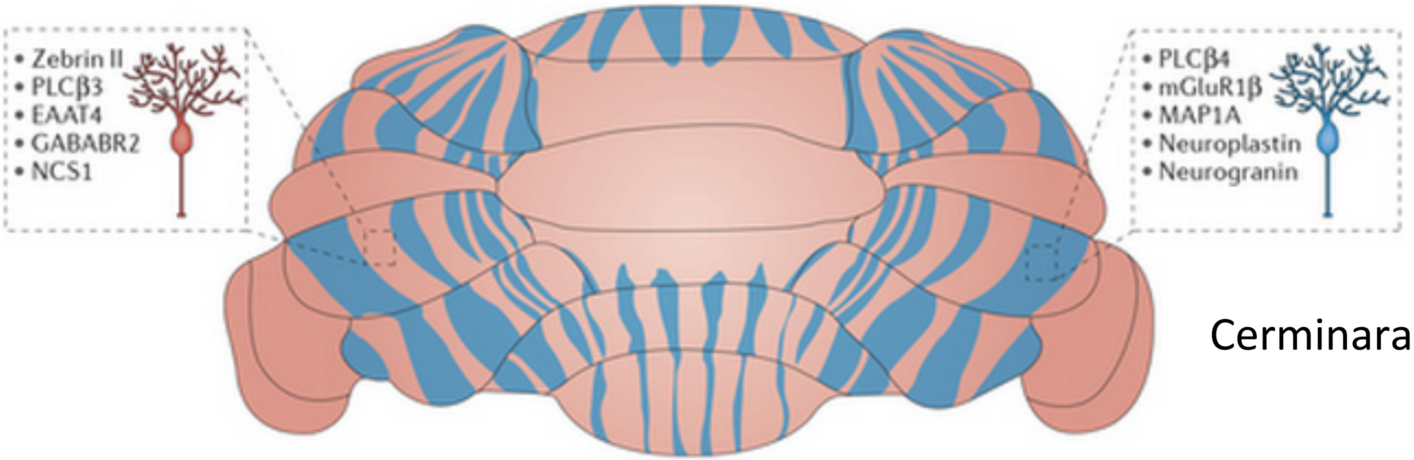


LTD of PF inputs

LTP of MF inputs

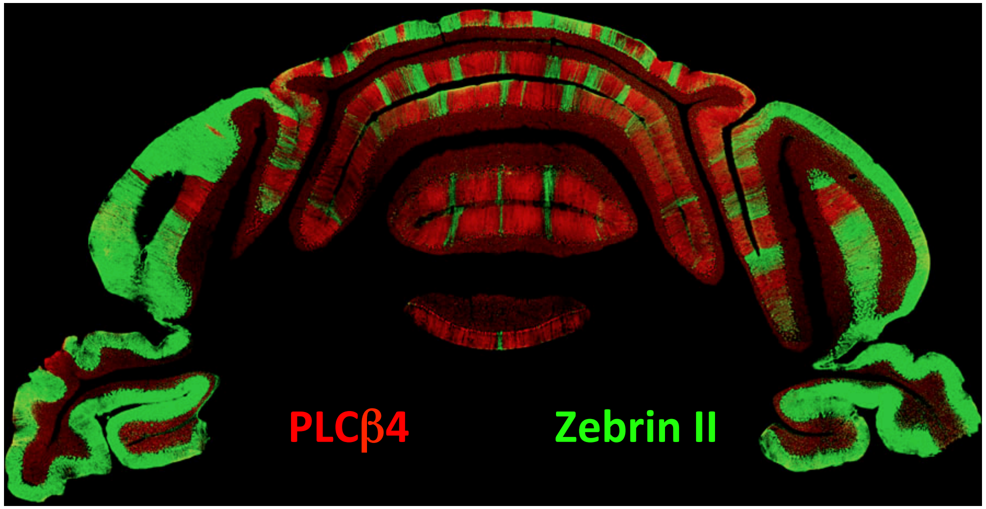
Purkinje Cell Compartmentation

Observations that the cerebellar cortex is not homogeneous has recently led to the discovery of microcircuits that are redefining the classical view of cerebellar circuitry.



Cerminara et al., 2015

From https://www.bcm.edu/research/labs/roy-sillitoe/gallery

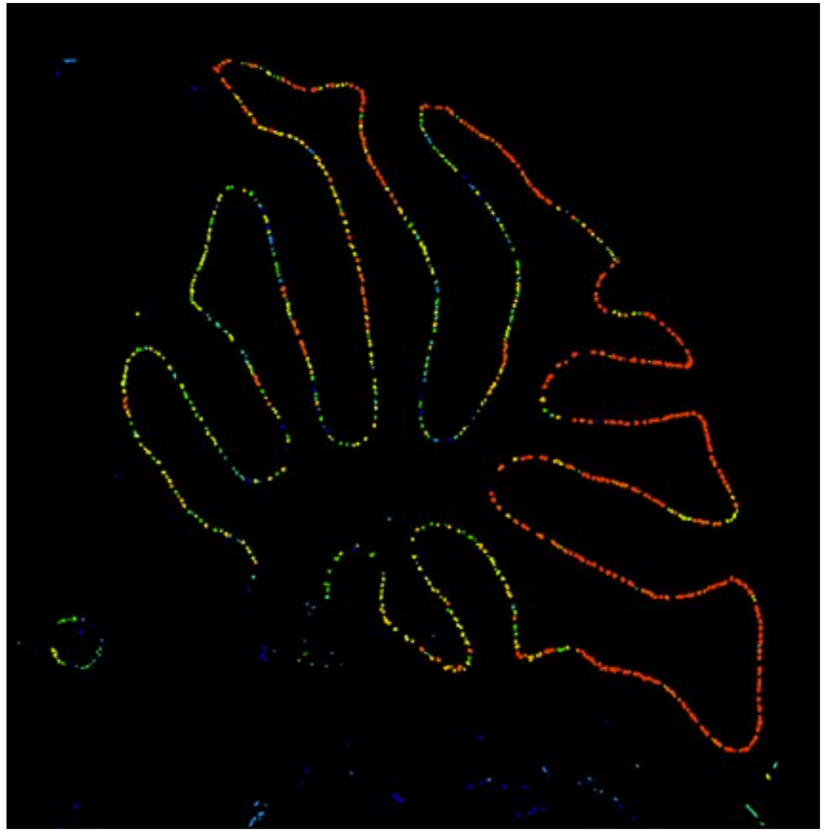


All Purkinje cells are not created equally. For anybody doing research on the cerebellum, this has relevance to how you preform your dissections.

Compartmentation seen for PLCβ3 in the Allen Brain Atlas

http://mouse.brain-map.org/

ISH veiw



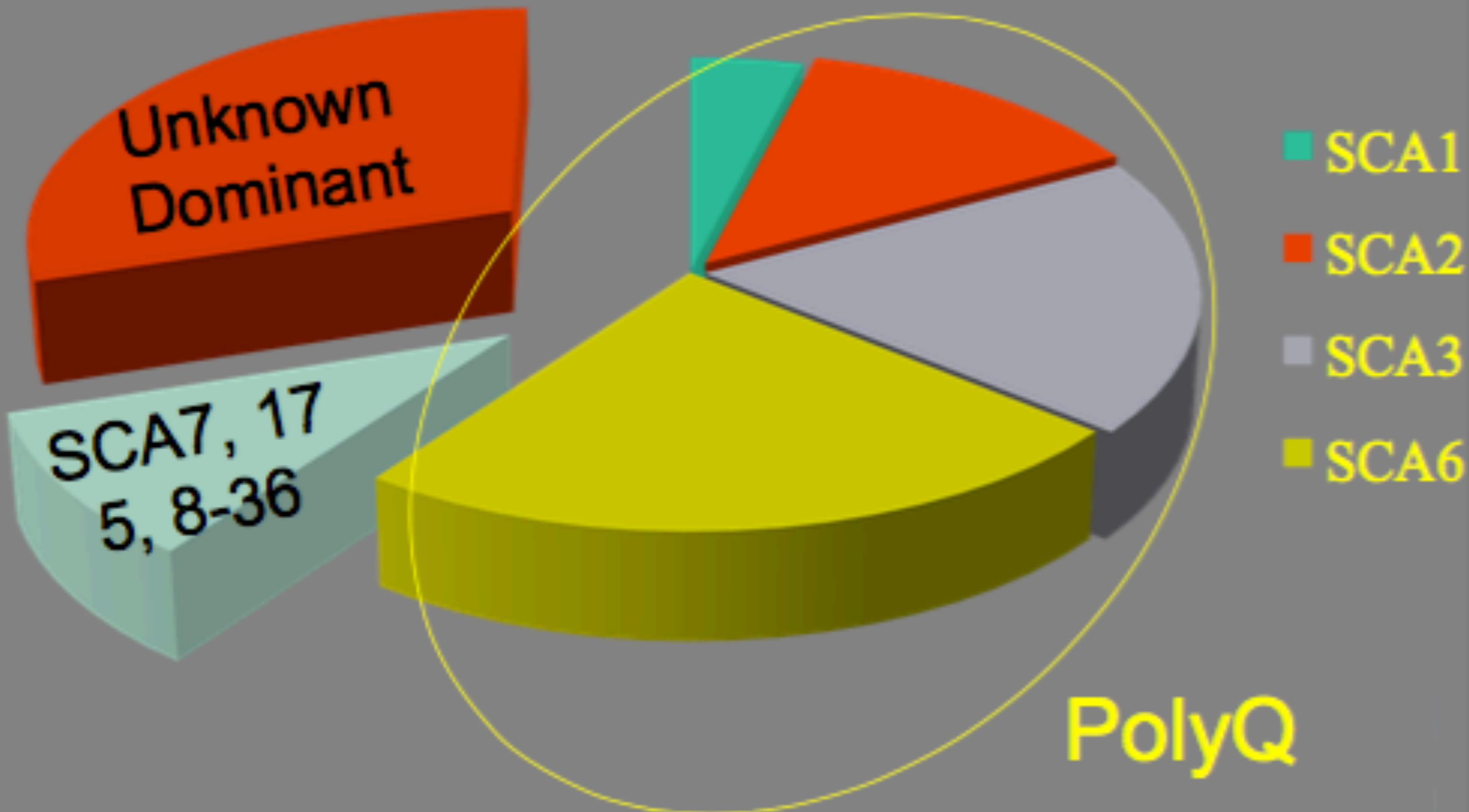
Expression mask

PART II

Spinocerebellar Ataxias

- Spinocerebellar Ataxias
- Neurodegenerative Disorders
- Affect primarily cerebellum
- Often Purkinje cells
- Autosomal dominant

Genetic Architecture of SCAs



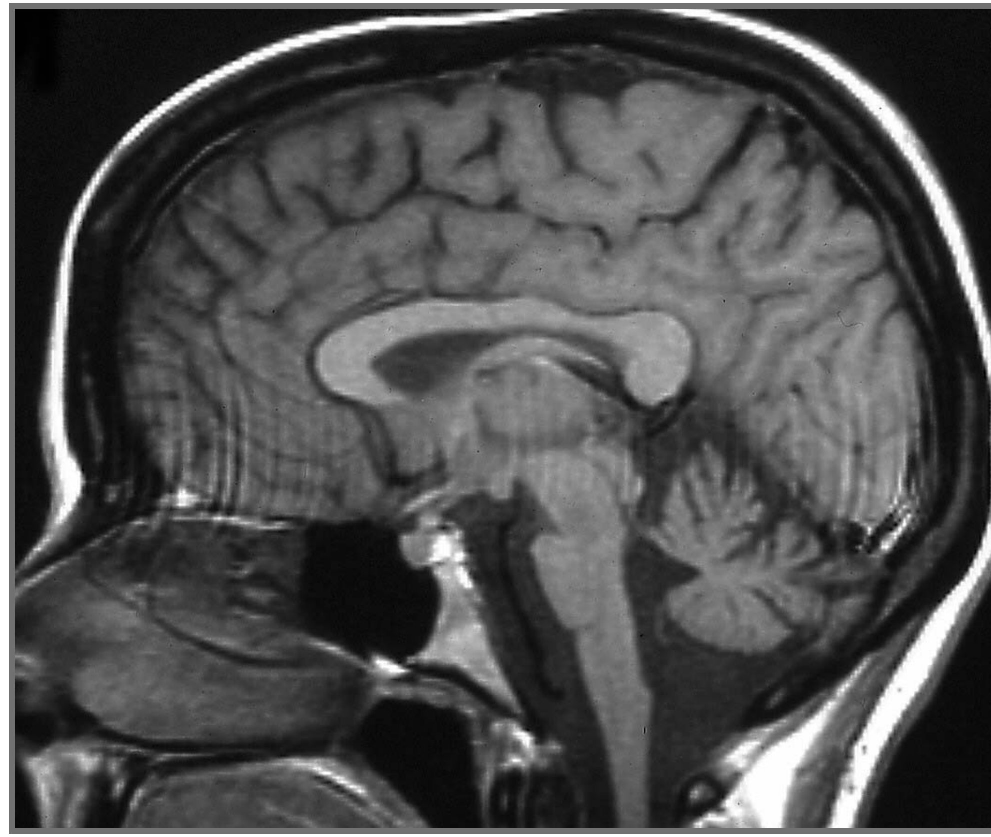
Non-polyQ Ataxias

• EA2	CACNA1a
• SCA5	beta3-spectrin
• SCA10	toxic RNA
• SCA11	Kinase (TTBK2)
• SCA13	Voltage- gated K^+ - channel (KCNC3)
• SCA14	Kinase (PKCγ)
• SCA15/16	Ca^{++} release (ITPR1 LoF)
• SCA19/22	Voltage- gated K^+ - channel (KCND3)
• SCA20	Dup 11q (260kb)
• SCA23	Prodynorphin
• SCA27	FGF14 LoF
• SCA28	mitochondrial AAA protease
• SCA31 & 36	toxic RNA
• SCA35	transglutaminase TGM6

The SCA2 Gene

Normal: 22Q
Mutant: ≥32Q

- CAG Repeat codes for glutamine (Q).



Ataxia Plus
Slow saccadic eye movements

More or less pure
DOPA-responsive PD

ALS-like

Pulst et al, Nature Genet 1996

SCA2

- Caused by CAG repeat expansion mutation in the *ATXN2* gene, encoding "ataxin-2"

- The normal gene has CAG repeat of structure interrupted by CAAs:

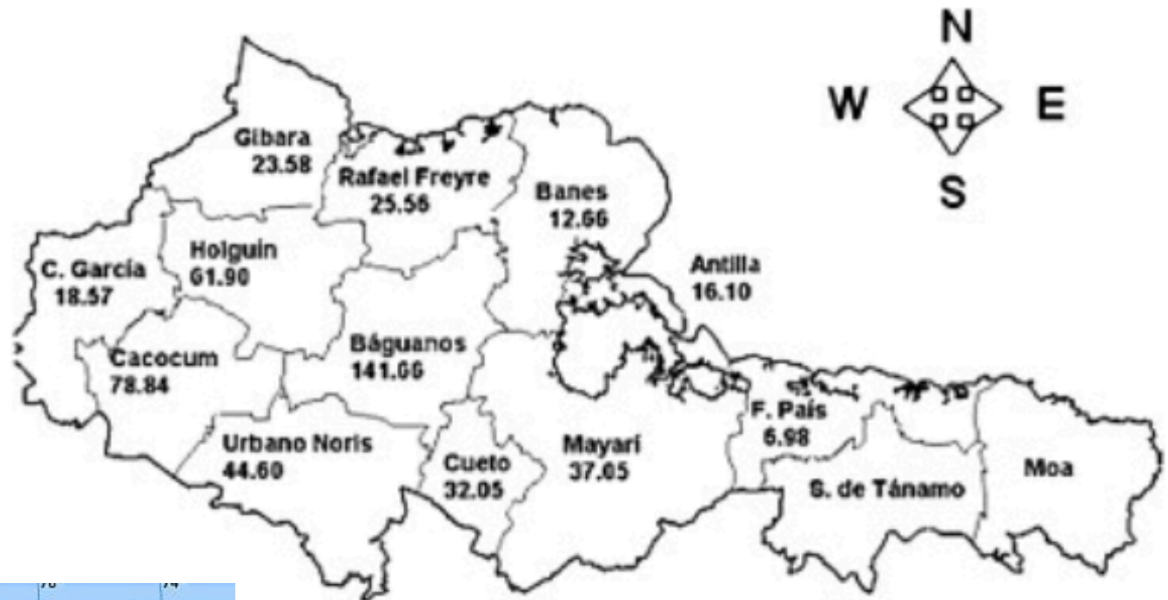
  $(CAG)_8CAA(CAG)_4CAA(CAG)_8$

- The mutant *ATXN2* has pure CAGs of lengths from 33 to >200 repeats

  $(CAG)_n$ Stablity is lost

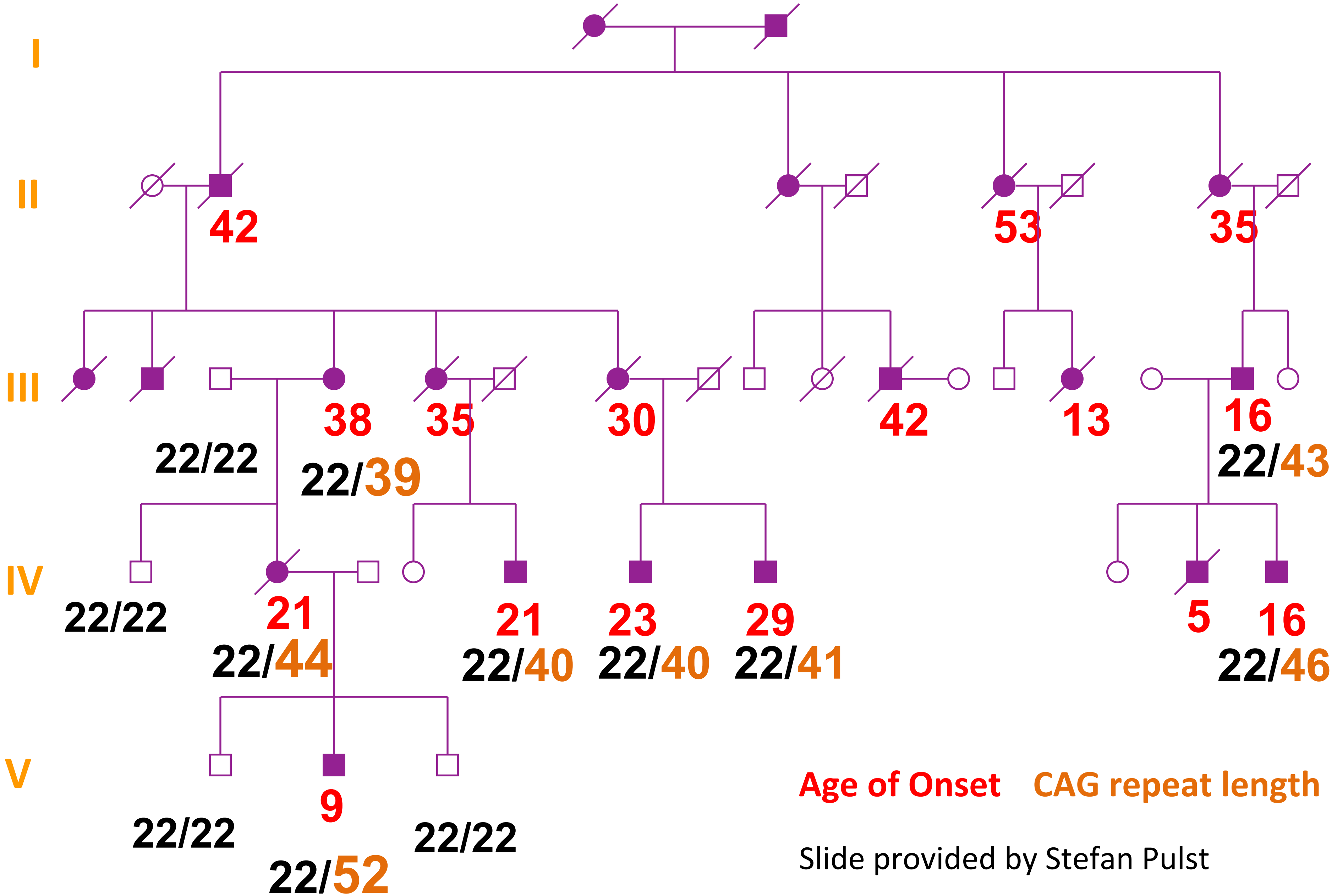
- The normal ataxin-2 interacts with RNA binding proteins: A2BP1/FOX1, PABP1, the ALS protein TDP-43

Prevalence rate in Eastern Cuba (per 100,000 people)



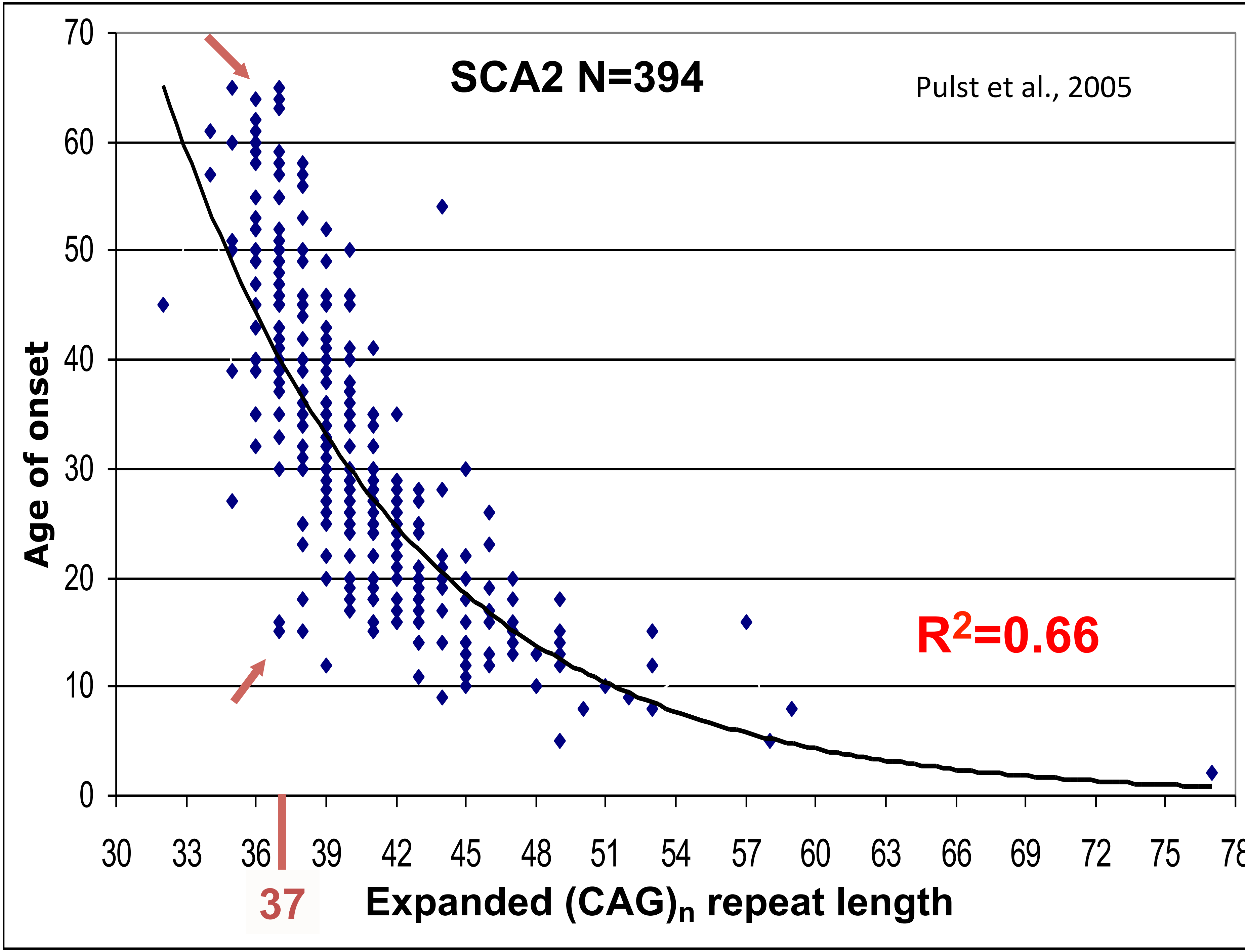
Velazquez Perez et al. Neurosci. Lett. 454:157-60; 2009.

Dynamic Mutation as the Cause of Anticipation

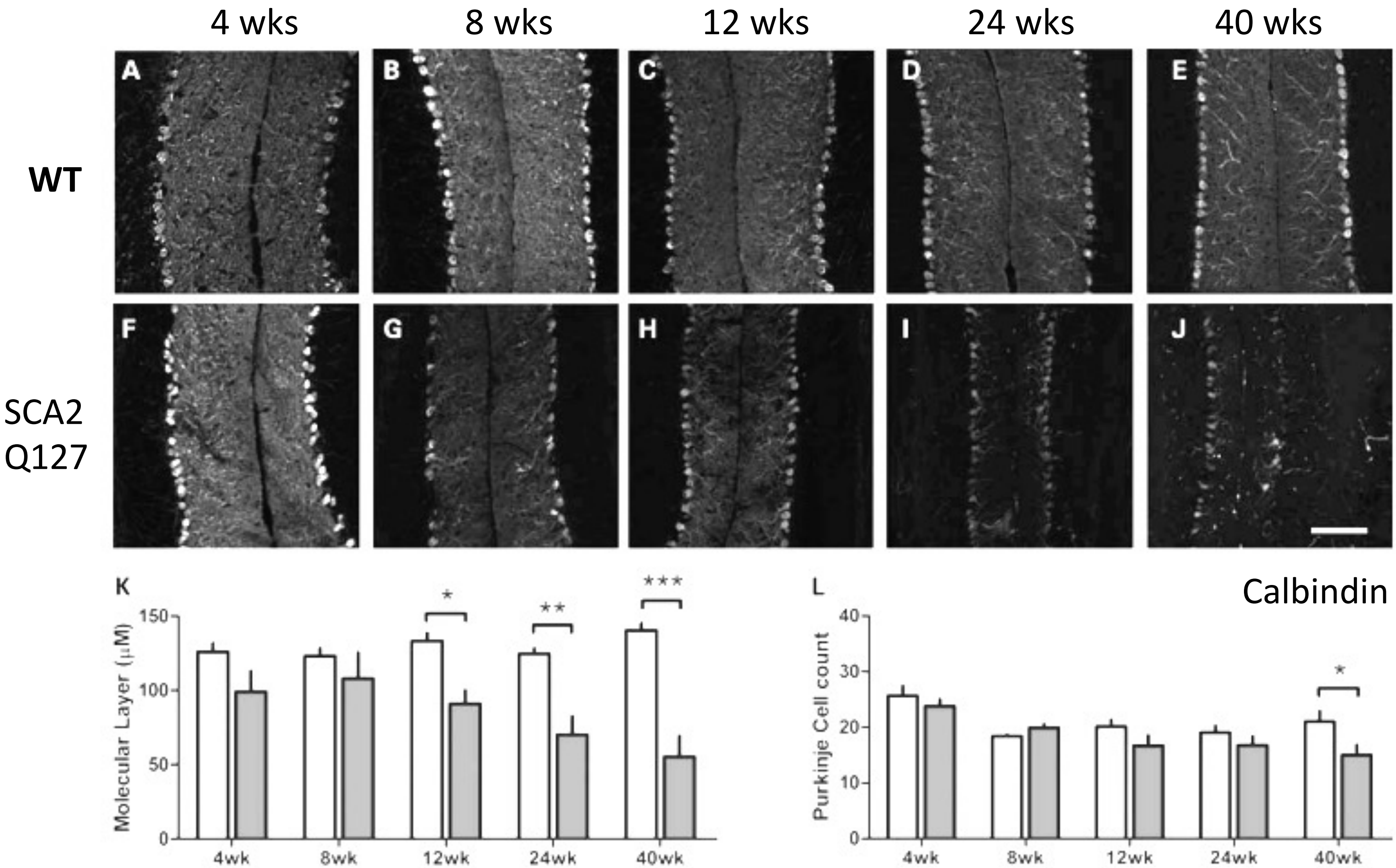


Age of Onset CAG repeat length

Slide provided by Stefan Pulst

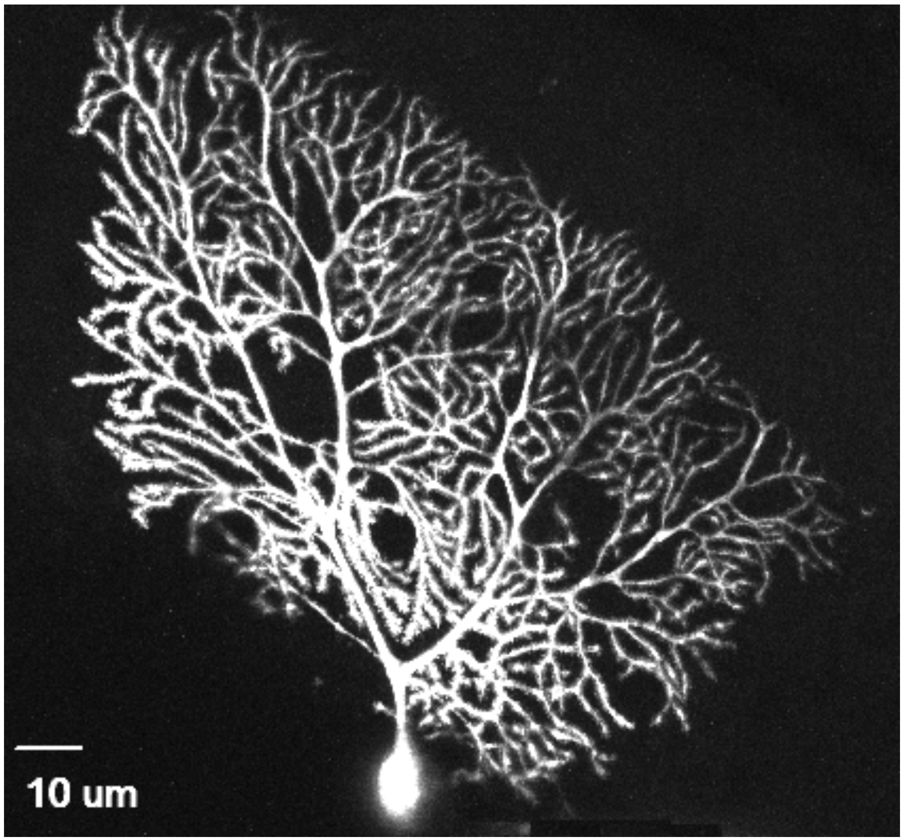

SCA2 N=394
Pulst et al., 2005
R^2=0.66
Age of onset
70
60
50
40
30
20
10
0
30
33
36
39
42
45
48
51
54
57
60
63
66
69
72
75
78
37
Expanded $(CAG)_n$ repeat length

Age dependent reduction of Cerebellar molecular layer thickness in SCA2 mice.

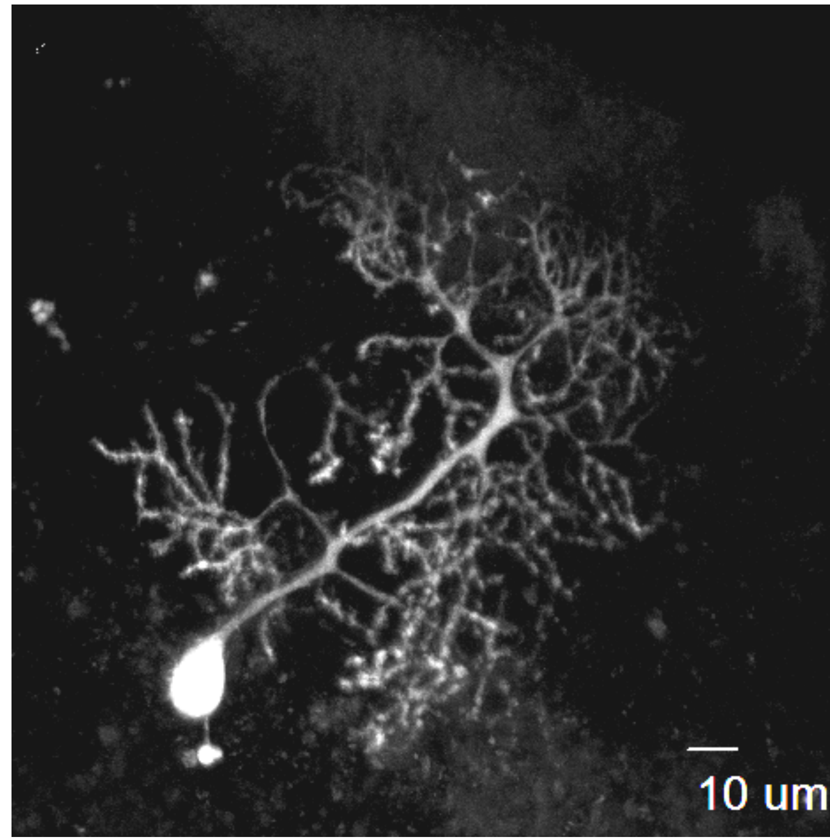


Hansen et al., 2012

Purkinje neuron morphology



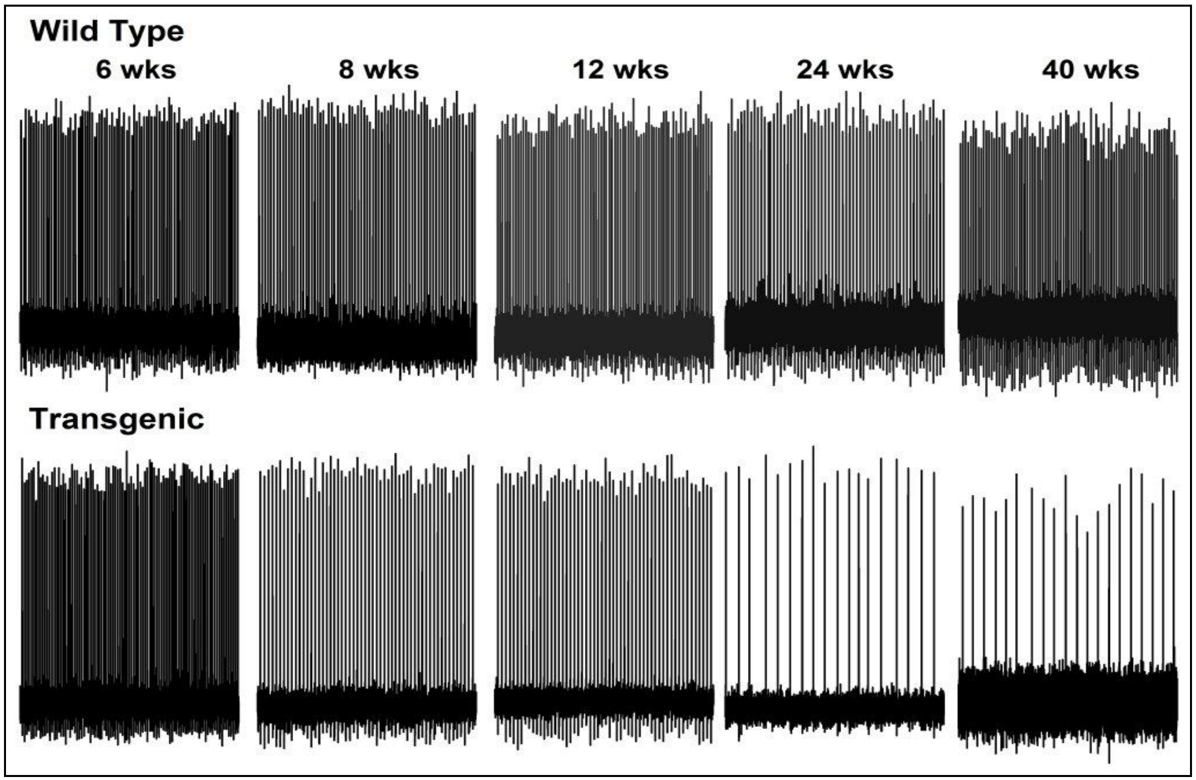
WILDTYPE

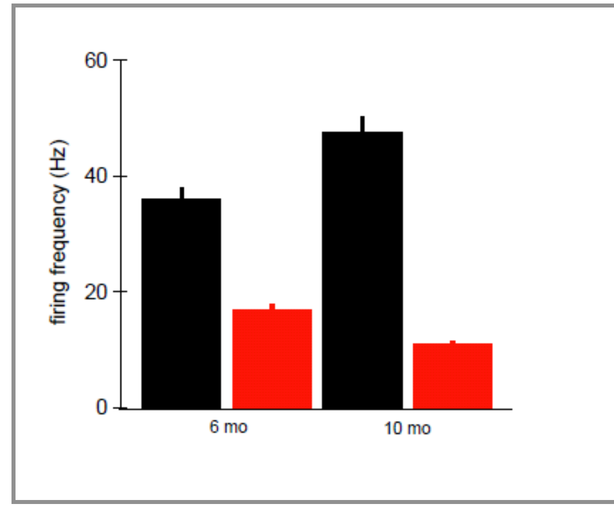


ATXN2-Q127, 24 wks

PC Firing is abnormal in SCA2 tg Mice.

Extracellular recordings from acute slices





Red=Q128
Black=wt

Tom Otis & Pratap Meera
Hansen et al., 2013

SCA2 Therapeutics

Spinocerebellar Ataxia Type 2 (SCA2)

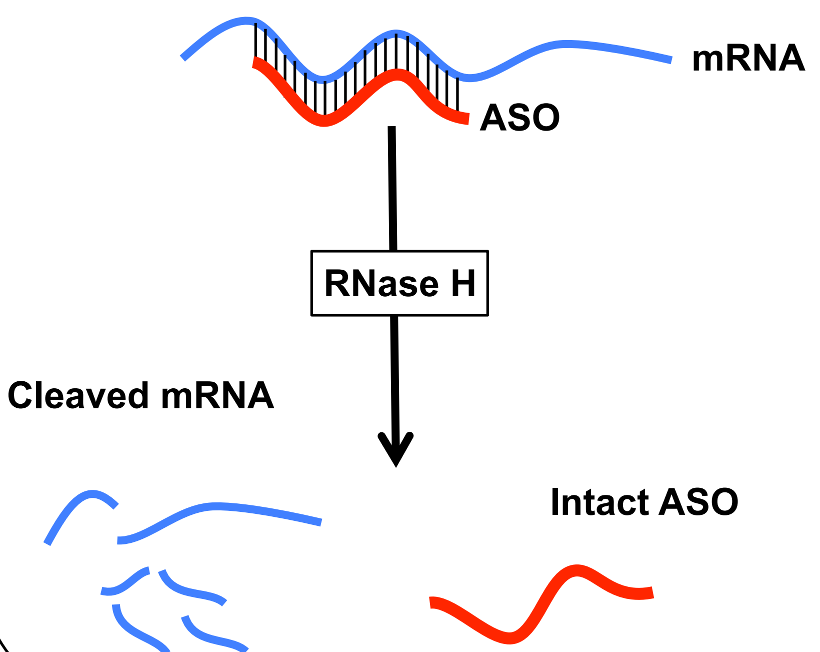
- **SCA2 is a dominantly inherited polyglutamine disorder caused by *ATXN2* mutation that causes ataxia.**
- **Characterized by gain of toxic function & Purkinje cell death.**
- **We hypothesize that lowering *ATXN2* expression will be therapeutic for SCA2**

Antisense oligonucleotide (ASO) structure and action

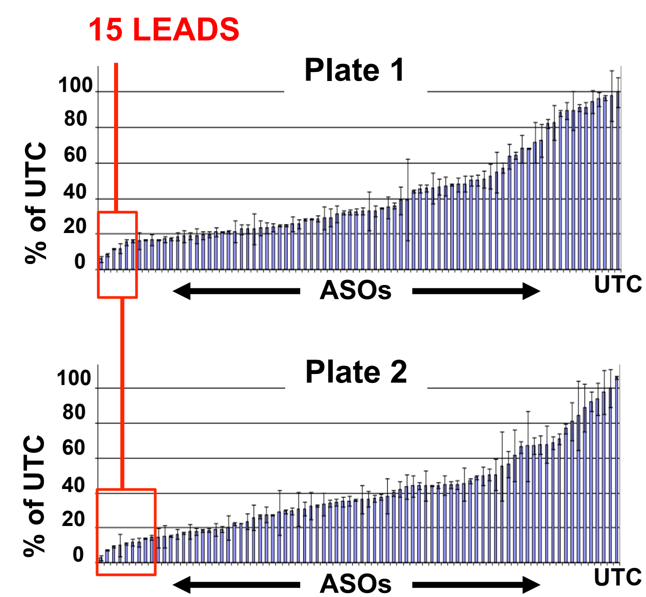
MOE Gapmer

MOE	PS	MOE
5 bp	10 bp	5 bp

ASOs stimulate RNase H



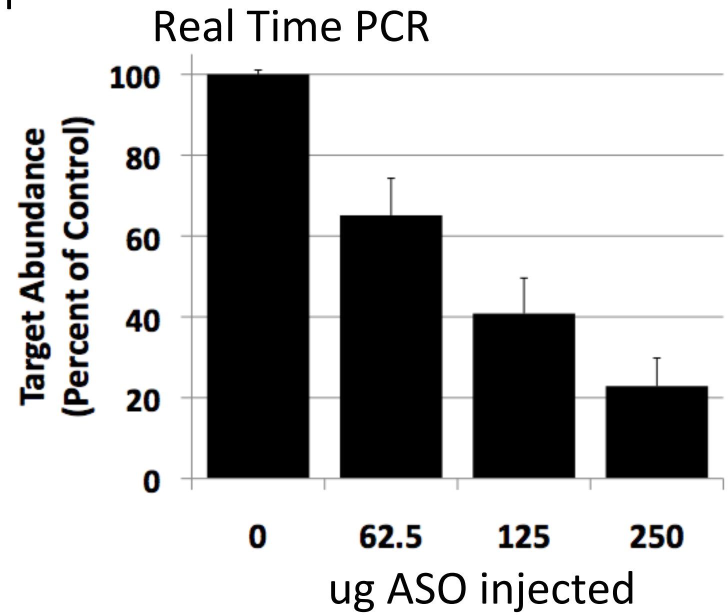
152 ASOs screened *in vitro* for lowering *ATXN2* expression

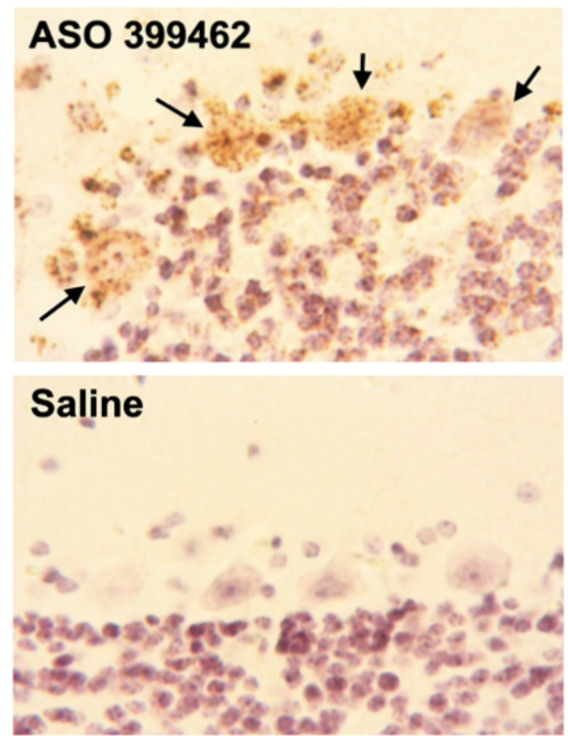


4.5 μM ASO in HepG2 cells

Isis Pharmaceuticals

Antisense oligonucleotides inhibiting *ATXN2* expression
Collaboration with ISIS Pharmaceuticals

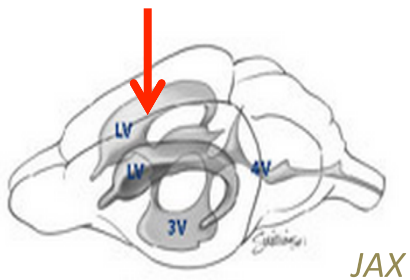




Clinical trials

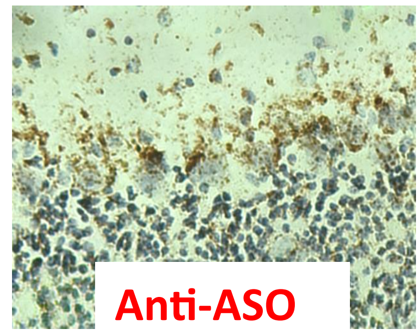
In vivo tests using *Pcp2-ATXN2*-Q127 mice

Intracerebroventricular (ICV) Injection



Right Lateral Ventricle

Injected ASOs accumulated in Purkinje cell layers

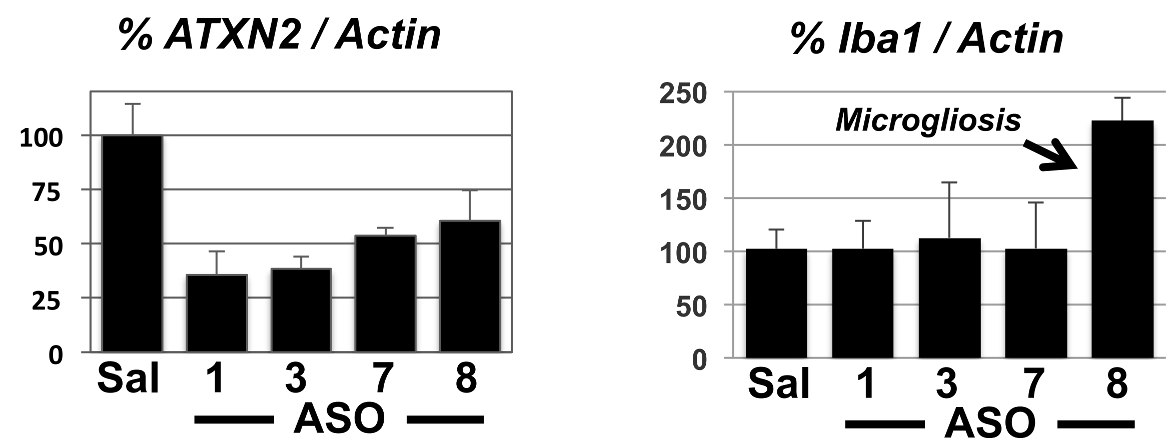


Molecular layer

Purkinje cell layer

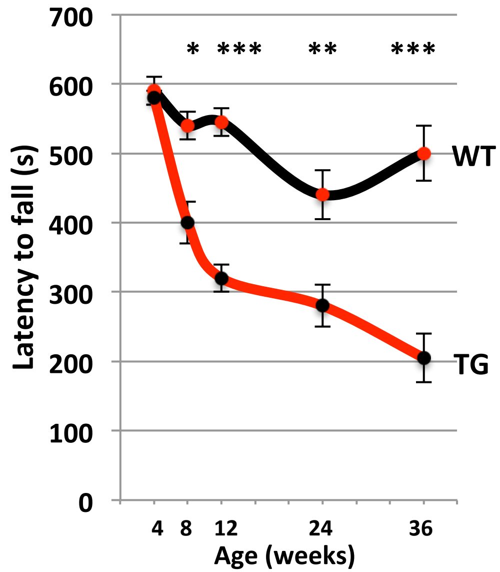
Granule layer

Cerebellar *ATXN2* and *Ibal by qPCR* after 7 days treatment with 250 μg ASO

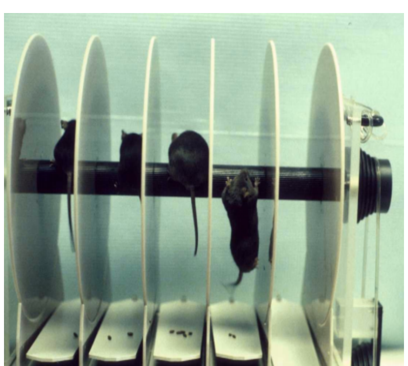


ATXN2 ASO reduced rotarod phenotype onset in *ATXN2*-Q127 mice

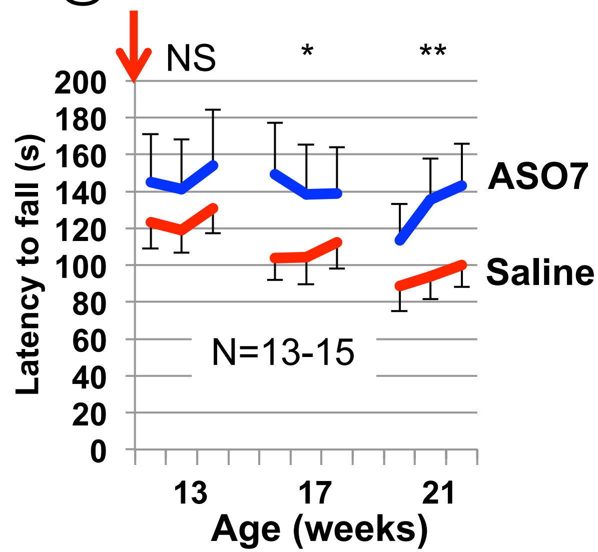
***ATXN2*-Q127**
Rotarod Phenotype



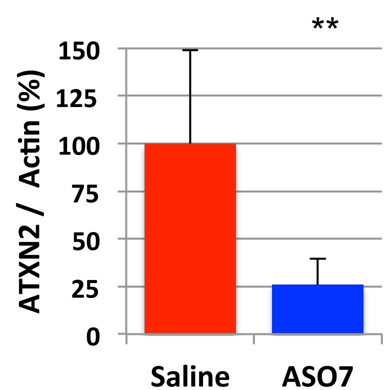
Hansen et al., 2012



210 µg ASO7
ICV@ 8wks



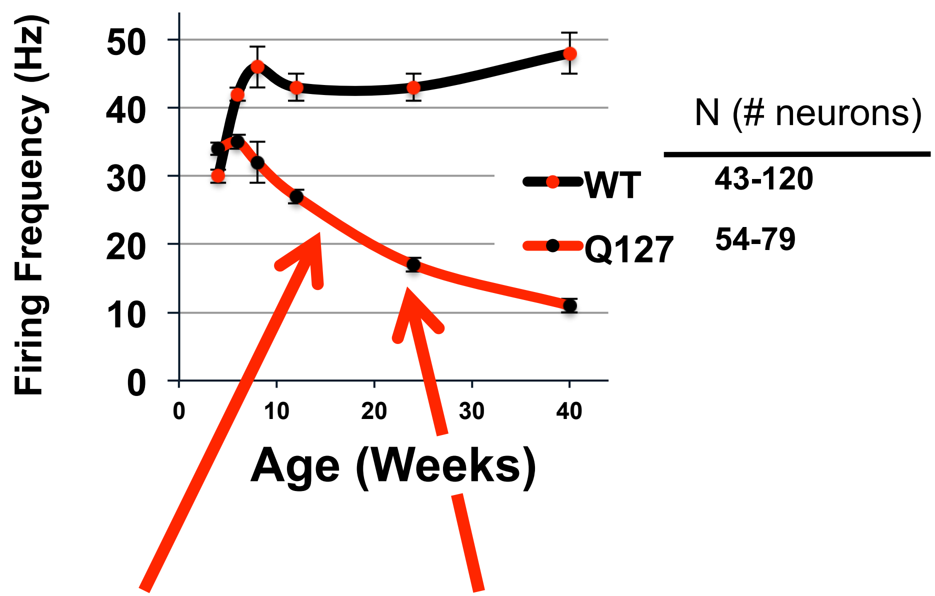
Endpoint cerebellar human *ATXN2* expression by qPCR



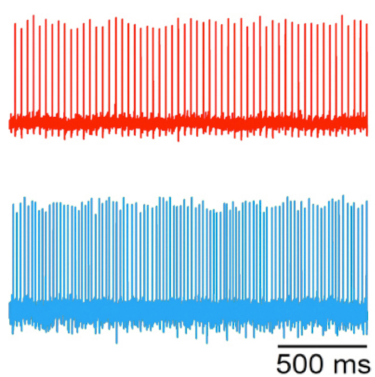
ASO7 restored the Purkinje cell firing frequency of Q127 mice

Extracellular Recordings – Meera Pratap & Tom Otis

Hansen et al., 2012



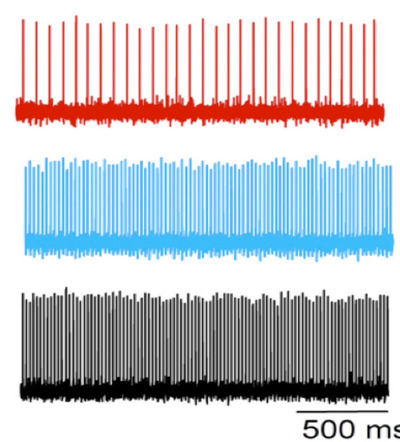
13 wk old mice treated 5 wks



Saline — 25±1 Hz, N=50 neurons

ASO7 — 46±2 Hz, N=88 neurons

21 wk old mice treated 13 wks



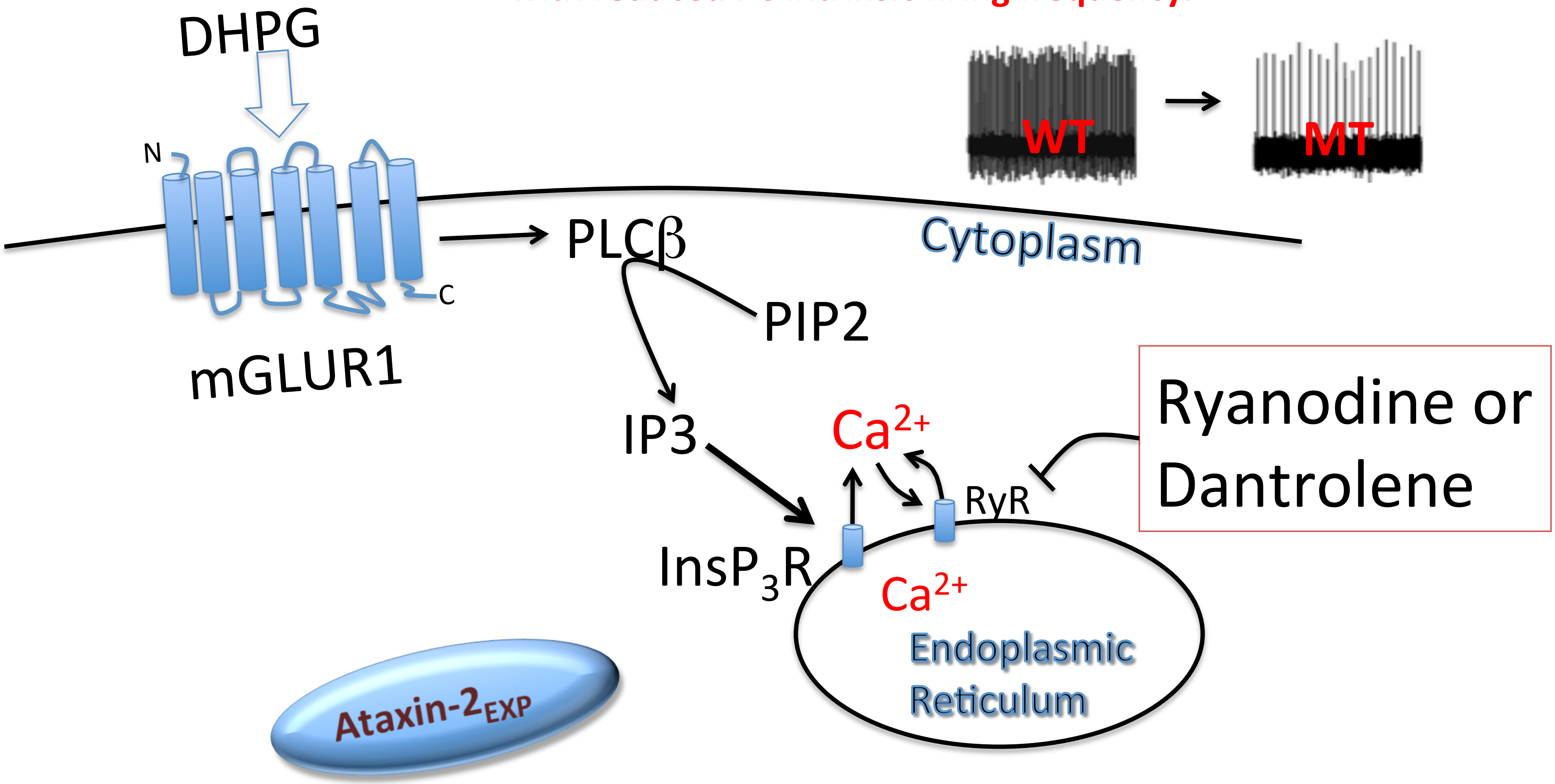
Saline — 16±1 Hz, N=107 neurons

ASO7 — 42±2 Hz, N=102 neurons

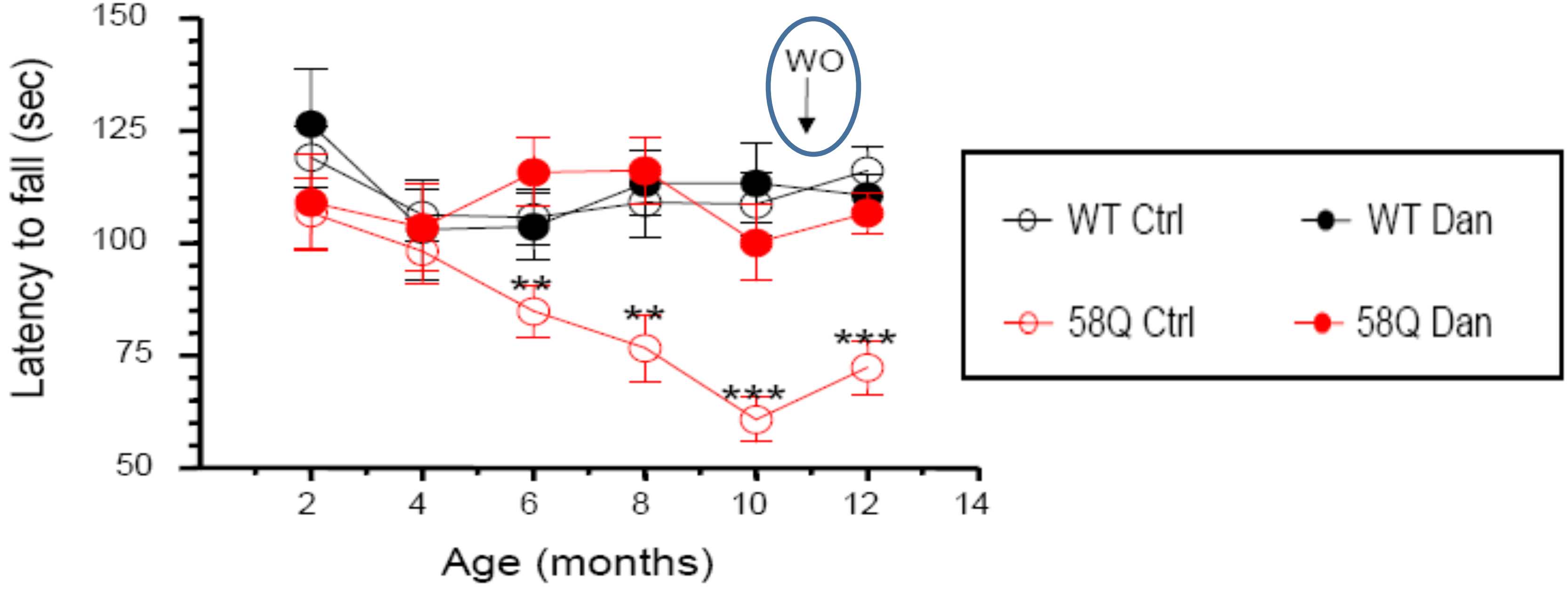
WT — 40±1 Hz, N=50 neurons

Ataxin-2 action on Ca^{2+} movement in Purkinje cells from *ATXN2* transgenic mice

Elevated cytoplasmic calcium is associated pathologically with reduced PC intrinsic firing frequency.



Liu J et al J Neurosci 2009


Latency to fall (sec)
150
125
100
75
50
WO
**
**
***
***
2
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14
Age (months)
WT Ctrl
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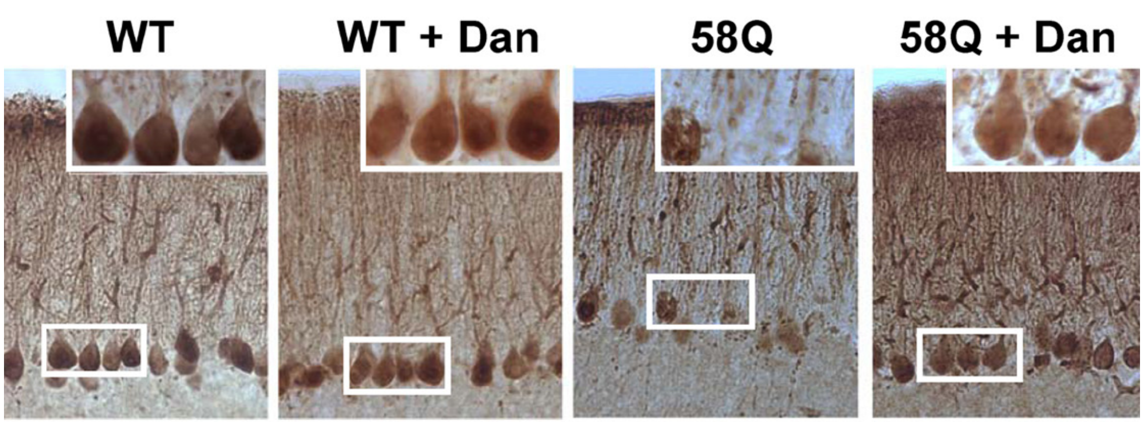
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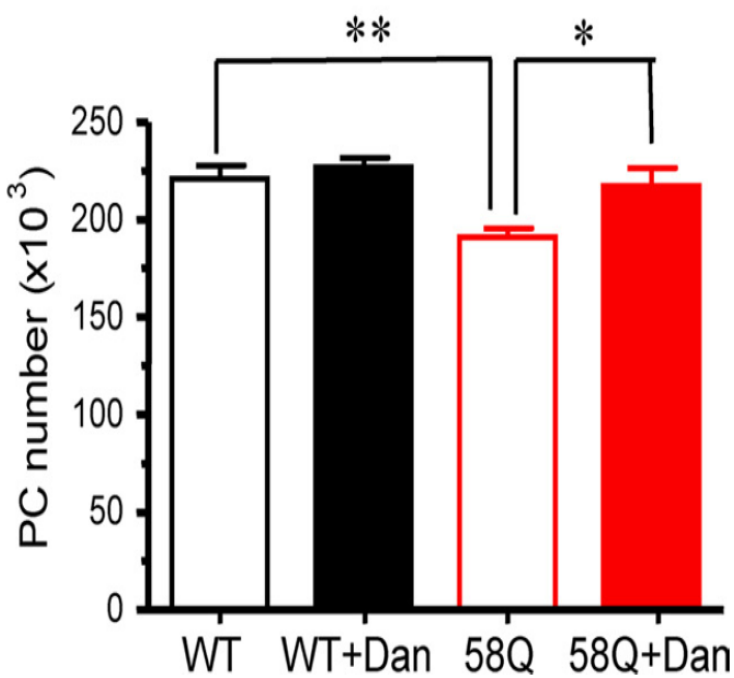
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Video of SCA2 Patients

(video will not play in this version)

Cerebellar disorders

- Spinocerebellar Ataxias
  - SCA1-SCA39,
  - Dominantly inherited
  - Polyglutamine: SCA1, 2, 3, 6, 7, 17
- Friedreich Ataxia
  - *FXN* mutation, encodes frataxin, GAA repeat in intron 1
  - Recessive
- Ataxia-telangiectasia
  - *ATM* gene, LOF point mutations
  - Recessive
  - Lymphoid and other tumors, p53 LOF related.
- Episodic Ataxias
  - Different genes
  - Most commonly recessive
- Paraneoplastic Cerebellar Degeneration
  - Commonly cancer related

The cerebellum contributes to cognition: Schmahmann Syndrome

Schmahmann JD. The role of the cerebellum in cognition and emotion: personal reflections since 1982 on the dysmetria of thought hypothesis, and its historical evolution from theory to therapy. (Schmahmann et al., 2010).

Abstract

The cognitive neuroscience of the cerebellum is now an established multidisciplinary field of investigation. This essay traces the historical evolution of this line of inquiry from an emerging field to its current status, with personal reflections over almost three decades on this journey of discovery. It pays tribute to early investigators who recognized the wider role of the cerebellum beyond motor control, traces the origins of new terms and concepts including the dysmetria of thought theory, the universal cerebellar transform, and the <u>cerebellar cognitive affective syndrome</u>, and places these developments within the broader context of the scientific efforts of a growing community of cerebellar cognitive neuroscientists. This account considers the converging evidence from theoretical, anatomical, physiological, clinical, and functional neuroimaging approaches that have resulted in the transition from recognizing the <u>cerebellar incorporation into the distributed neural circuits subserving cognition and emotion</u>, to a hopeful new era of treatment of neurocognitive and neuropsychiatric manifestations of cerebellar diseases, and to cerebellar-based interventions for psychiatric disorders.

Assigned Reading

This is a short essay that discusses the cerebellum role in cognition and challenges the concept:

> Glickstein M. What does the cerebellum really do? Current Biology 17(19): R824–R827 (2007).

Compared to other parts of the brain the cerebellar circuit is simple. But the overall function of the cerebellum is complex. This discusses the cerebellum differences from other parts of the brain and its function in an “overall” context:

> Heck D & Sultan F. Cerebellar structure and function: Making sense of parallel fibers. Human Movement Science 21:411-421 (2002).

Citations

Cerminara NL, Lang EJ, Sillitoe RV & Apps R. Redefining the cerebellar cortex as an assembly of non-uniform Purkinje cell microcircuits. Nature Reviews Neuroscience 16, 79–93 (2015).

Chao DL, Ma L, Shen K. Transient cell–cell interactions in neural circuit formation. Nature Reviews Neuroscience 10, 262-271 (April 2009).

Hansen ST, Meera P, Otis TS, Pulst SM. Changes in Purkinje cell firing and gene expression precede behavioral pathology in a mouse model of SCA2. Hum Mol Genet. 2013 Jan 15; 22(2): 271-83.

Heck D & Sultan F. Cerebellar structure and function: Making sense of parallel fibers. Human Movement Science 21 (2002) 411–421.

Liu J, Tang TS, Tu HP, Nelson O, Herndon E, Huynh DP, Pulst SM, Bezprozvanny, I (2009) Deranged calcium signaling and neurodegeneration in spinocerebellar ataxia type 2. J Neurosci, 29:9148-9162.

Meera P, Pulst S, Otis TS. "Cellular and circuit mechanisms underlying spinocerebellar ataxias." Journal of Physiology Special Issue on "Ageing and Neurodegeneration: A Physiological Perspective", submitted 07-Jul-15.

Citations (cont.)

Napper RM & Harvey RJ. Number of parallel fiber synapses on an individual Purkinje cell in the cerebellum of the rat. J Comp Neurol. 1988 Aug 8;274(2):168-77.

Nicholls JG, Martin AR, Fuchs PA, Brown DA, Diamond ME, Weisblat DA. From Neuron to Brain. Sinauer Associates; 2012.

Pulst SM, Nechiporuk A, Nechiporuk T, Gispert S, Chen XN, Lopes-Cendes I, Perlman S, Starkman S, Rouleau GA, Auburger G, Korenberg, J.R., Figueroa C, Sahba S. (1996) Identification of the SCA2 gene: Moderate expansion of a normally biallelic trinucleotide repeat. Nature Genetics, 40:269-276.

Pulst SM, Santos N, Wang D, Yang HY, Huynh D, Velazquez L, Figueroa KP (2005) Spinocerebellar ataxia type 2: polyQ repeat variation in the CACNA1a channel modifies age of onset. Brain, 128:2297-2303.

Purves D, Augustine GJ, Fitzpatrick D, Hall WC, LaMantia AS, White WE. Neuroscience, 5th edition. Sinauer Associates; 2012.

Schmahmann JD. The role of the cerebellum in cognition and emotion: personal reflections since 1982 on the dysmetria of thought hypothesis, and its historical evolution from theory to therapy. Neuropsychol Rev. 2010 Sep;20(3):236-60.

Wadiche JI & Jahr CE. Multivesicular release at climbing fiber-Purkinje cell synapses. Neuron 32 (2): 301–13; 2001.

Citations of web information

List of animals by number of neurons.
http://en.wikipedia.org/wiki/List_of_animals_by_number_of_neurons

Anatomy Explorer. This directs to the cerebellar peduncle:
http://www.innerbody.com/image_nerv02/nerv62-new.html

Some zebrin stains:
https://www.bcm.edu/research/labs/roy-sillitoe/gallery

Any papers by Stefan Pulst and colleagues can be downloaded from here:
http://pulstlab.genetics.utah.edu/Publications.html

eDoctor Online medical atlas. Gross anatomy on the cerebellum.
http://www.edoctoronline.com/medical-atlas.asp?c=4&id=21803

Glossary Terms

From:

http://sites.sinauer.com/neuroscience5e/flashcards19.html

Asterisks represent the more important terms:

- *Basket Cells*

Inhibitory interneurons in the cerebellar cortex whose cell bodies are located within the Purkinje cell layer and whose axons make basketlike terminal arbors around Purkinje cell bodies.

- *Cerebellar Ataxia* *

A pathological inability to make coordinated movements, associated with lesions to the cerebellum.

- *Cerebellar Peduncles* *

Three bilateral pairs of axon tracts that carry information to and from the cerebellum. The superior cerebellar peduncle, or brachium conjunctivum, is an efferent motor pathway; the middle cerebellar peduncle, or brachium pontis, is an afferent pathway arising from the pontine nuclei. The smallest but most complex is the inferior cerebellar peduncle, or restiform body, which encompasses multiple pathways.

- *Cerebrocerebellum* *

The part of the cerebellar cortex that receives input from the cerebral cortex via axons from the pontine relay nuclei.

- *Clarke's Nucleus*

A group of relay neurons located in the medial aspect of the dorsal spinal column. Component of a cerebellar motor pathway important in processing proprioceptive input. Also called the dorsal nucleus of Clarke.

- *Climbing Fibers* *

Axons that originate in the inferior olive, ascend through the inferior cerebellar peduncle, and make terminal arborizations that invest the dendritic tree of Purkinje cells.

- *Cuneate Nuclei*

Sensory relay nuclei that lie in the lower medulla; they contain the second-order sensory neurons that relay mechanosensory information from peripheral receptors in the upper body to the thalamus.

- *dysdiadochokinesia*

Difficulty performing rapid alternating movements.

- *dysmetria*

Inaccurate movements due to faulty judgment of distance, especially over- or underreaching. Characteristic of cerebellar pathology.

- *Inferior Olive *

Prominent nucleus in the medulla; a major source of input to the cerebellum. Also called inferior olivary nucleus.

- *Intention Tremor*

Tremor that occurs while performing a voluntary motor act. Characteristic of cerebellar pathology. Also called action tremor.

- *Nystagmus *

Literally, nodding. Refers to repetitive movements of the eyes normally elicited by large-scale movements of the visual field (optokinetic nystagmus). Nystagmus in the absence of appropriate stimuli usually indicates brainstem or cerebellar pathology.

- *Pontine Nuclei *

Collections of neurons in the pons that receive input from the cerebral cortex and send their axons across the midline to the cerebellar cortex via the middle cerebellar peduncle.

- *Red nucleus *

A midbrain structure involved in motor coordination, especially in non-human mammals.

- *Spinocerebellum *

Region of the cerebellar cortex that receives input from the spinal cord, particularly Clarke's nucleus in the thoracic spinal cord.

- *Vestibulocerebellum*

The part of the cerebellar cortex that receives direct input from the vestibular nuclei or vestibular nerve.

Other Terms to recognize in the context of this lecture *

Efferent
Afferent
Ispilateral signs
Molecular layer
Granule layer
Purkinje cell
Arbor vitae
Deep cerebellar nucleus
Glutamate
GABA
Stellate cell
Golgi cell

Parallel fiber
Climbing fiber
Mossy fiber
Superior peduncle
Middle peduncle
Inferior peduncle
Pons
Pontine nuclei
Thalamus
Motor cortex
Inferior olive
Bergmann glia
Beam

Calcium
Intrinsic firing
Compartmentation
Zebrin
Spinocerebellar ataxia
Polyglutamine disease
Anticipation
Gain of toxic function
Rotarod
Schmahmann
Spinocerebellar tract
Nystagmus
Slow Saccades