

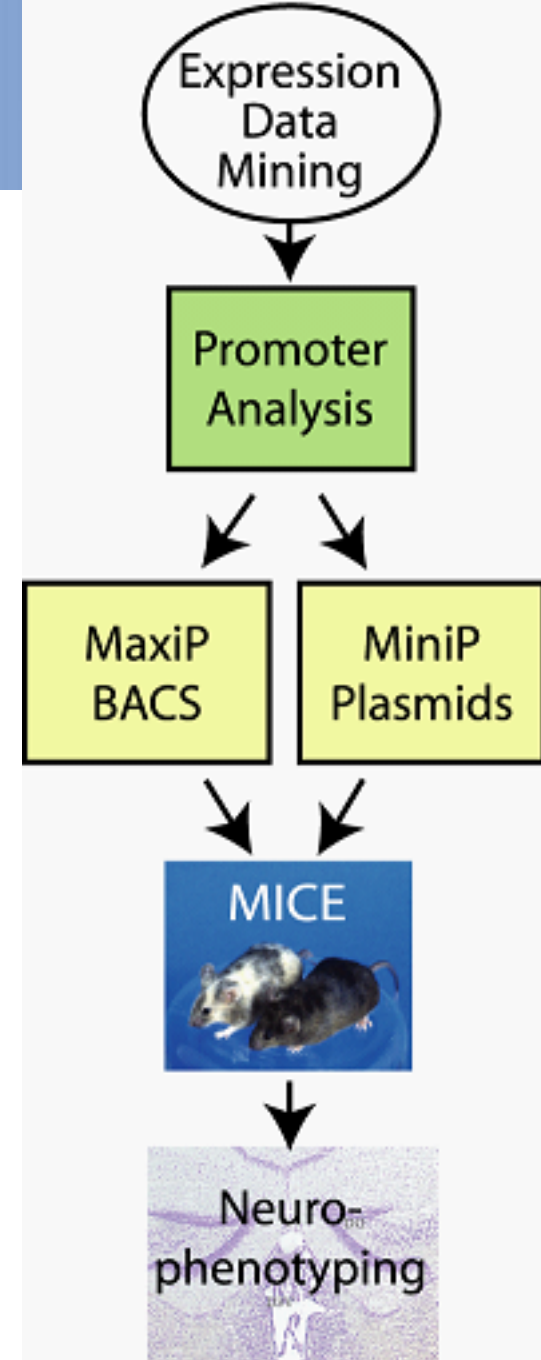
Cis-Regulatory Analysis for Purkinje Cell-Specific Transcriptional Control

Warren Cheung

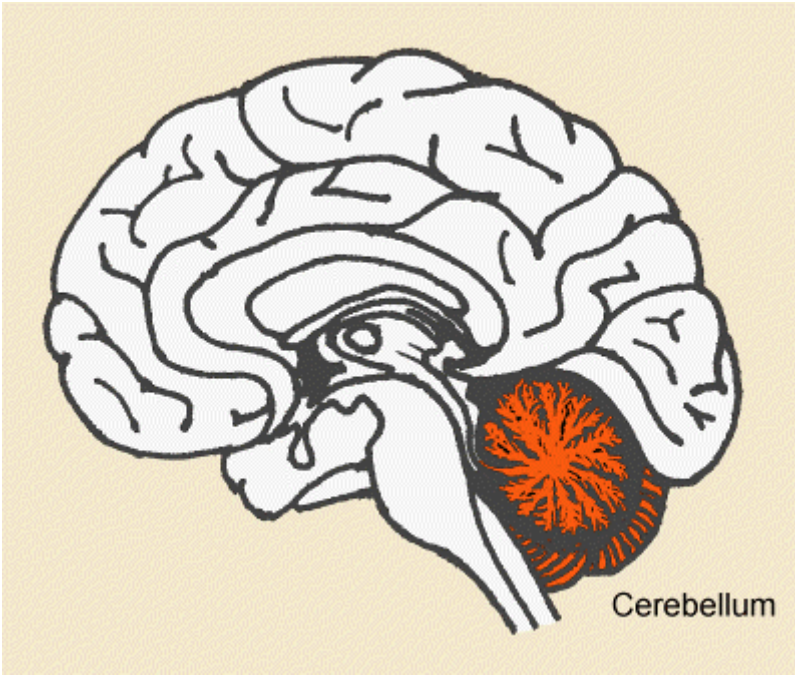
Wyeth Wasserman



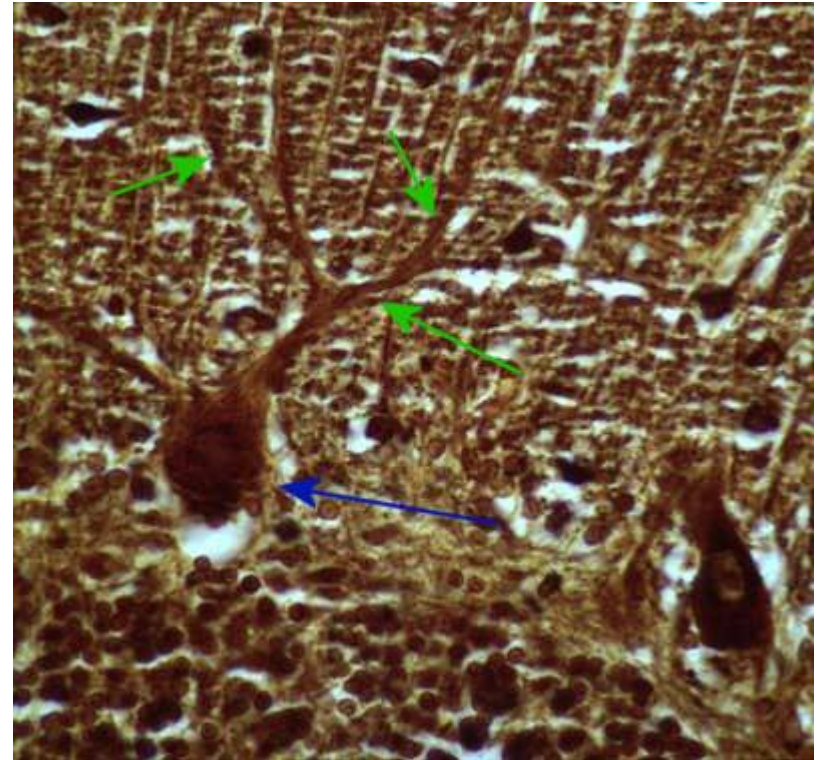
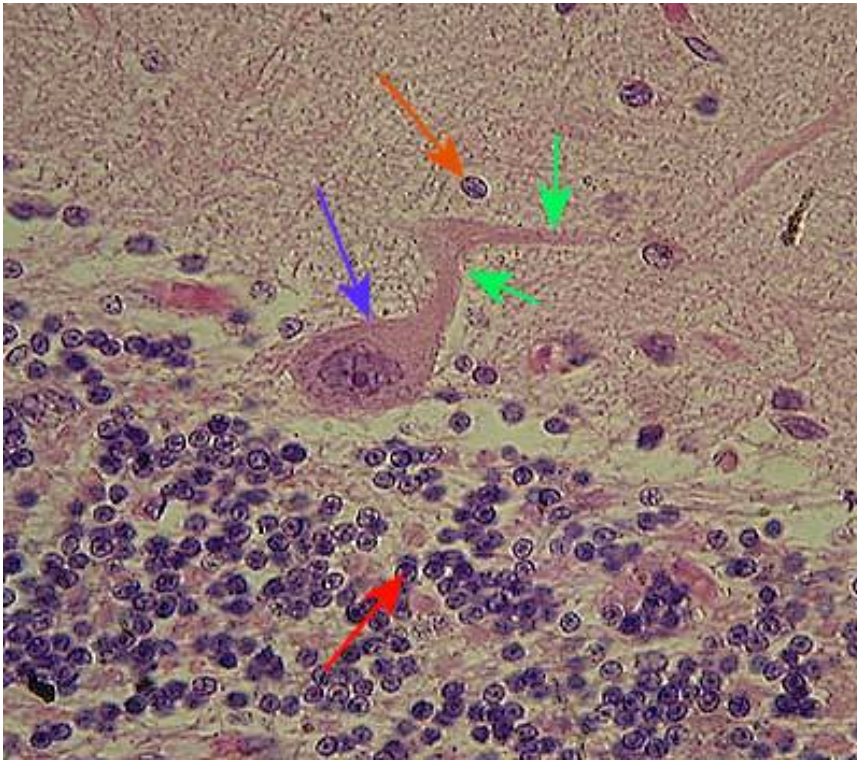
- 160 DNA MiniPromoters
- Drive gene expression in specific brain regions and cell types



Purkinje Cells



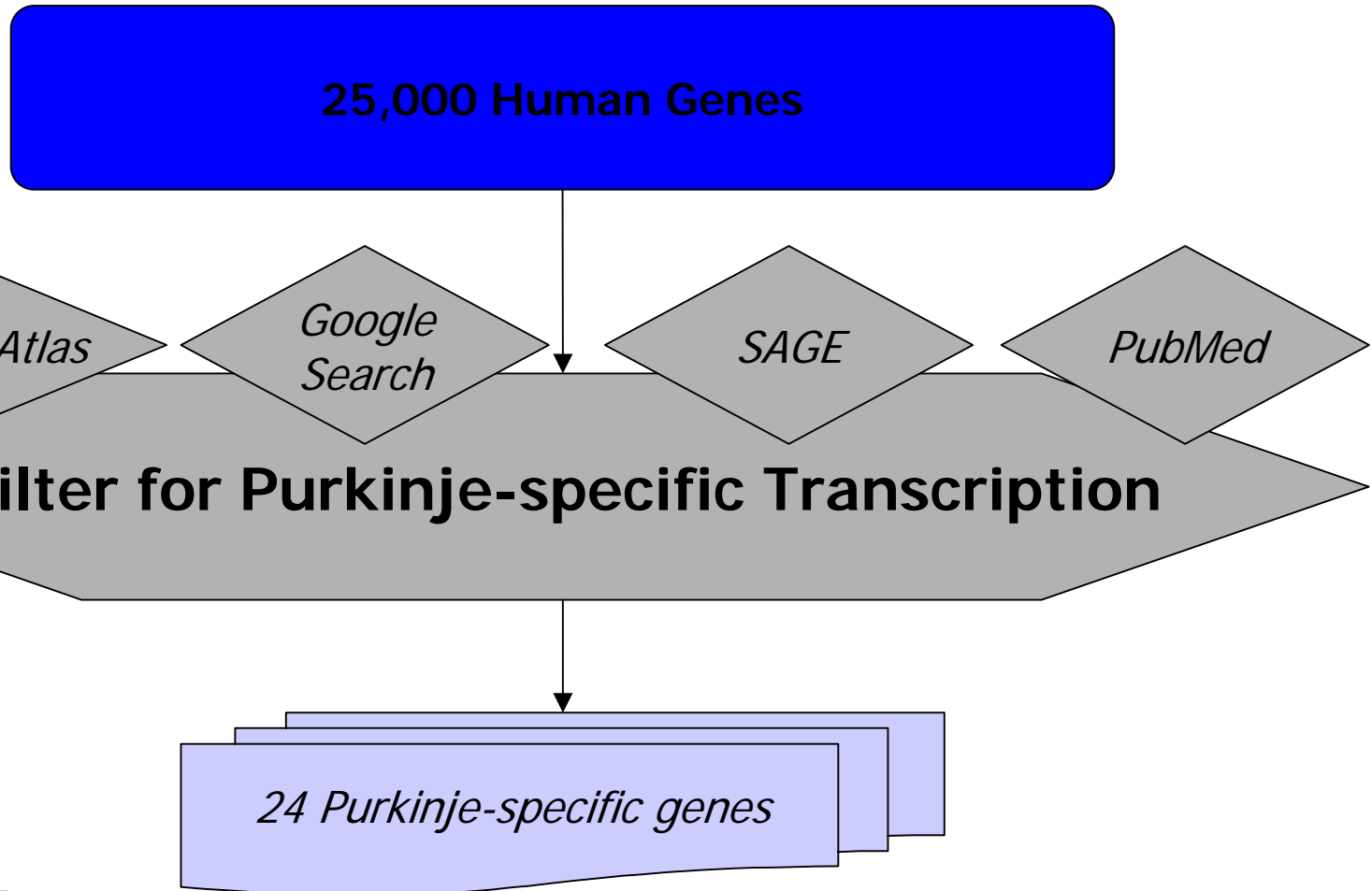
Purkinje Cells In Vivo



Goals

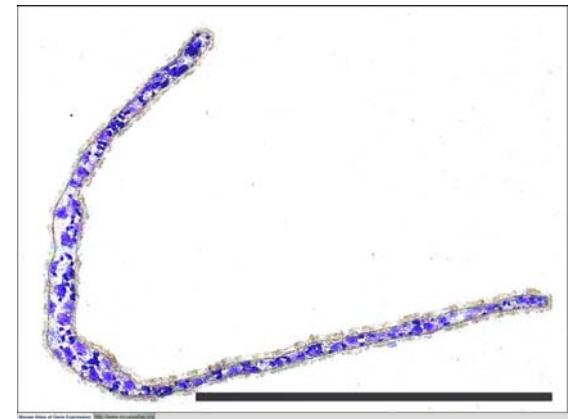
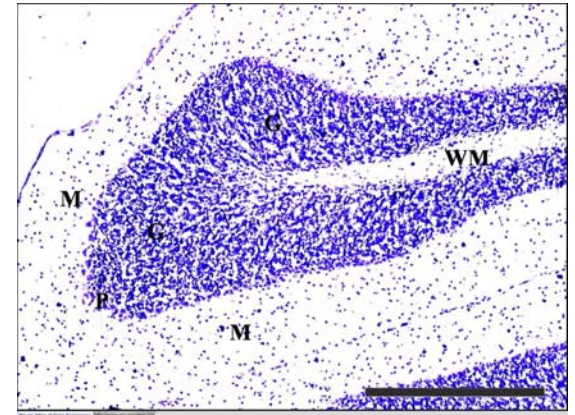
- Pleiades Promoter Project Genes
 - PCP2
 - HBEGF
 - ICMT
- Identify Purkinje cell-specific genes
- Find Overrepresented Promoter Elements

Hunting For Genes

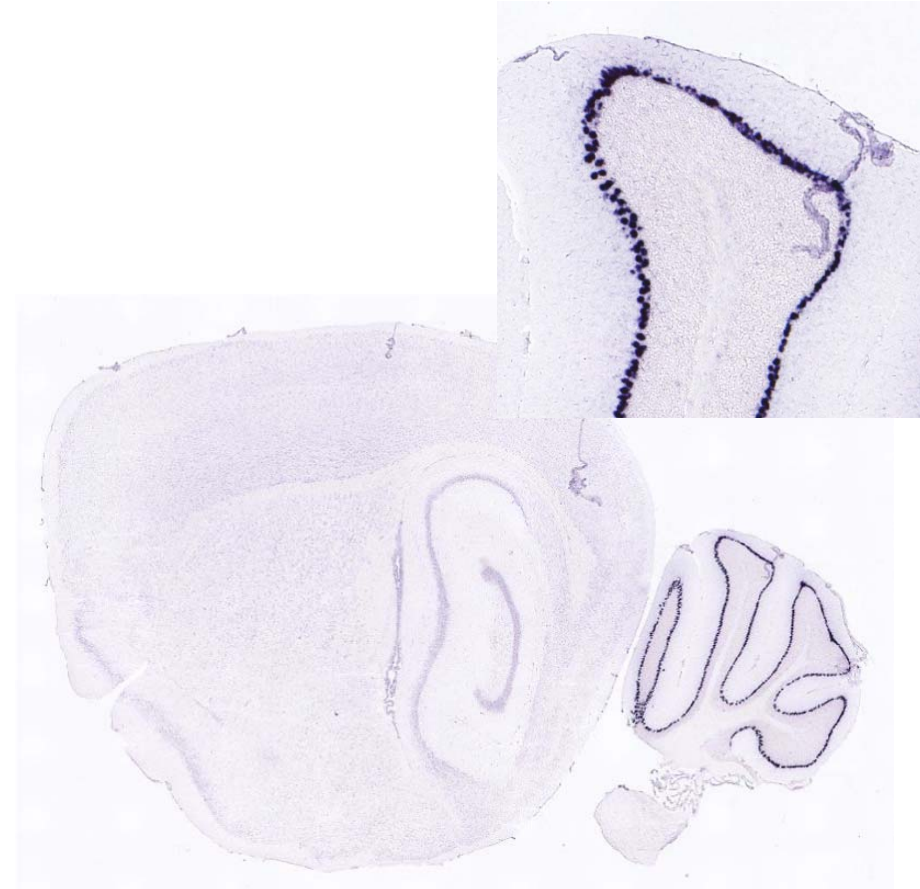
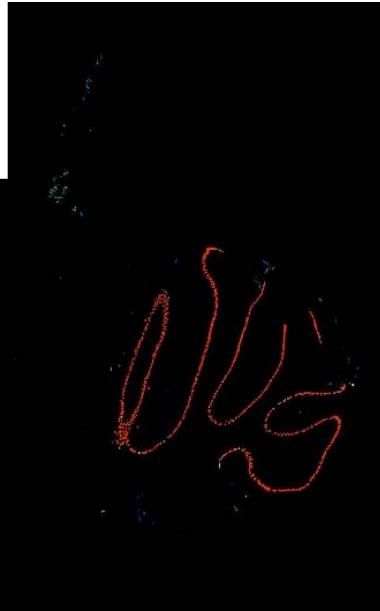
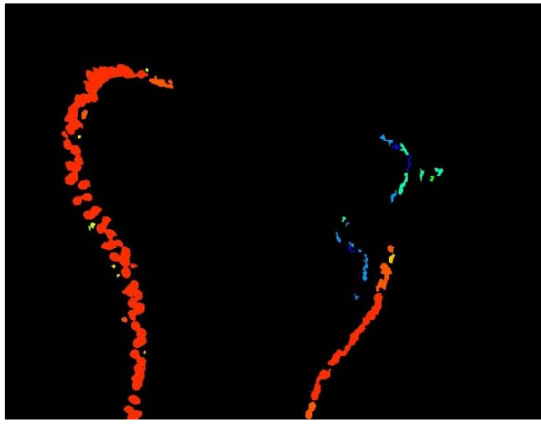


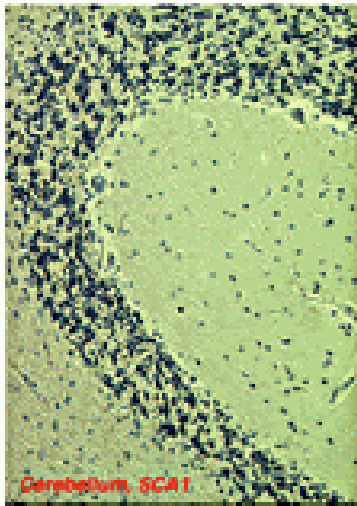
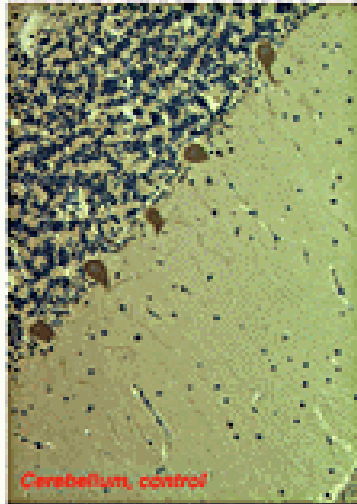
SAGE analysis

- Approximately 25,000 human genes
- ~2000 genes in Pleiades Promoter Project Database with SAGE data
- 60 genes where expression in Purkinje cell layer is at least 2x next highest expression target region



ICMT – Allen Brain Atlas





ICMT

- Also known as PCCMT
- PMID 10649571
- Lin et al. show abundant expression in normal Purkinje cells
- Lack of Purkinje cells and PCCMT expression in SCA1 disease mice

Overrepresentation Analysis



oPOSSUM

- Input: 24 genes
- Uses transcription factor binding site profiles
- Examine mouse-human conserved regions
- Identify commonly-occurring profiles

oPossum Scoring

- **One-tailed Fisher Exact Probability**
 - Hypergeometric probability distribution
 - Proportion of genes in set with binding sites
 - Compute probability of non-random association
- **Z-score**
 - Binomial distribution model
 - Number of predicted binding site nucleotides
 - Compare background rate to gene set rate

Human oPossum Results

	All Genes Background	
Rank	<i>Fisher P-value</i>	<i>Z-score</i>
1	Myf	S8
2	MEF2	E4BP4
3	RORalpha-2	SQUA
4	Agamous	AGL3
5	bZIP910	RORalpha-2
6	RORalpha-1	HNF-1
7	c-FOS	RORalpha-1
8	Broad-complex_1	RXR-VDR
9	Gfi	E74A
10	TBP	HLF

Human Overrepresented TFBS

	Cerebellar White Matter Genes Background	
<i>Rank</i>	<i>Fisher P-value</i>	<i>Z-score</i>
1	MEF2	S8
2	Myf	RORalpha-2
3	Agamous	SQUA
4	c-FOS	HMG-IY
5	bZIP910	Broad-complex_4
6	Athb-1	AGL3
7	Broad-complex_1	HLF
8	ATHB5	Dof2
9	RORalpha-2	Hunchback
10	Hunchback	Dof3

Known Motif Verification

- MEF2C
 - linked to Purkinje cell development
- ROR α
 - Transcriptionally regulates PCP2
- Gfi
 - Maximally expressed in Purkinje cells
- S8
 - Localised in Purkinje cell dendrites

Endgame

- **Meta-Analysis**
 - Identify Purkinje-specific TF binding sites
 - Analyse Known Promoter Elements
 - Comparison against background
 - Comparison with mouse
- **Other Promoter Analyses**
 - cisRED
 - Augure

Wasserman Lab

Thanks

Staff

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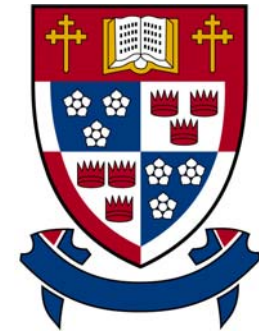
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CIHR IRSC

Canadian Institutes of Health Research
Institute of Genetics

Instituts de recherche
en santé du Canada
L'Institut de génétique



Michael Smith Foundation for
Health Research

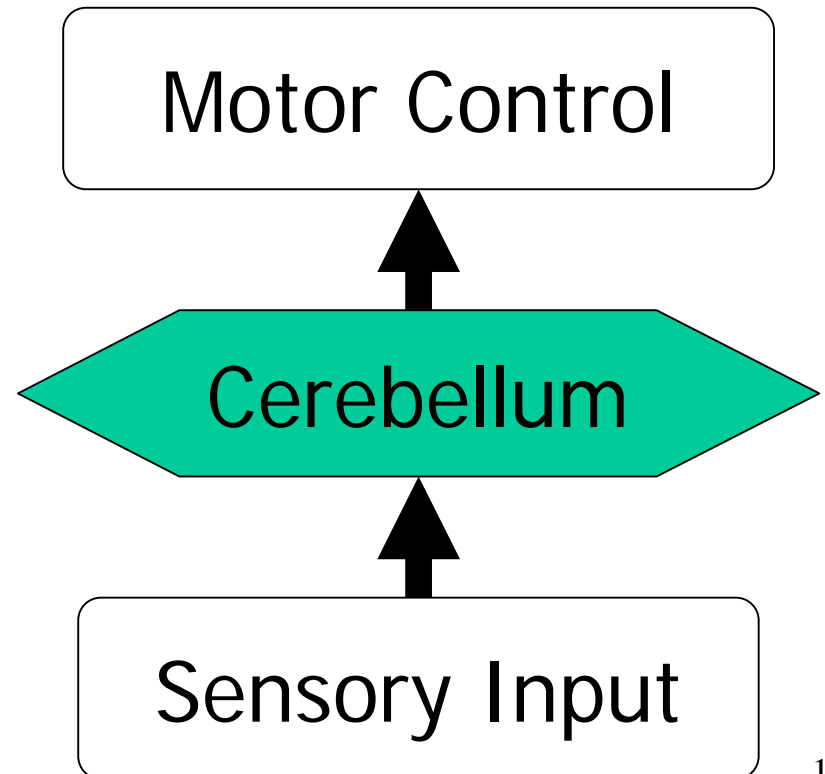
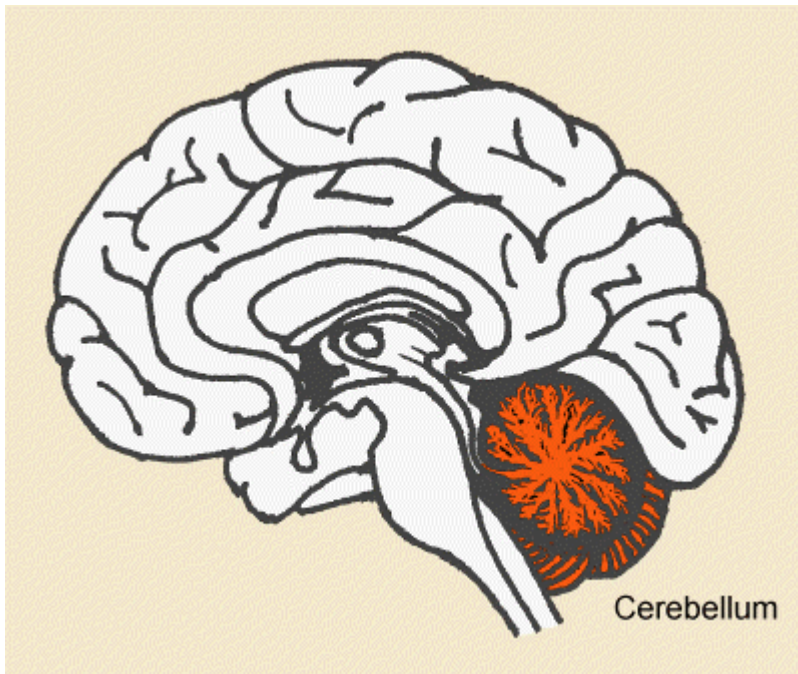


CIHR/MSFHR Strategic Training Program in

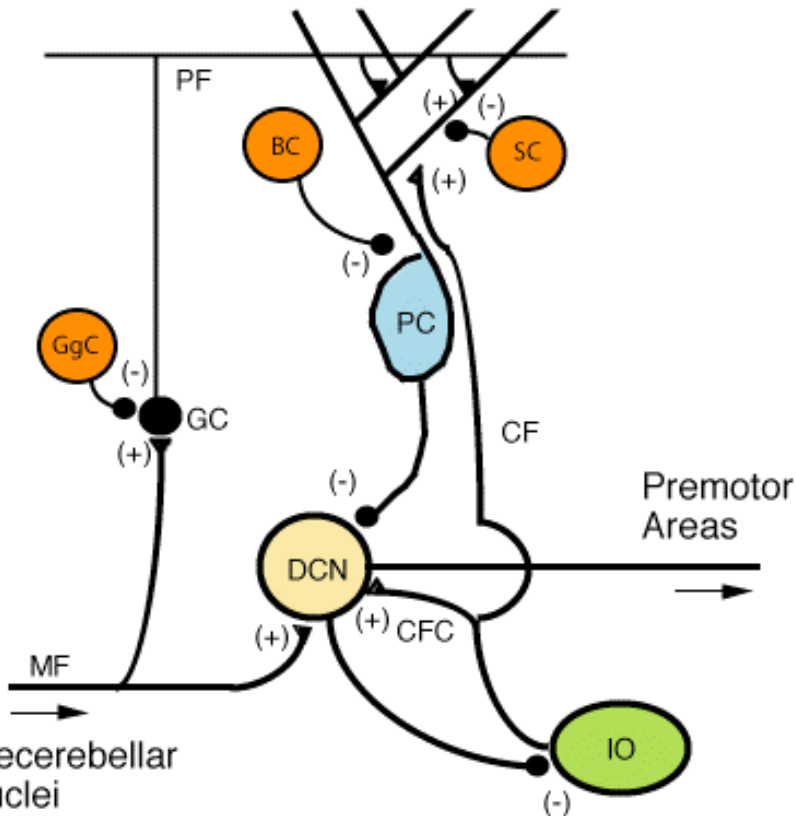
BIOINFORMATICS

Cerebellum

- All Vertebrates



Purkinje Cells



- Purkinje Cell Layer
- Climbing Fiber
 - Single Strong Input
- Many dendritic spines
 - Parallel fibers
 - Many Weak Inputs

MF: Mossy fibers. DCN: Deep cerebellar nuclei. IO: Inferior Olive. CF: Climbing fiber. GC: Granule Cell. PF: Parallel fiber. PC: Purkinje Cell. GgC: Golgi Cell. SC: Stellate Cell. BC: Basket Cell.

oPossum-TF Classes

Human TF analysis		
Rank	<i>Fisher P-value</i>	<i>Z-score</i>
1	Myf (bHLH)	S8 (HOMEO)
2	MEF2 (MADS)	E4BP4 (bZIP)
3	RORalpha-2 (Nuclear Receptor)	SQUA
4	Agamous	AGL3
5	bZIP910	RORalpha-2
6	RORalpha-1	HNF-1
7	c-FOS	RORalpha-1
8	Broad-complex_1 (Zn Finger)	RXR-VDR
9	Gfi	E74A (ETS)
10	TBP (TATA Box)	HLF