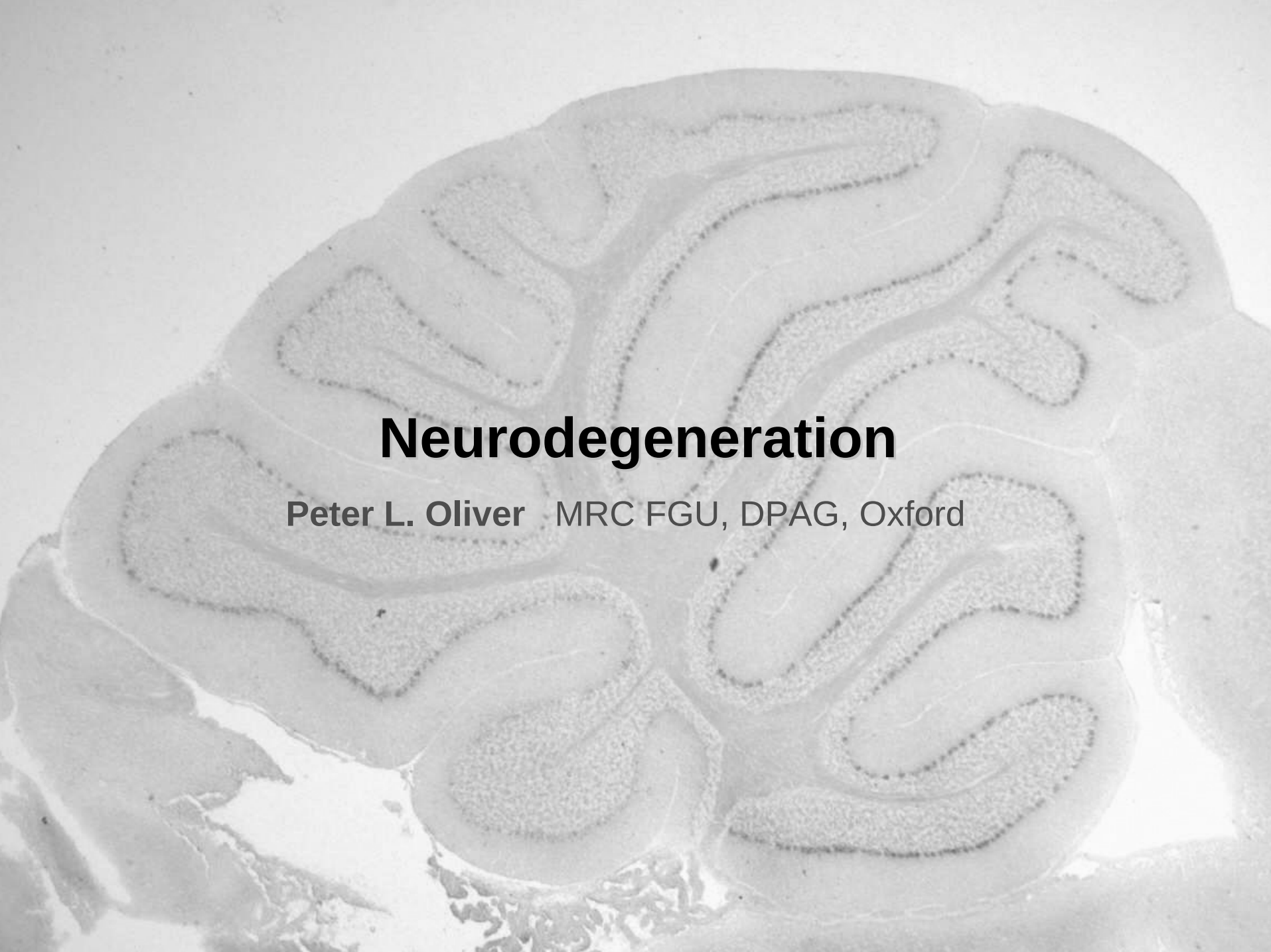


Neurodegeneration

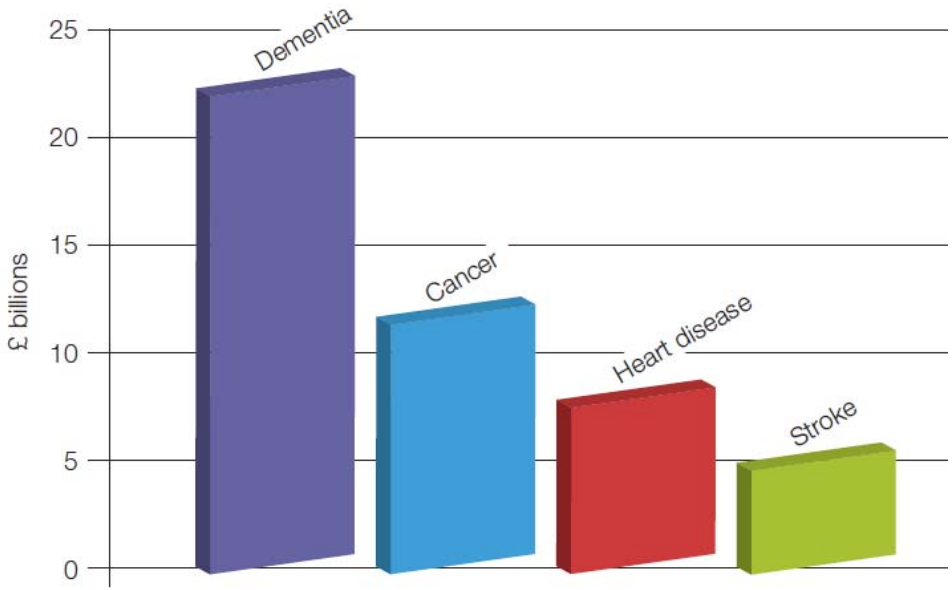
Peter L. Oliver MRC FGU, DPAG, Oxford

Health implications of neurodegenerative disease

- Prevalence of dementia rises from 5% to over 30% between the ages of 65 and 85 year old
- Each year 150,000 people in UK develop cognitive impairment and memory loss; within 5 years half will have dementia
- Alzheimer's disease costs the country ~£23 billion per year, approximately 20% of the UK health budget
- Number of people in UK over 60 now out-number those under 16
- Worldwide problem: e.g. China
    - 2009: 130 million people over 60
    - 2040 Estimated to have more people with dementia than the entire developed world put together

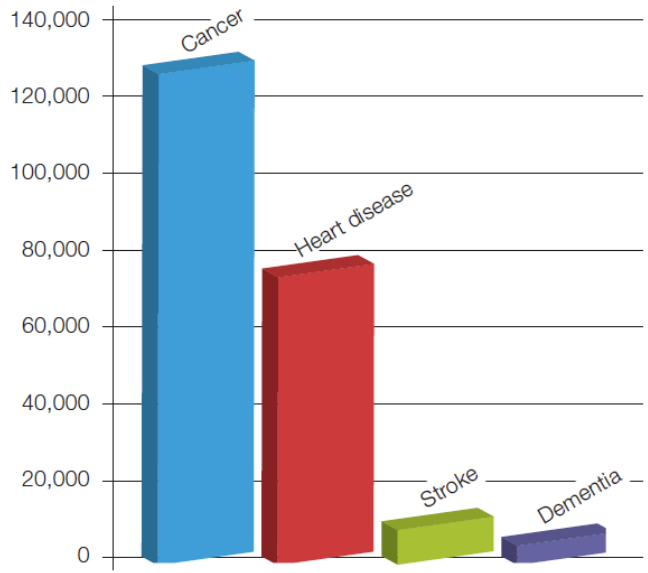
Dementia funding does not match the costs

Costs



Dementia costs more than cancer + heart disease

Research spending



For every £1 million in care costs for the disease:
£129,269 is spent on cancer research
£73,153 on heart disease research
£8,745 on stroke research
just £4,882 on dementia research.

Mouse modelling of neurodegenerative disease

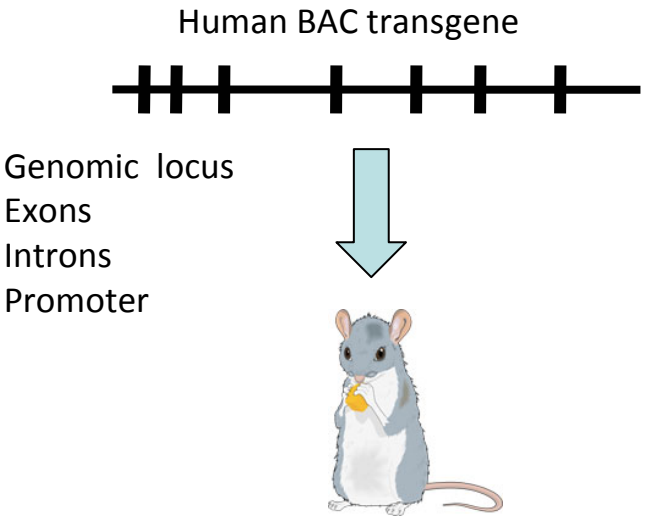
- AIM: Understand molecular mechanisms / identify therapeutic targets

- Advantages
  - Brain structure / behaviour / *in vivo*
  - Genetics / manipulation (KO / KI / KD / conditional / inducible)
  - Fast generation times - access to tissue / pre-symptomatic

- Disadvantages
  - Models reflect human pathology?
  - Timing – late-onset disease – expensive

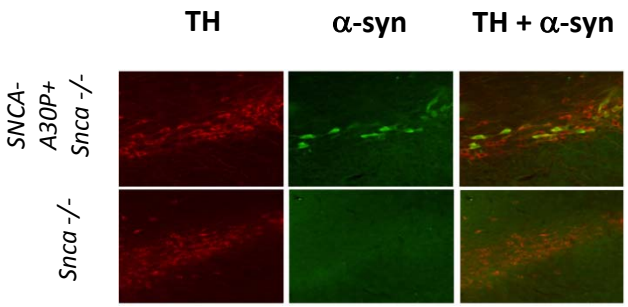
Disease → mouse / mouse → disease

BAC transgenics as more 'physiological' genetic models



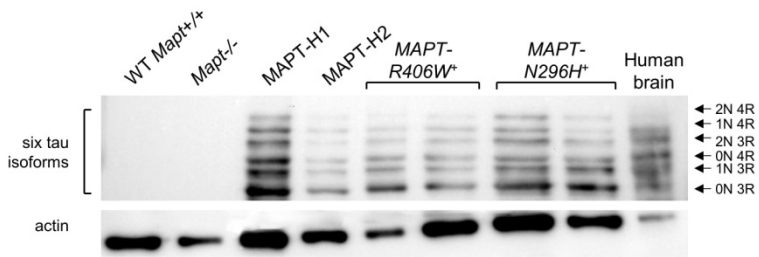
- **"Humanised" transgenic mouse models**
  Human disease mutation on mouse KO
- **Spatial and temporal regulation of expression**
  e.g. our *SNCA* Tg mice
- **Multiple splice variants expressed**
  e.g. our *MAPT* Tg mice
- **Physiological levels of transgene expression**

Substantia nigra pars compacta



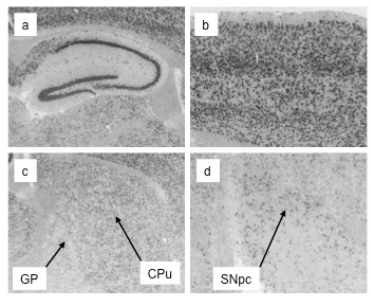
Tissue-specific expression

MAPT



Correct protein splicing

MAPT

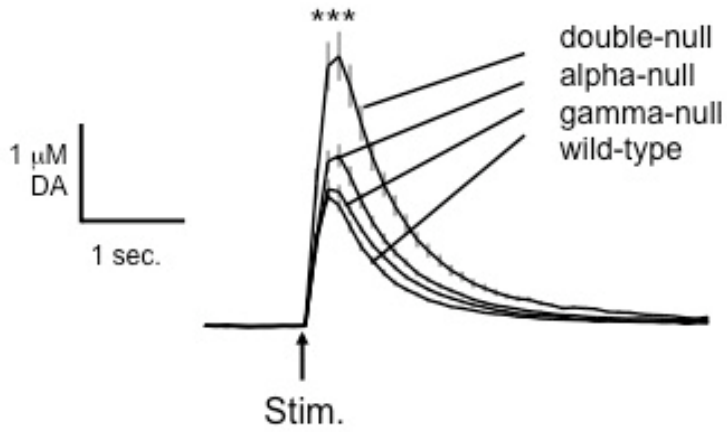


RNA *in situ*

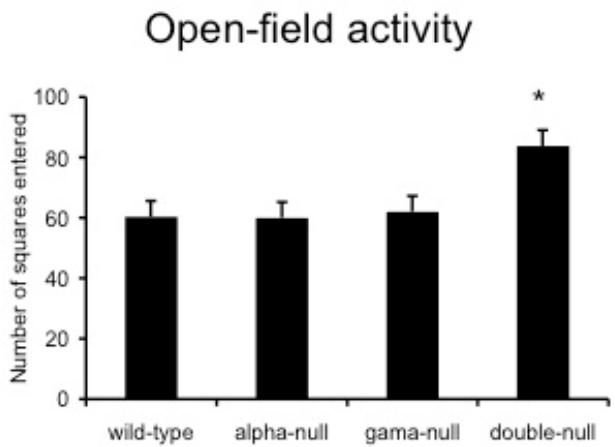
Anwar, Denk, Senior, Johnson, Oliver, Cragg, Wade-Martins

α- γ- synuclein double knockout mice show increased dopamine release in striatum

Fast scan cyclic voltametery

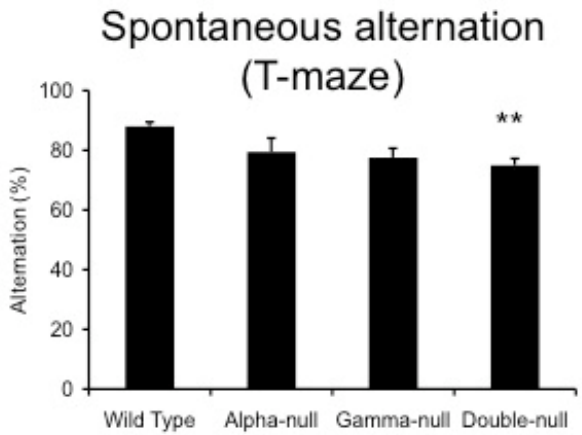


Behaviour



α- γ- synuclein double knockouts:

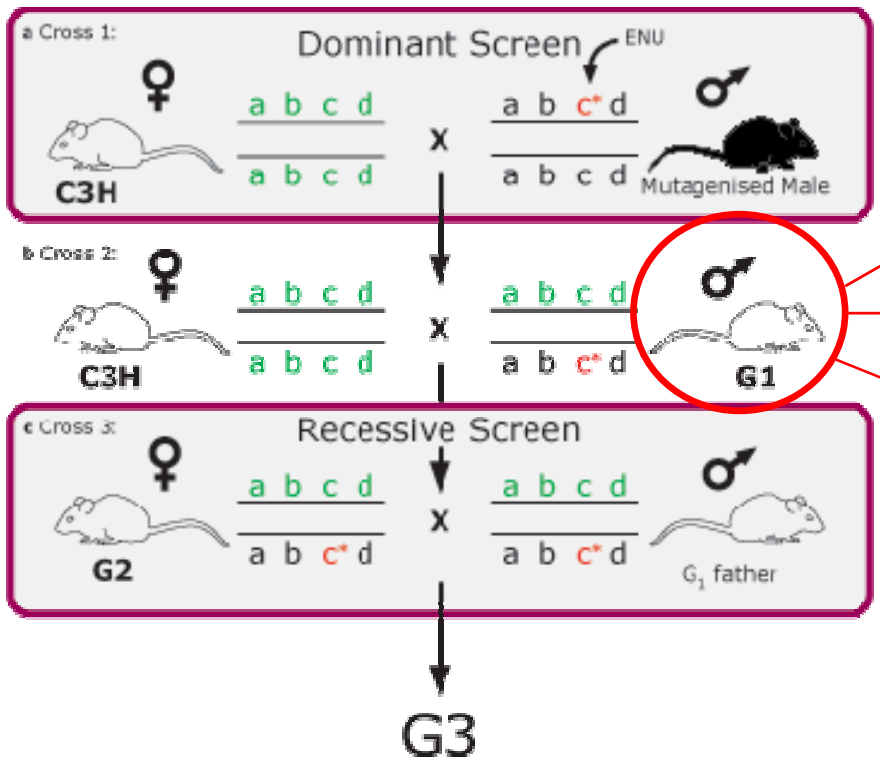
- Two-fold increase in electrically-evoked dopamine release
- No change in dopamine content in striatum
- Increased release probability from synapses
- Hyperactive in novel environment, deficit in spatial working memory



- Triple KOs - TBA

Senior, Anwar, Cragg, Buchman, Wade-Martins

Harwell ENU mutagenesis – from mouse to disease gene



- Deafness
- Diabetes
- Development
- Circadian
- Bone
- Muscle
- Behaviour

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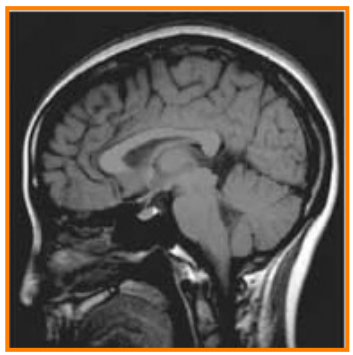
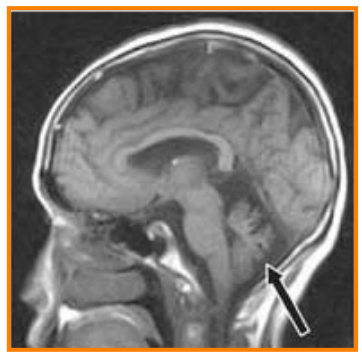
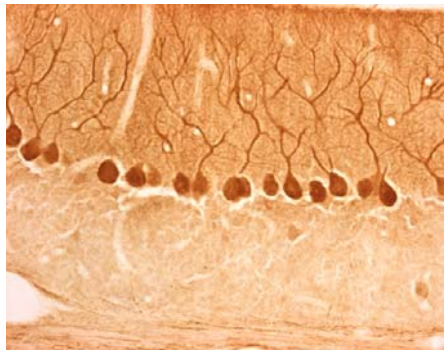
Ataxia and movement disorders

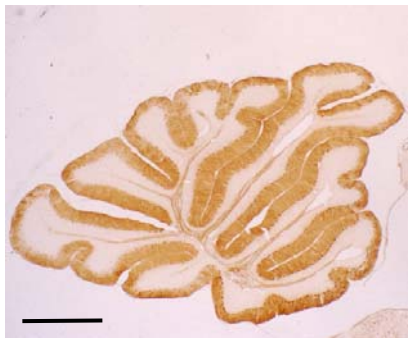
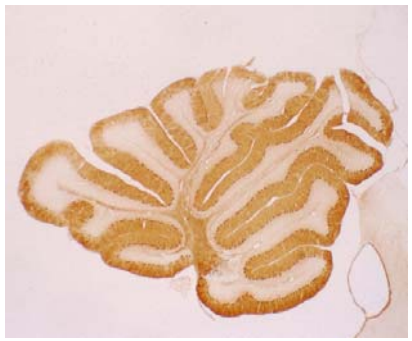
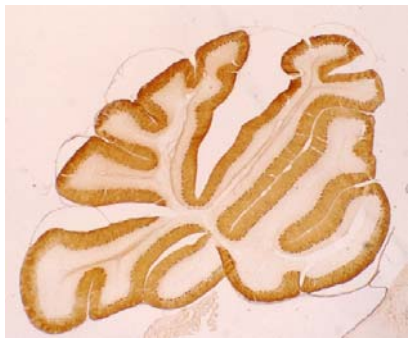
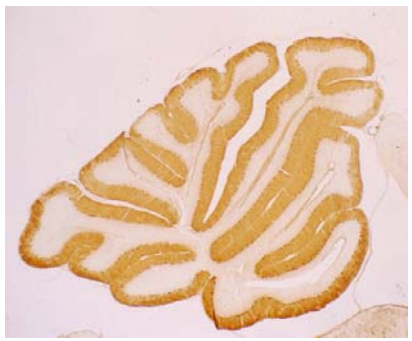
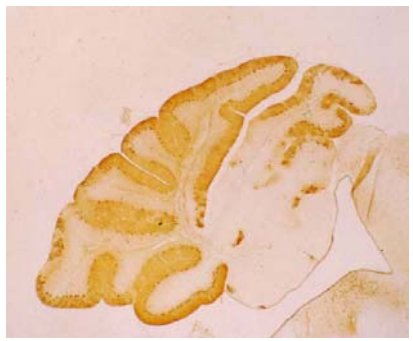
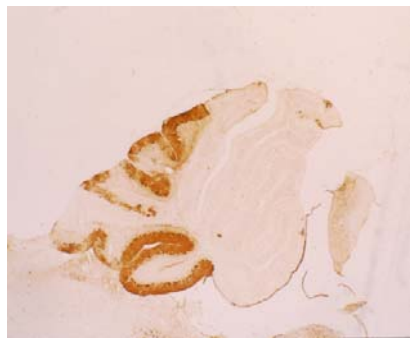
- Heterogeneous group of neurological diseases - symptoms often progressive and range from minor co-ordination difficulties to an inability to walk
- Prevalence: up to 5 in 100,000
- >50 forms of inherited ataxia
- Genetic cause only identified in one-third of ACDA/SCAs
- Mouse models of ataxia have provided some new insights into disease mechanisms, however no treatments are available





Anti-calbindin



	3 weeks	10 weeks	20 weeks	42 weeks
control				
robotic				

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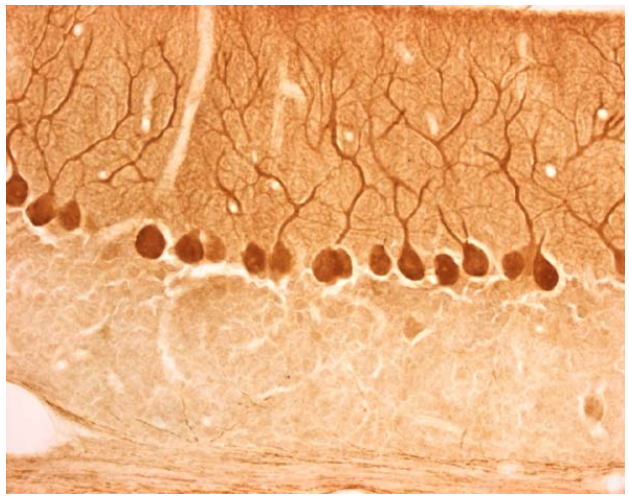
Isaacs & Oliver et al. *J.Neurosci*

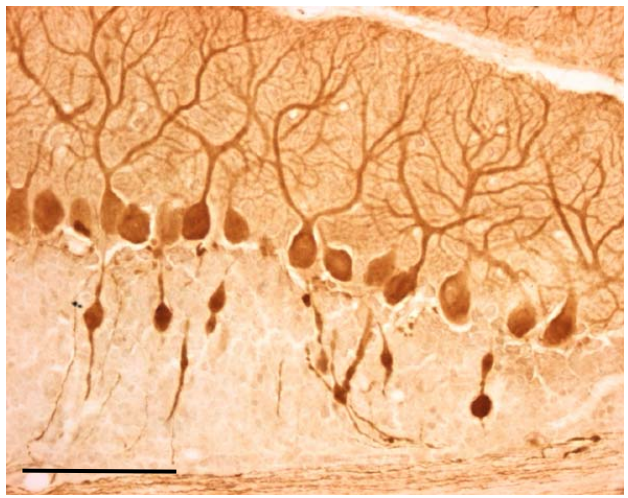
Anti-calbindin

5 weeks

control

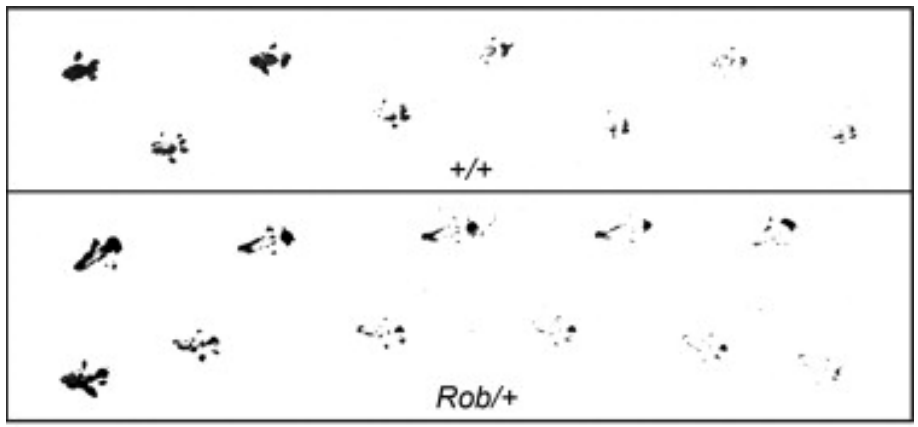


robotic



Behavioural testing

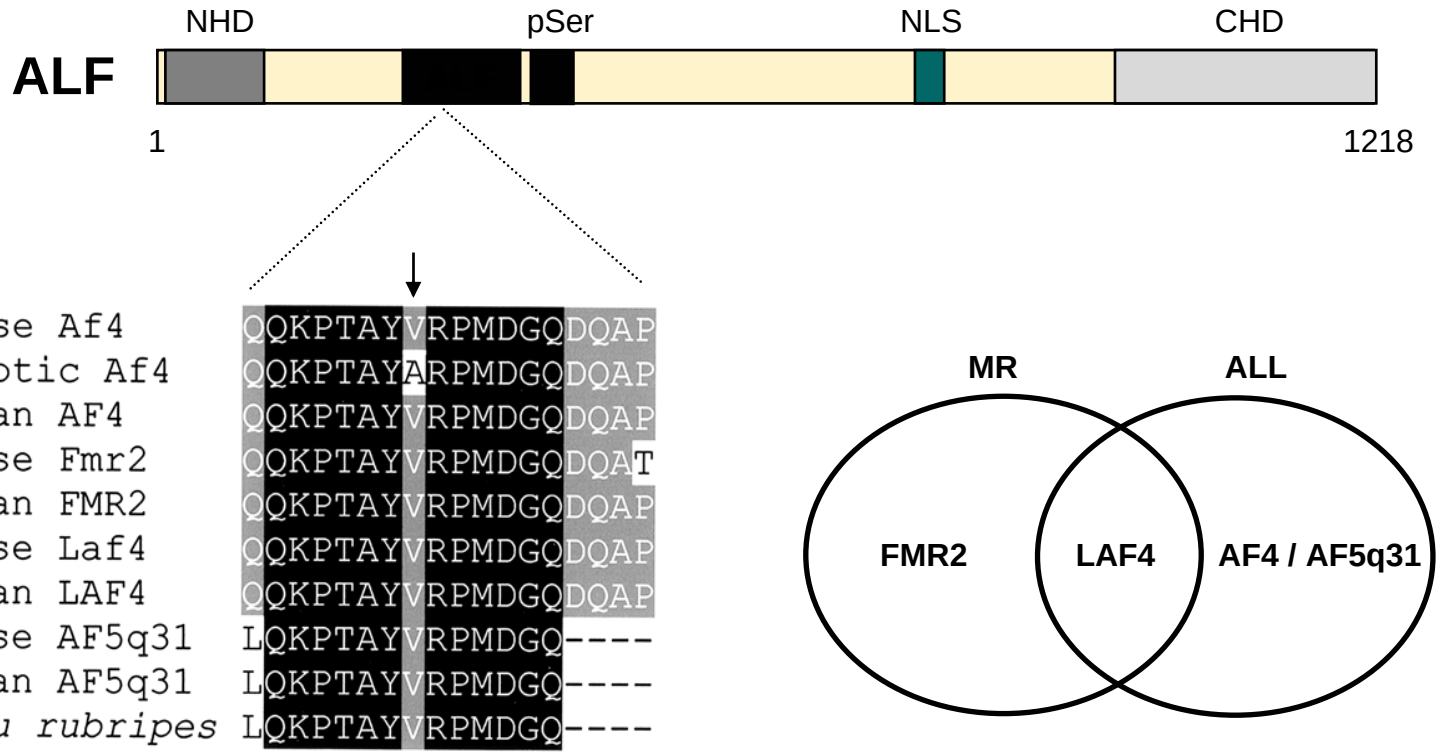
- Ataxia is progressive (6, 10, 20, 40w testing)
  - Accelerating rotarod
  - Static beam
  - Footprint analysis



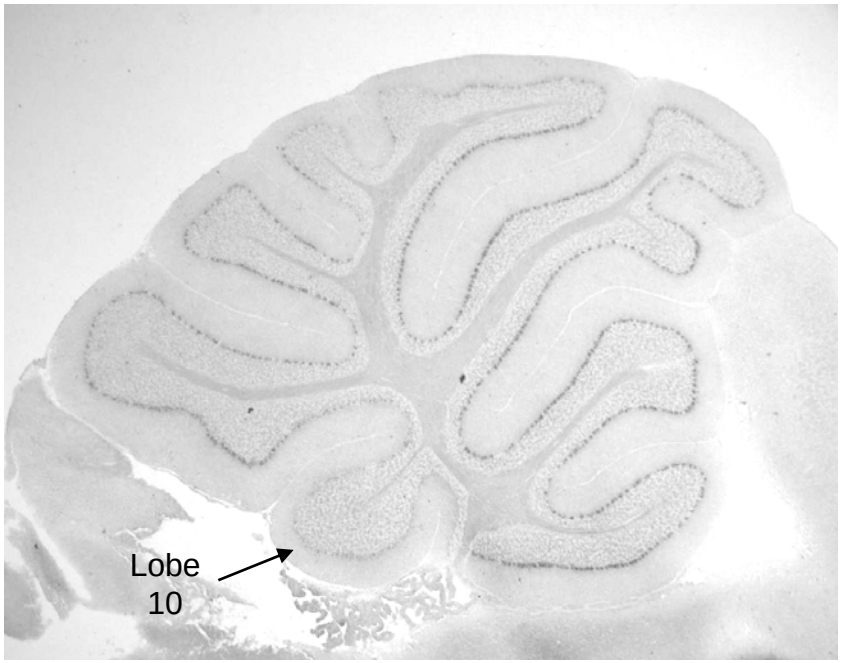
Oliver et al. *Behav Brain Res*

Mutation detection

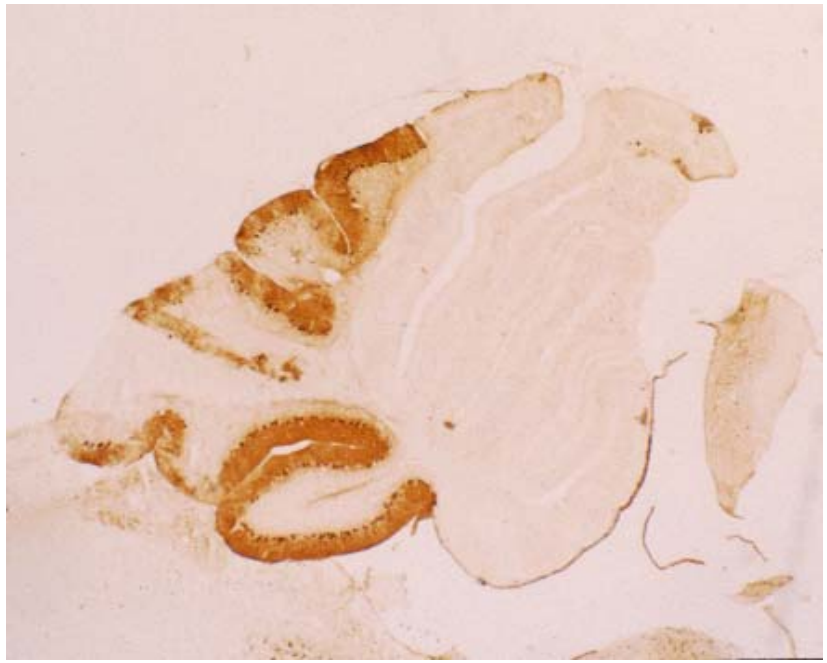
- Mutation identified in conserved region of Af4:



Expression of *Af4*



robotic, 8 weeks



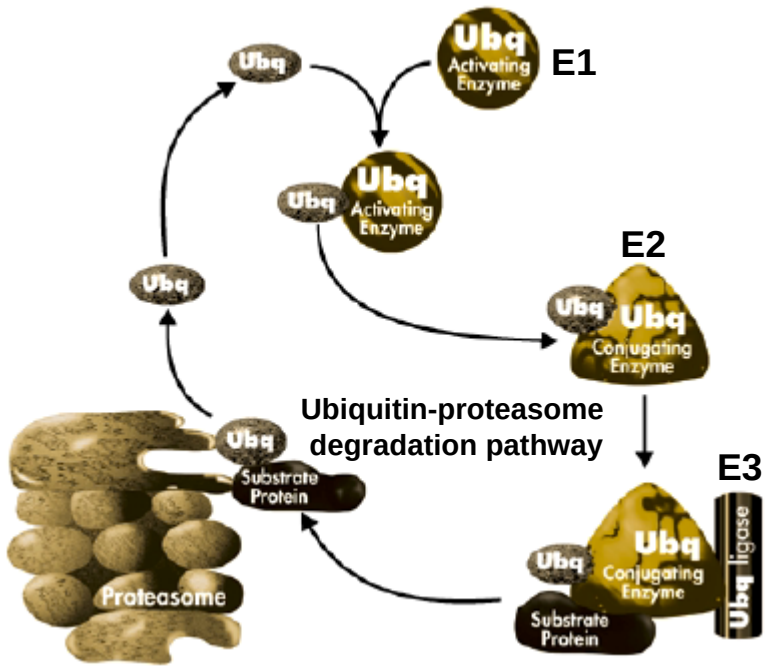
robotic, 42 weeks, anti-calbindin

Robotic - questions

- No obvious link between mutant gene (*Af4*) and phenotype
- *Af4* knockout mouse: No ataxia or cataracts or CNS lesions
  - Subtle T-cell developmental defects
  - Robotic therefore gain-of-function?
- ALF proteins putative transcription factors
  - Direct effect of mutation on TA activity?
  - Targets of Af4? / cause of cell death?
  - Expression profiling of *Rob*/+ and +/+ cerebellum
- Protein binding partners of Af4 in the brain
  - Yeast two-hybrid screen with wild-type and mutant Af4 baits
  - Different +ves? / affinity?

Siah proteins

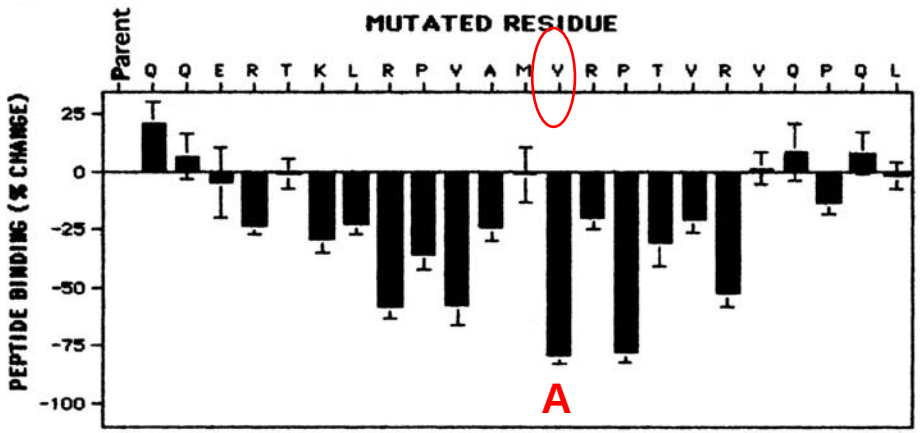
- **E3 ubiquitin ligase**
- **Homologues of *Drosophila seven in abstentia* (sina)**
- **Substrate recognition / facilitiates transfer of ubiquitin to target protein for degradation by the proteasome**



The robotic mutation is predicted to impair the interaction Siah-1 / Af4

PROTEIN	PEPTIDE	SEQUENCE
PHYL	(108-130)	QQERTKLRFVAMVRITVRVQPQL
OBF-1	(39-61)	ASSGAAPAPTAVVLLHQPLATYT
SIP	(52-74)	AELLDNEKPAAVVAIITTGYTVK
DCC	(1325-1347)	EEAPSRTIPTACVRITHPLRSFA
TIEG1	(193-215)	VEAARKNIPCAASSINRSKCERN
NUMB	(334-356)	FSSAPMTKPVTVTAQSPTFQAN
EF1-δ	(126-148)	APQTQHVSPMRQVEIPAKKPATP
Vav	(640-662)	ATNEVGWFPCNRVRIYVHGPPQD
Kid	(518-540)	TVTGAKPLKKAVTMLQLIQEQA
N-CoR	(131-153)	VSDSHFQRISAAVLLLVHTLPEG
FIR	(353-375)	VMAAQAPGVITGVTIARPPIPVT
consensus motif		--------P-A-**V**-**P**--------

mouse Af4	QQKPTAYVRPMDGQDQAP
robotic Af4	QQKPTAYARPMDGQDQAP
human AF4	QQKPTAYVRPMDGQDQAP
mouse Fmr2	QQKPTAYVRPMDGQDQAT
human FMR2	QQKPTAYVRPMDGQDQAP
mouse Laf4	QQKPTAYVRPMDGQDQAP
human LAF4	QQKPTAYVRPMDGQDQAP
mouse AF5q31	LQKPTAYVRPMDGQ----
human AF5q31	LQKPTAYVRPMDGQ----
Fugu rubripes	LQKPTAYVRPMDGQ----



House et al. PNAS 2003

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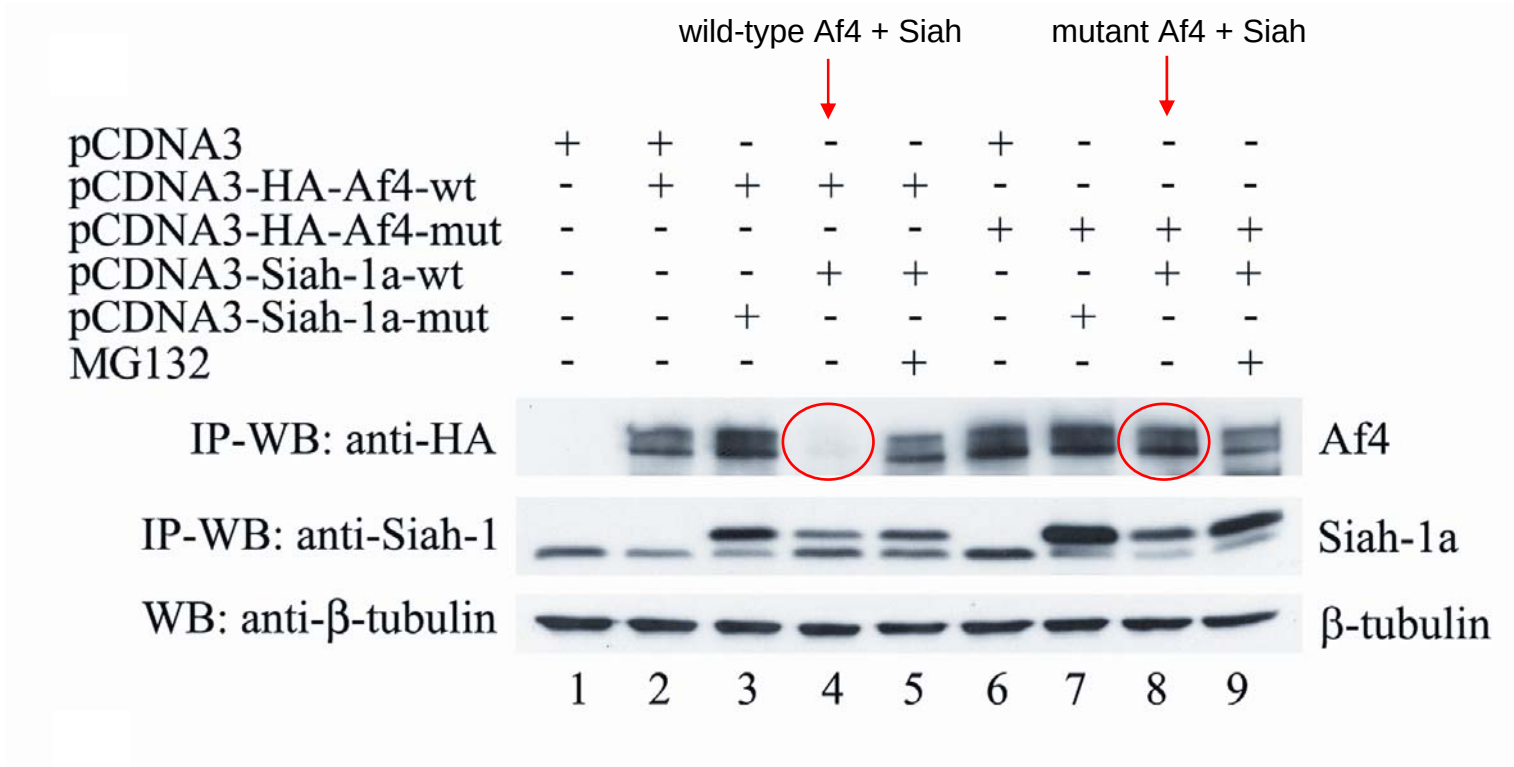
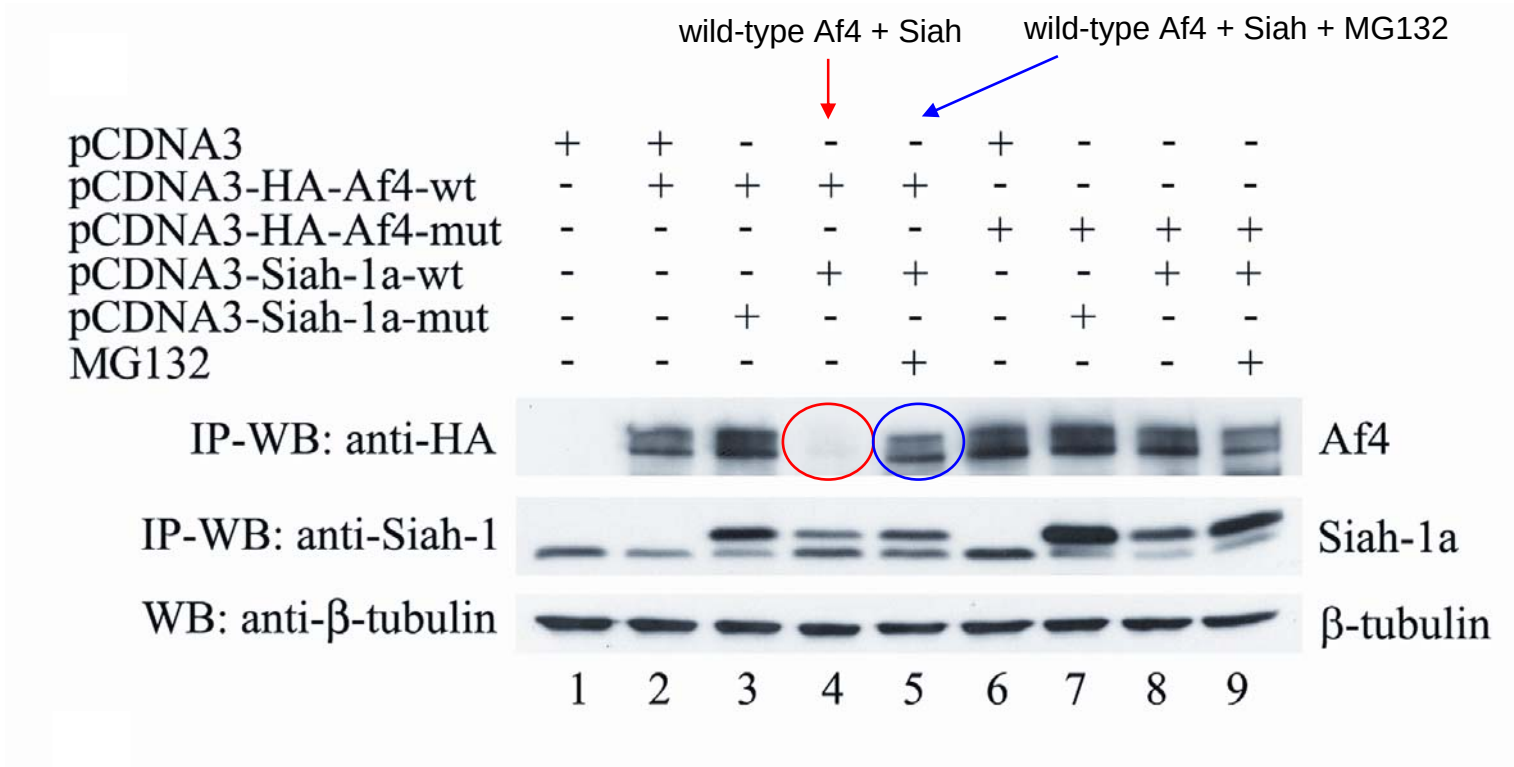
Robotic mutation blocks degradation of Af4 by Siah

Co-IP in HEK293T cells



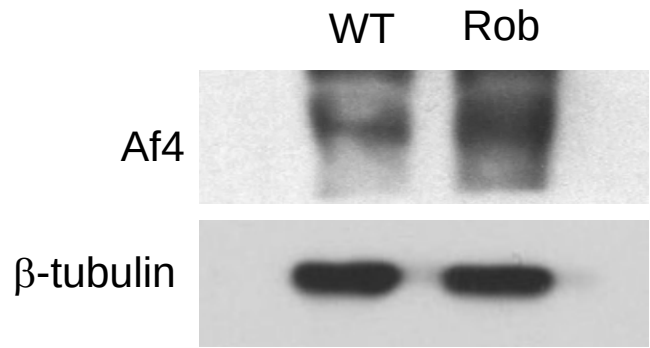
Siah mediates Af4 degradation through Ub-proteasomal pathway

Co-IP in HEK293T cells



Oliver & Bitoun et al. *PNAS*

Accumulation of Af4 in robotic tissues

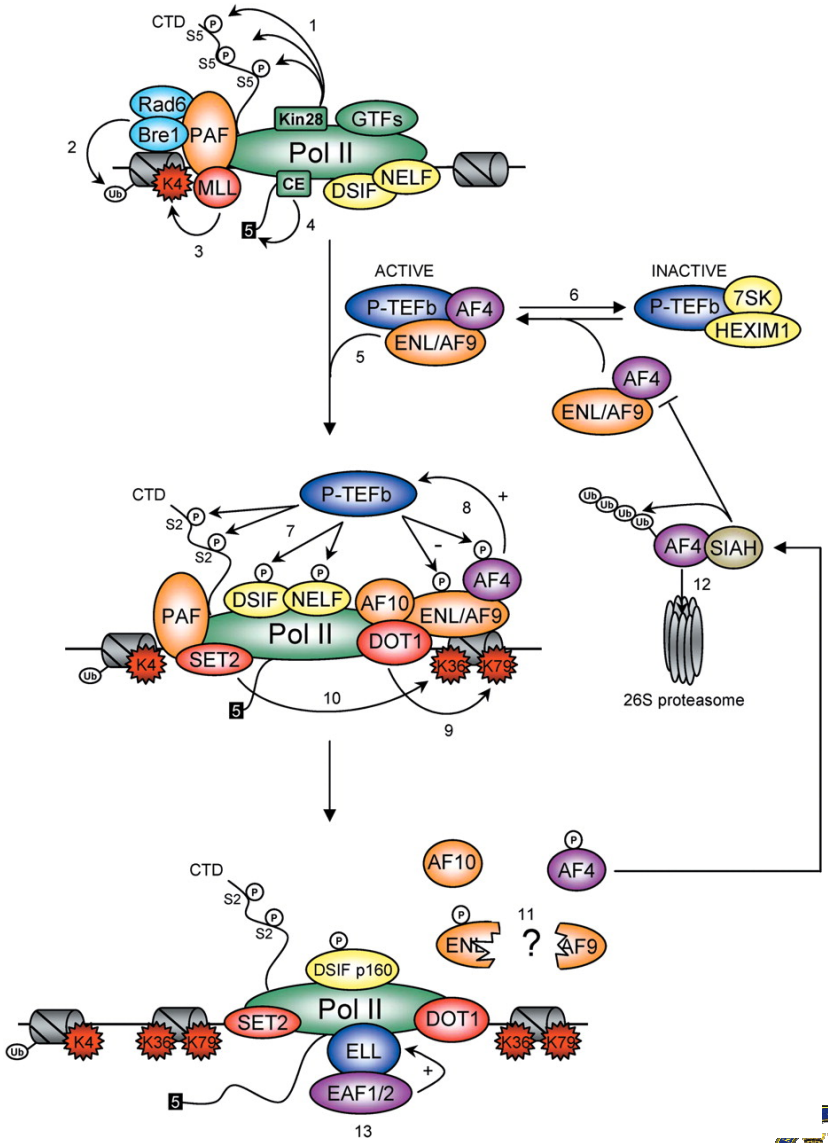


- How does Af4 regulate transcription?
- How does an increase in Af4 cause neurodegeneration?

Af4: links to chromatin remodelling

1) AF4 forms a complex with ENL/AF9 to activate P-TEFb
2) P-TEFb phosphorylates Pol II and DSIF/NELF to allow elongation – AF4 also phosphorylated
3) ENL/AF9 associates with H3
4) Recruitment of DOT1 for H3 methylation K79 – Pol II elongation through open chromatin
5) ENL/AF9 phosphorylated and degraded releasing AF4

- **Transcriptional targets?**
- **Cause of cell death in robotic?**



Bitoun et al. HMG

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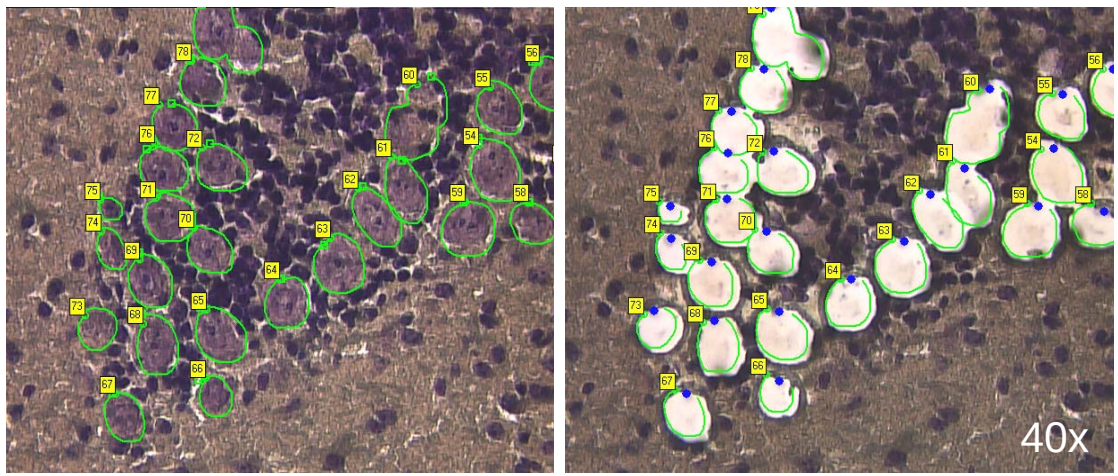
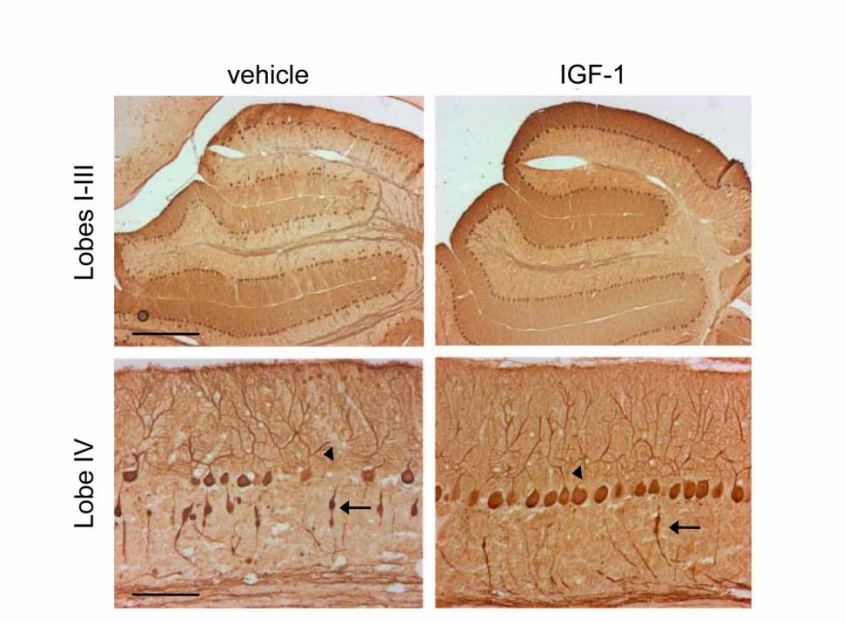
Expression profiling of the robotic cerebellum

- RNA purified from laser-capture micro-dissected Purkinje cells:
  - *Af4* specifically expressed in Purkinje cells
  - 4 +/+ 4 *Rob*/+ mice used at 3 weeks of age
  - 2,500 cells (8 hours+) individually dissected from cerebellar sections
  - Total of 8-12ng of RNA purified
  - Affymetrix whole genome chips hybridised (*Sheena - OXION*)
  - Data / pathway analysis



New targets regulated by Af4

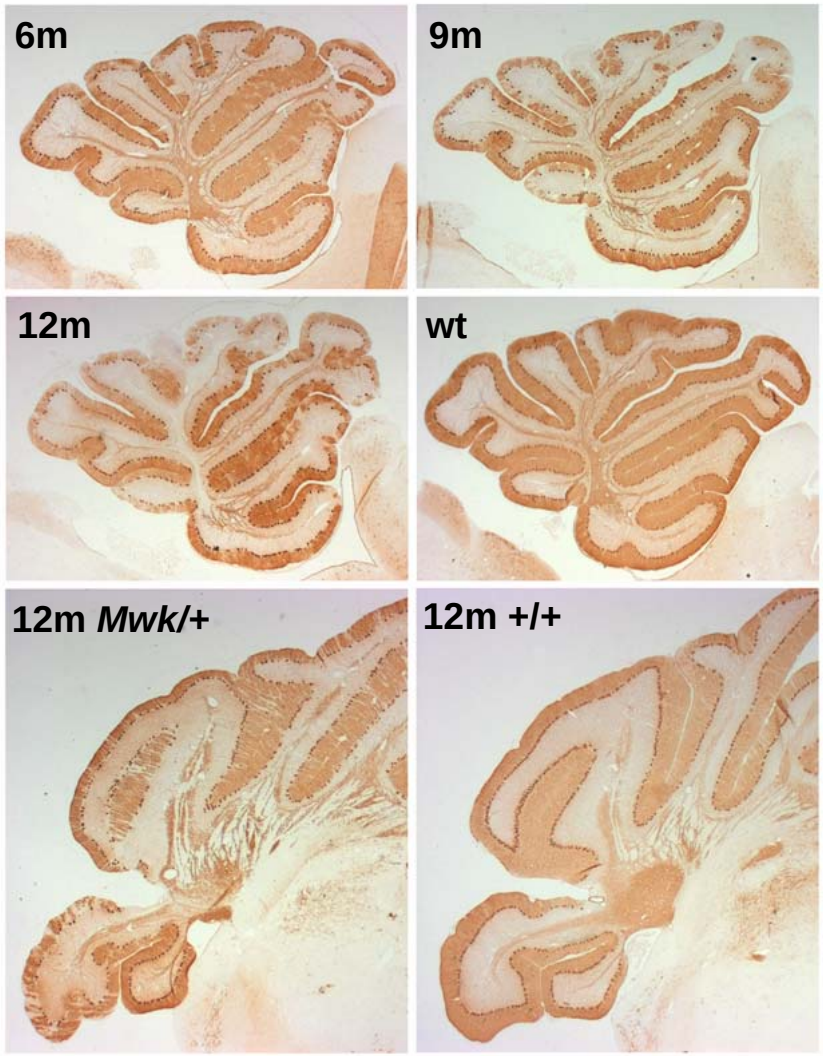
- **Expression changes confirmed by qRT-PCR – IGF-I down-regulated**
- **IGF-I confirmed as target for Af4 (repression)**
- **Af4 critical regulator of IGF-I signalling pathway**



Bitoun & Finelli et al. *J.Neurosci*

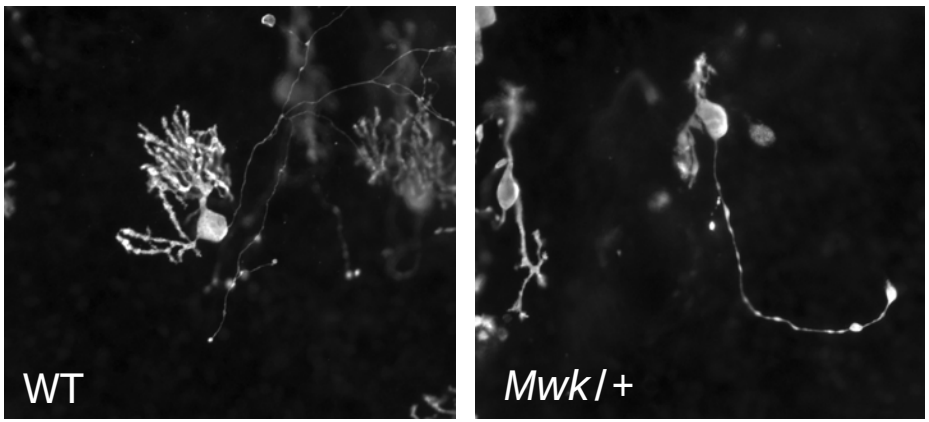
Moonwalker

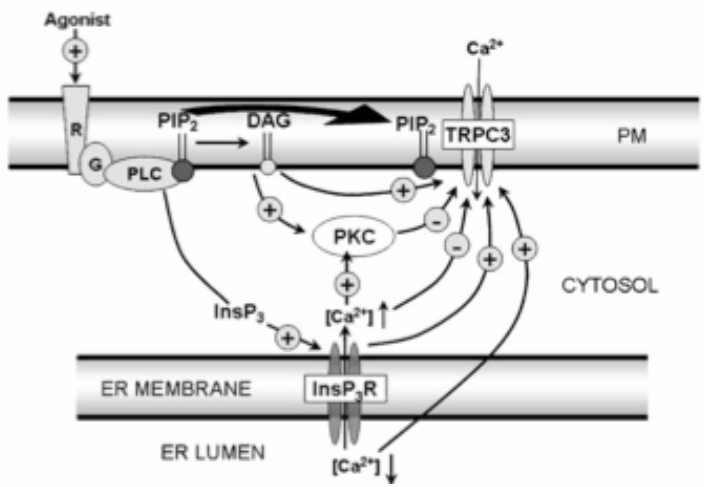
- Ataxic gait, retropulsion on smooth surface
- Smaller size (60-70%)
- Late-onset PC loss – hemispheres more susceptible
- Rotarod testing: similar performance to WT
- Static rod testing: performs badly



Moonwalker - characterisation and cloning

- Point mutation identified in conserved residue of Trpc3 cation channel - highly expressed in Purkinje cells
- Electrophysiology – channel is over-active (Maike Glitsch)
- Organotypic slice cultures – defects in PC development
- Ataxia patient screening
- Role of TRPC3 in calcium signalling





Becker et al. *PNAS*

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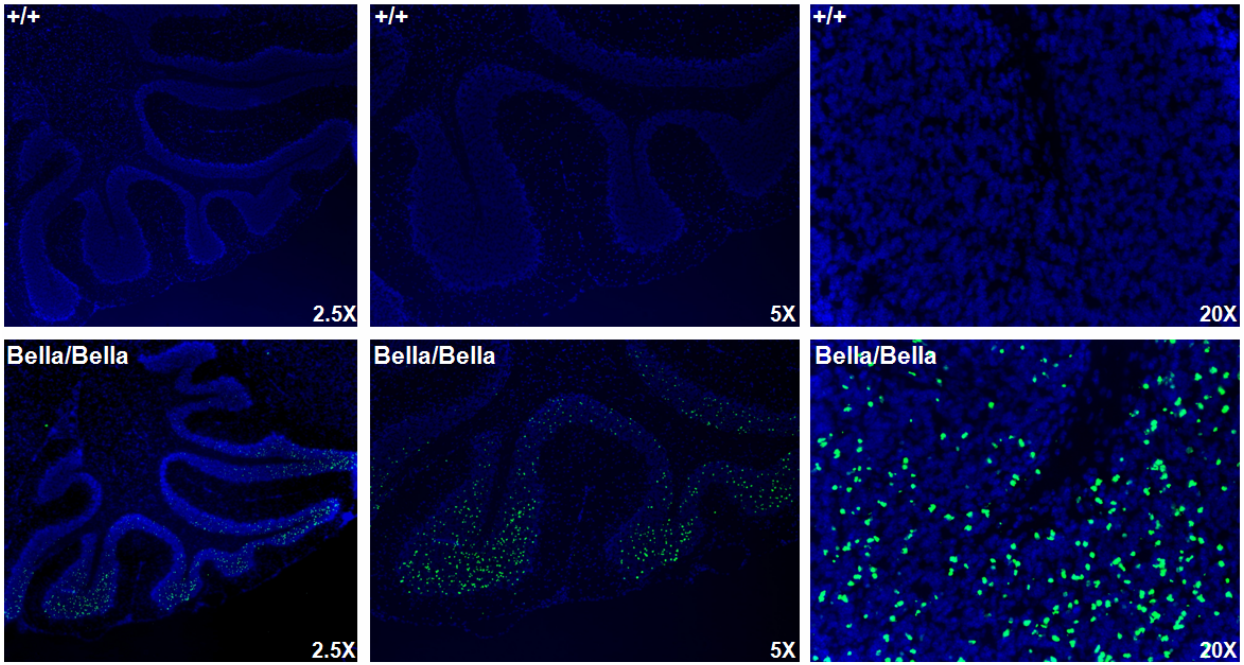
Bella

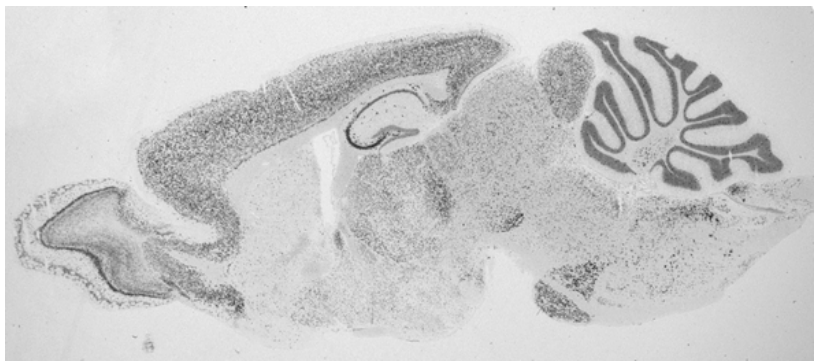
- Severe ataxia
    - Recessive
    - Onset 16-18 days
    - Rapid progression of ataxia – tiptoe gait / limb grasping
    - Paralysis 24-25 days – death

    - No phenotype in heterozygous animals (18m)

Bella - apoptotic cells in GCL



Blue – DAPI, Green - TUNEL



3Rs in mouse modelling

- Reduction
  - Animals used for multiple purposes – e.g. behav/path
  - Genomics / SNPs for genetic mapping
- Refinement
  - Optimisation of behavioural methods
  - Monitoring of new mutants / crosses
- Replacement
  - Cell culture modelling / primary cell culture
  - e.g. culture GCs longer than lifespan of Bella mice

ENU mutagenesis resource - future

- Continuing to generate dominant and recessive mutants
- Modifier screens (HD / ALS)

- Generate null / gain / loss-of function mutations – allelic series
- Endogenous loci – no copy number / promoter problems
- Gene-driven screen
  - Screen gene of interest from 9000 DNA samples
  - Rederive mouse from frozen sperm
  - Assay for mutation function?

- Sequence genomes?
- Ageing programme

Conclusions

- Generating new insights into neurodegeneration using mouse models
- Validity of model
- Mutant lines are complementary to KO studies
- Future:
  - Off-the-shelf KOs?
  - Human cell lines (iPS cells)
  - Non-coding sequences (more functional DNA is nc than pc)

Acknowledgements

Robotic:
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Lucie Vizor, Michelle Stewart, MLC staff, FESA, Steve Brown

DPAG AH staff

Kay Davies
Richard Wade-Martins

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http://www.neuroscience.ox.ac.uk/

Oxford Parkinson's Disease Centre

http://opdc.medsci.ox.ac.uk/

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