# Deranged calcium signaling and neurodegeneration in spinocerebellar ataxia type 2

Jing Liu, Tie-Shan Tang, Huiping Tu, Omar Nelson
Emily Herndonm, ….., Stefan Pulst, Ilya Bezprozvanny

# Ilya Bezprozvanny PhD

- $Ca^{++}$ signaling
  - Structure, function and modulation of the IP3R
  - Synaptic voltage-gated $Ca^{++}$ channels
  - Deranged $Ca^{++}$ signaling and neurodegenerative disorders: AD, HD, SCA

# Ilya Bezprozvanny PhD

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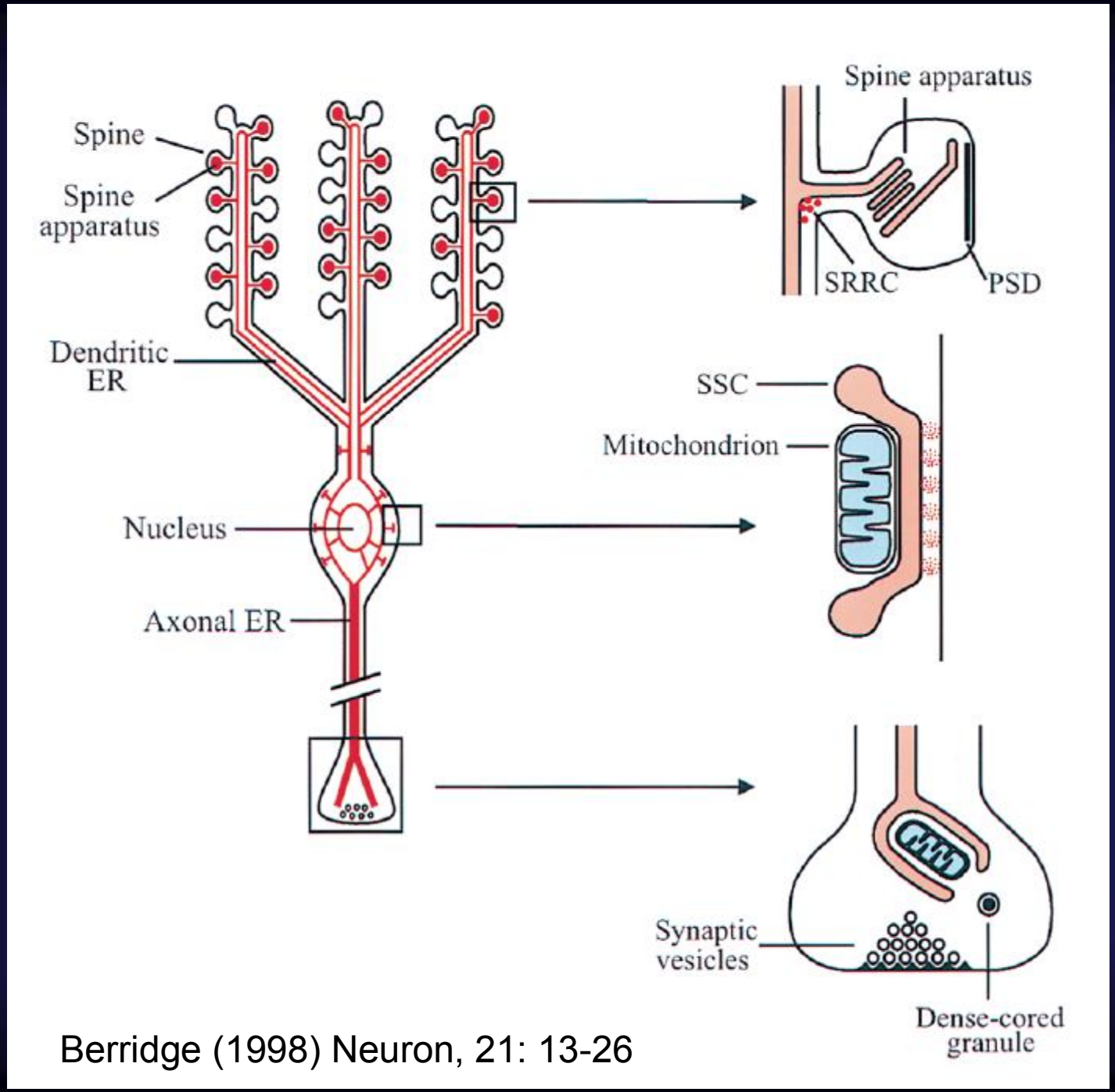
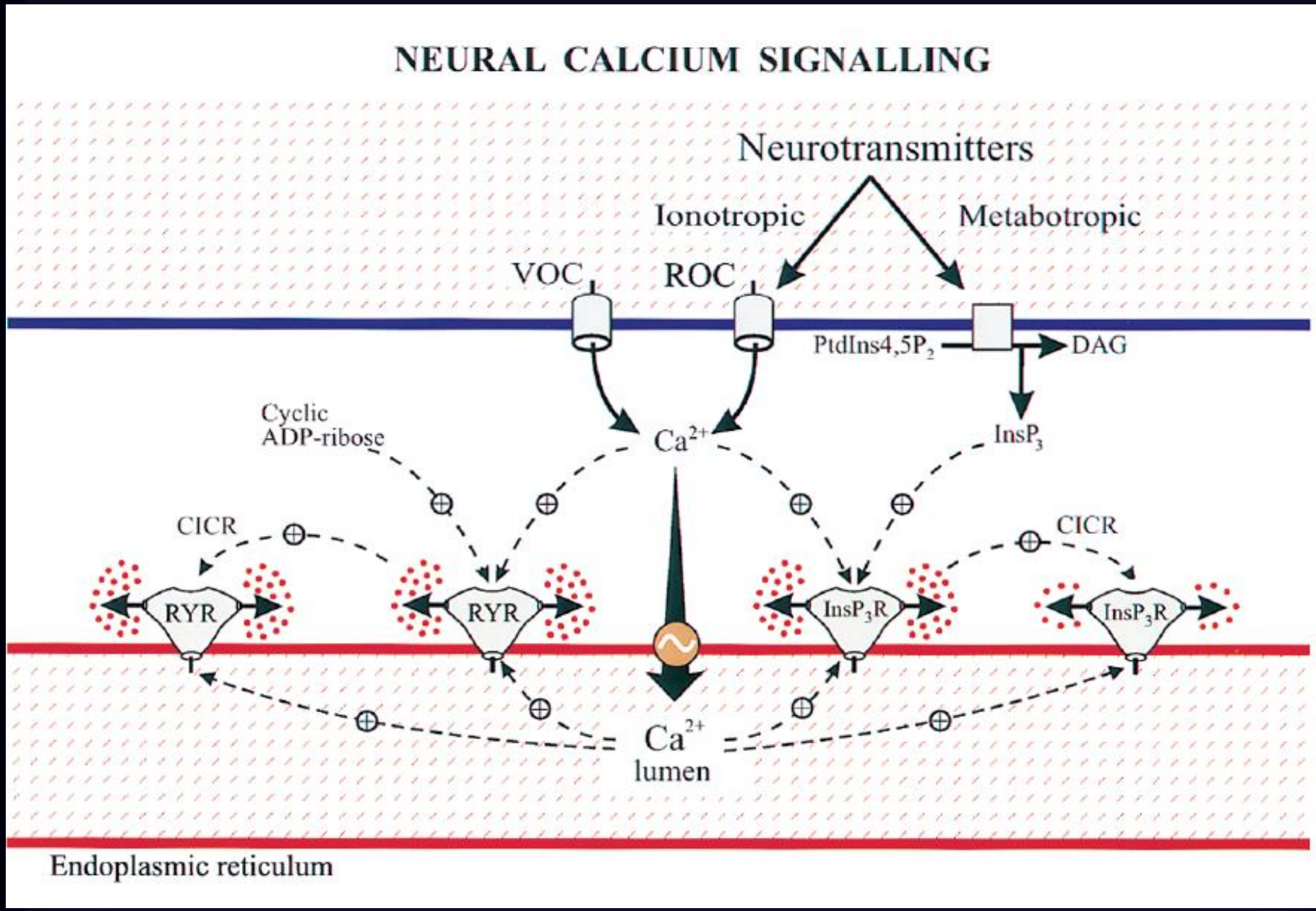



Berridge (1998) Neuron, 21: 13-26

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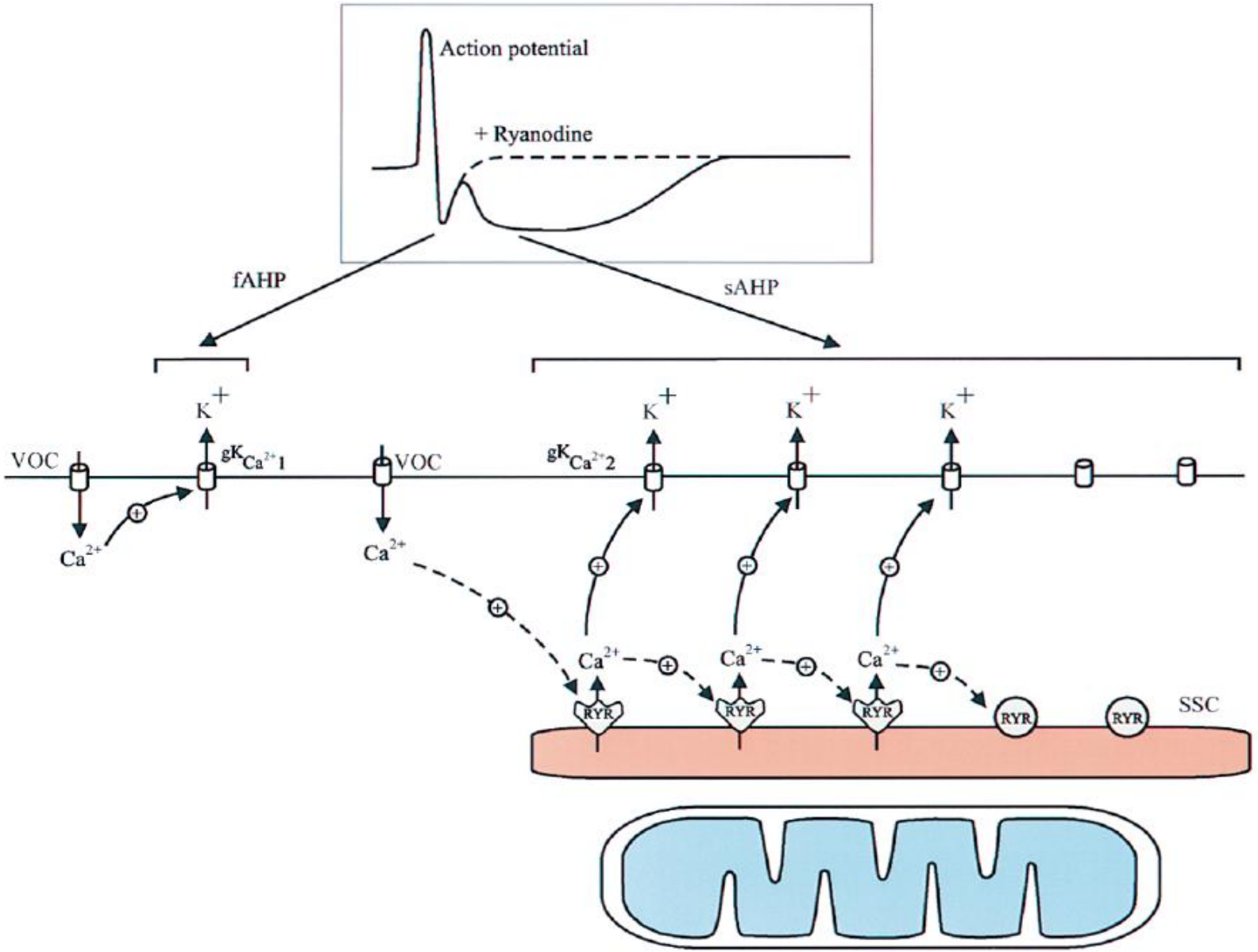


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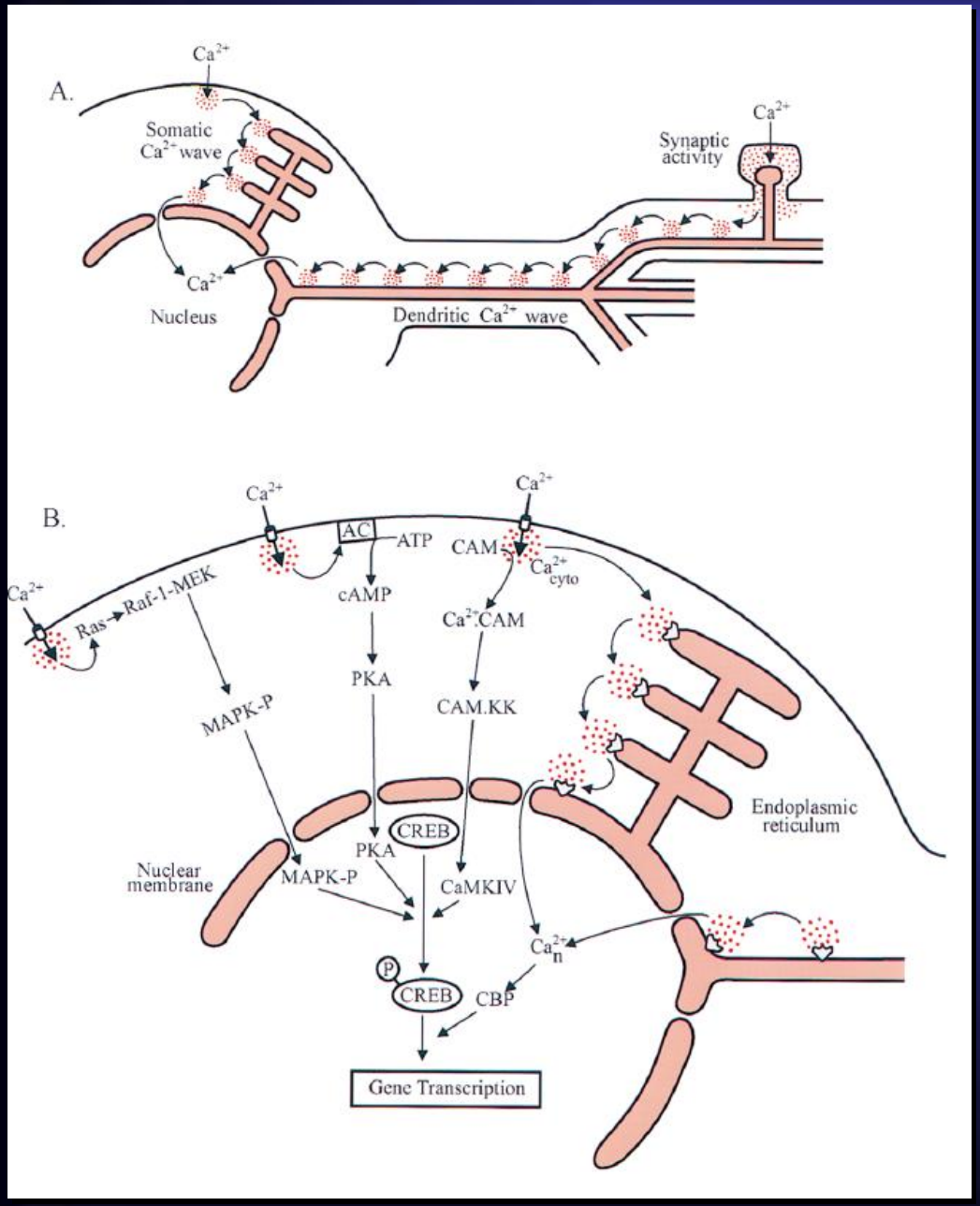


# $Ca^{++}$ signaling

- Fast neurotransmitter release
- Mediates timing of after-hyperpolarization
- Gene transcription
- Synaptic plasticity

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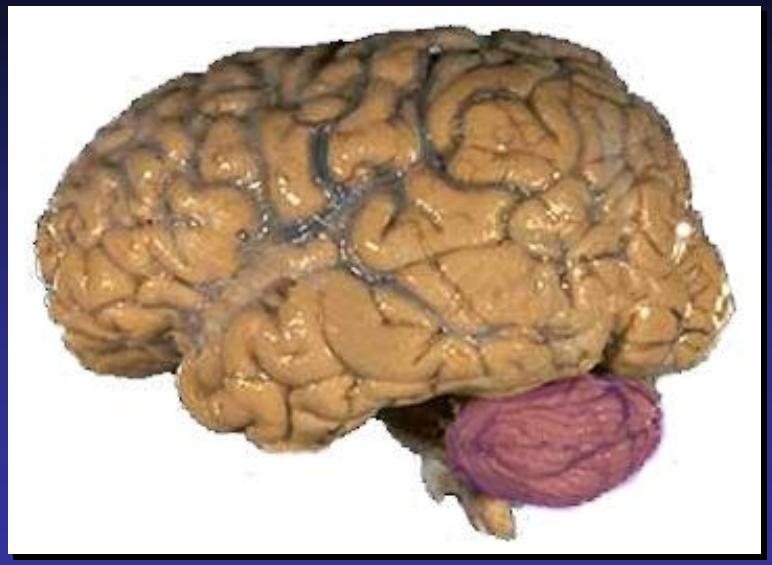
- SCA 2 and Purkinje cell degeneration
  - Expanded polyQ repeat (>32) results in pathological state
  - PC degeneration occurs earliest
- Internal Ca++ mediated by the IP3R & RyR
  - IP3R and RyR extensively expressed in ER membrane :
    - most CNS neurons express cardiac RYR2
    - Purkinje cells express skeletal muscle RyR1



# Hypothesis

Does mutant ATX-2 result in abnormal Ca++ signaling in Purkinje cells?

# Experimental Design

- Animals: WT & Q58 TG
- Cellular
  - *In vitro* binding assay
  - PC Cell culture and Immunostaining
  - *In Vitro* apoptosis cell death
- Physiological
  - E-phys recording
  - Calcium imaging
- Pharmacology
  - Dantrolene feeding
- Behavioral
  - Motor coordination: Beam cross; accelerating rotarod
- Anatomical
  - Stereological analysis
  - Pathology
- Statistics: two-tailed Student's unpaired t-test…AND?

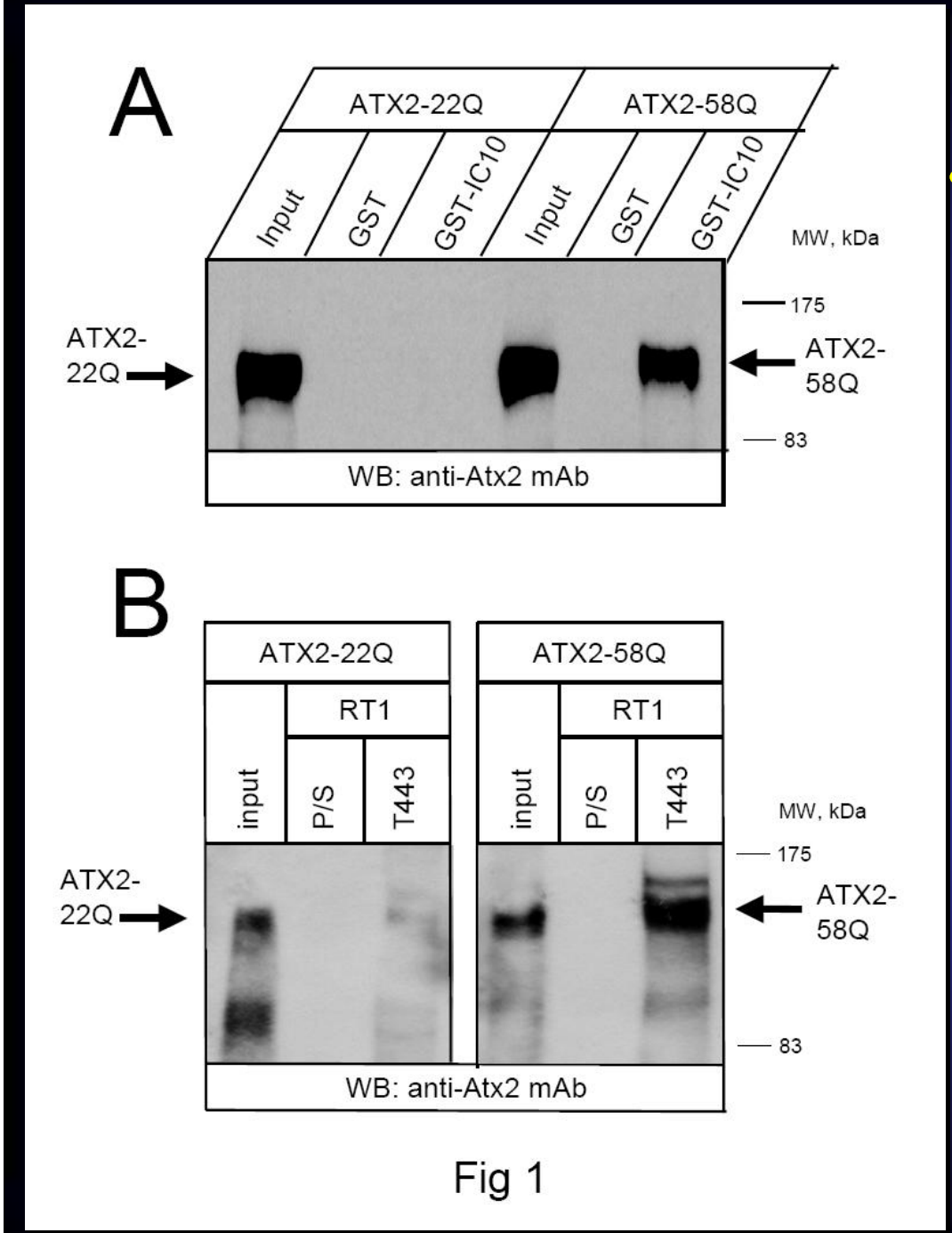



Fig 1

- In vitro binding assay
  - Pull down exp indicates association of proteins
  - Atxn2 plasmids transformed into COS7)-mixed with purified C-Terminal IP3 fragments (GST-IC10)
  - Full length IP3 fragmment (T443)
  - Western-blot of lysate

- Rat IP3R1 and Atxn 22/ 58 proteins in sf9 cells
- ER purified and fused to planar lipid bilayers
- Channels activated by 100nm or 2μM of IP3
- Recording currents?

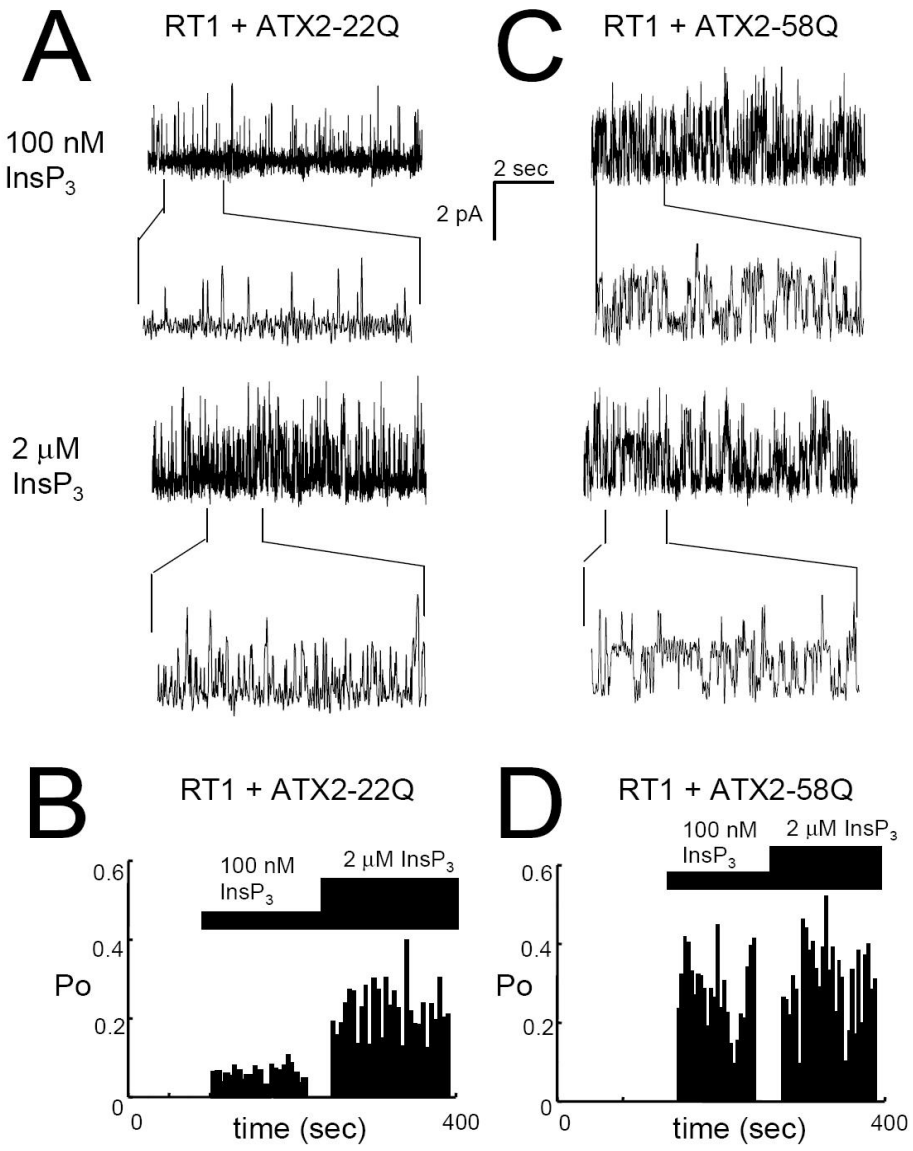
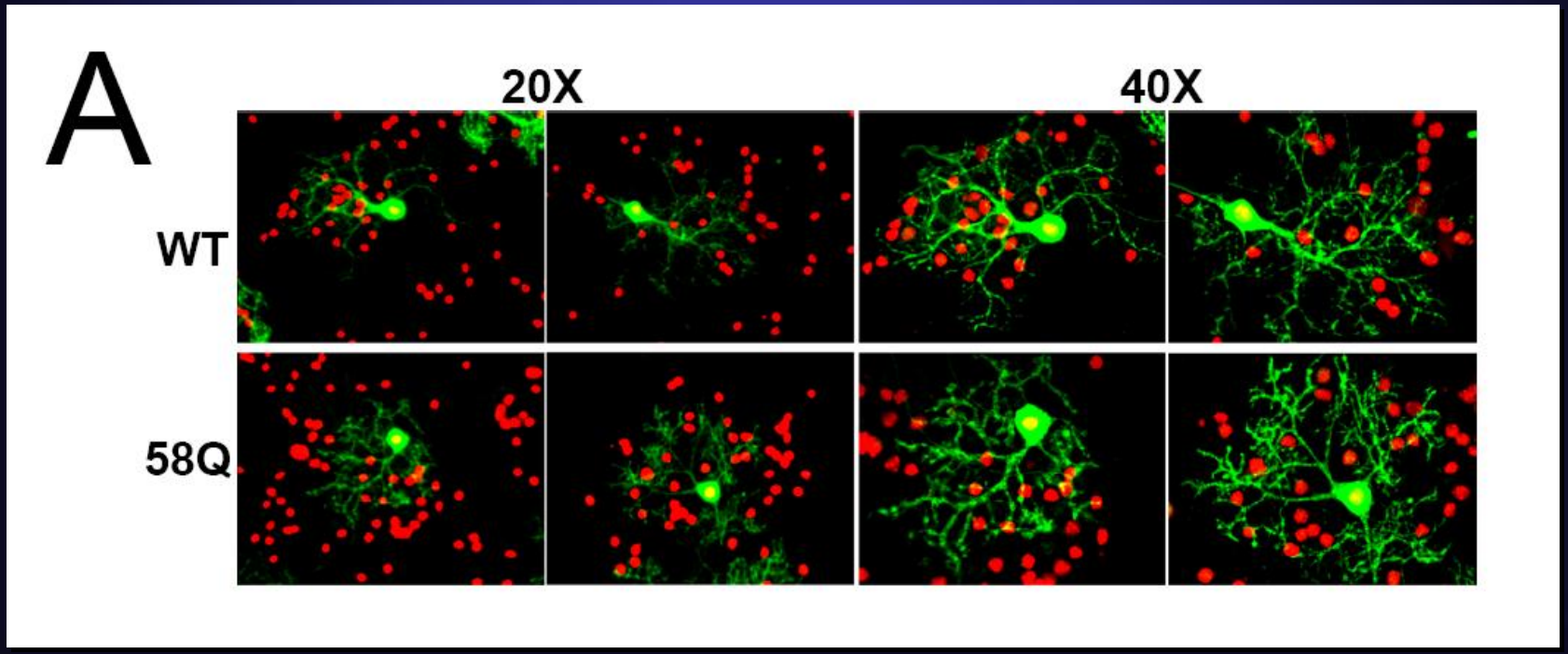
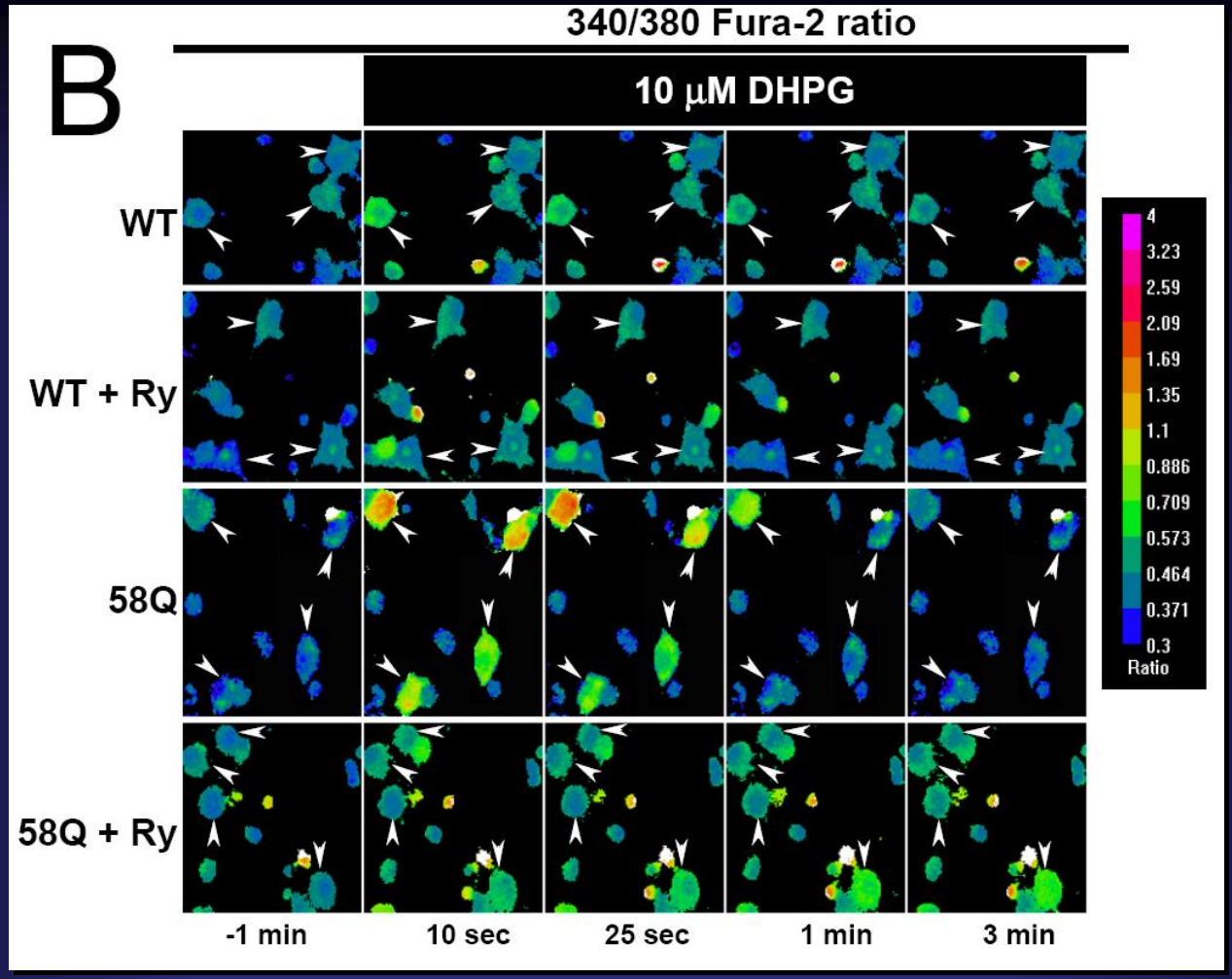



Fig 2

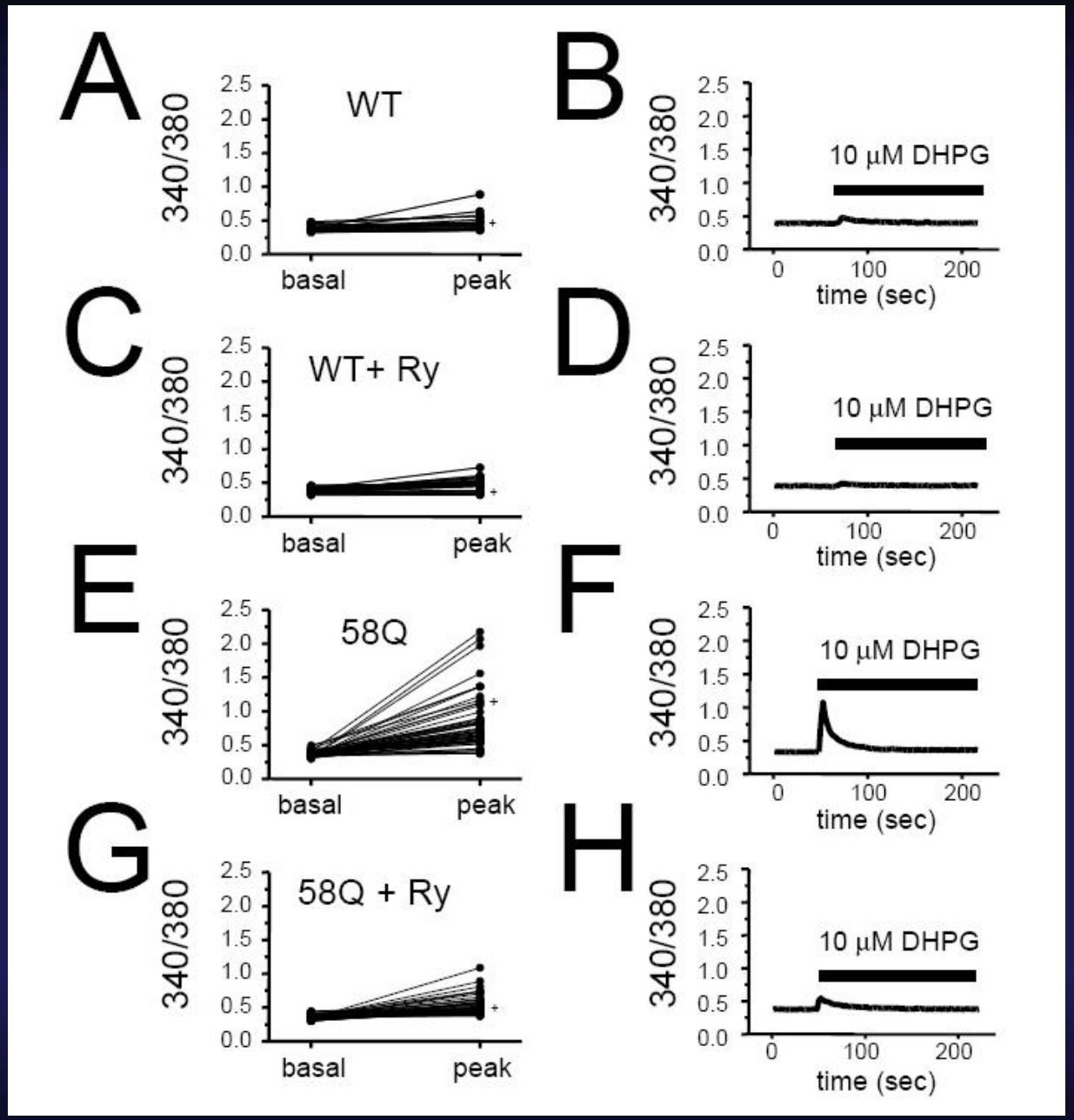
- Purkinje Cell culture

- Neonatal cerebellar dissected
- Tissue digested and PC cultured
  - Day 14 cells stained with anti-IP3R1 (T443,green) and DAPI (red)






- DHPG stimulated calcium release mGLUR (5)
- Fura-2 CA++ sensitive dye


A
WT
340/380
basal
peak
B
10 μM DHPG
time (sec)
C
WT+ Ry
D
E
58Q
F
G
58Q + Ry
H

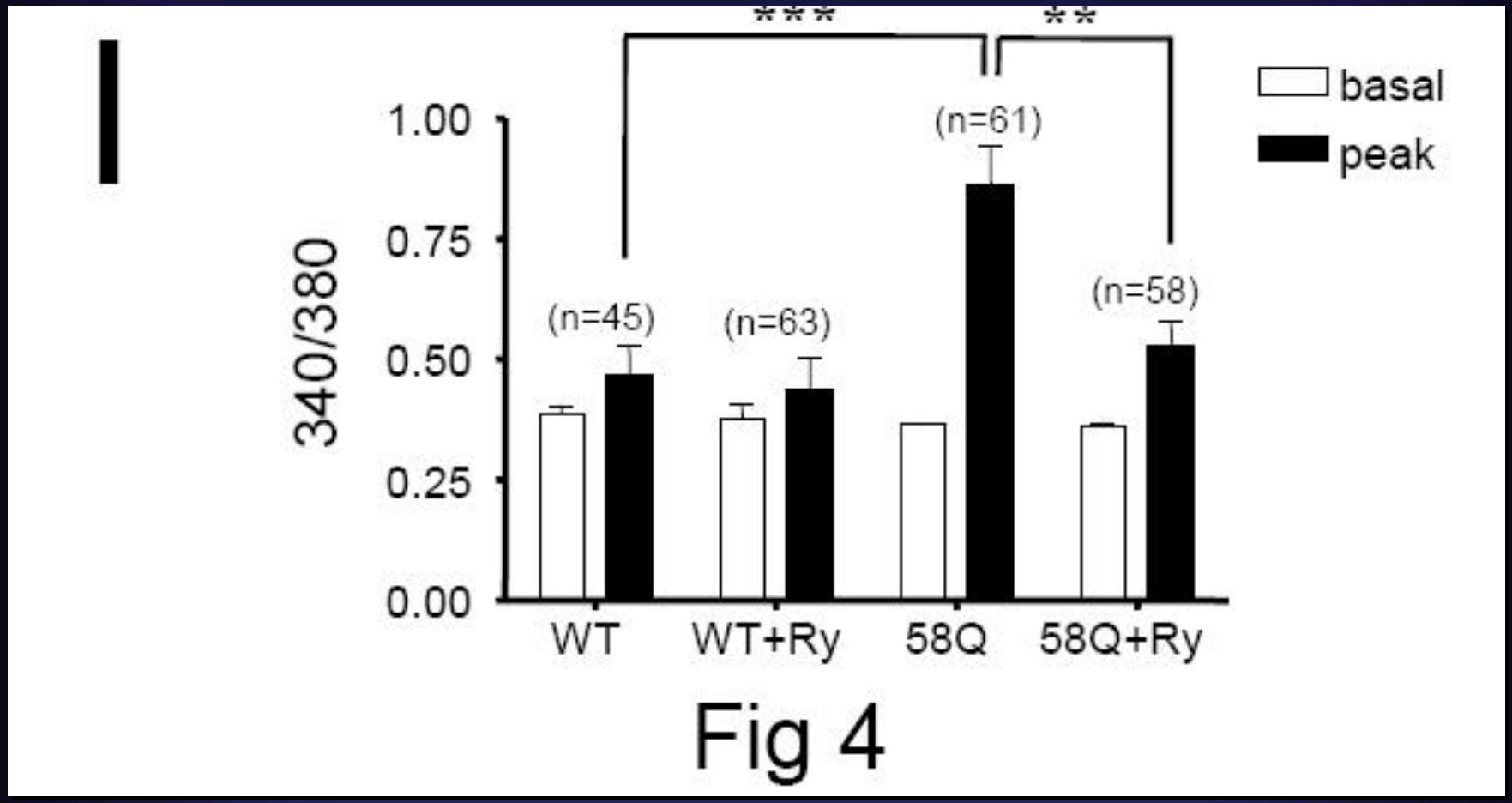



Fig 4

- Note: this should not be a t-test

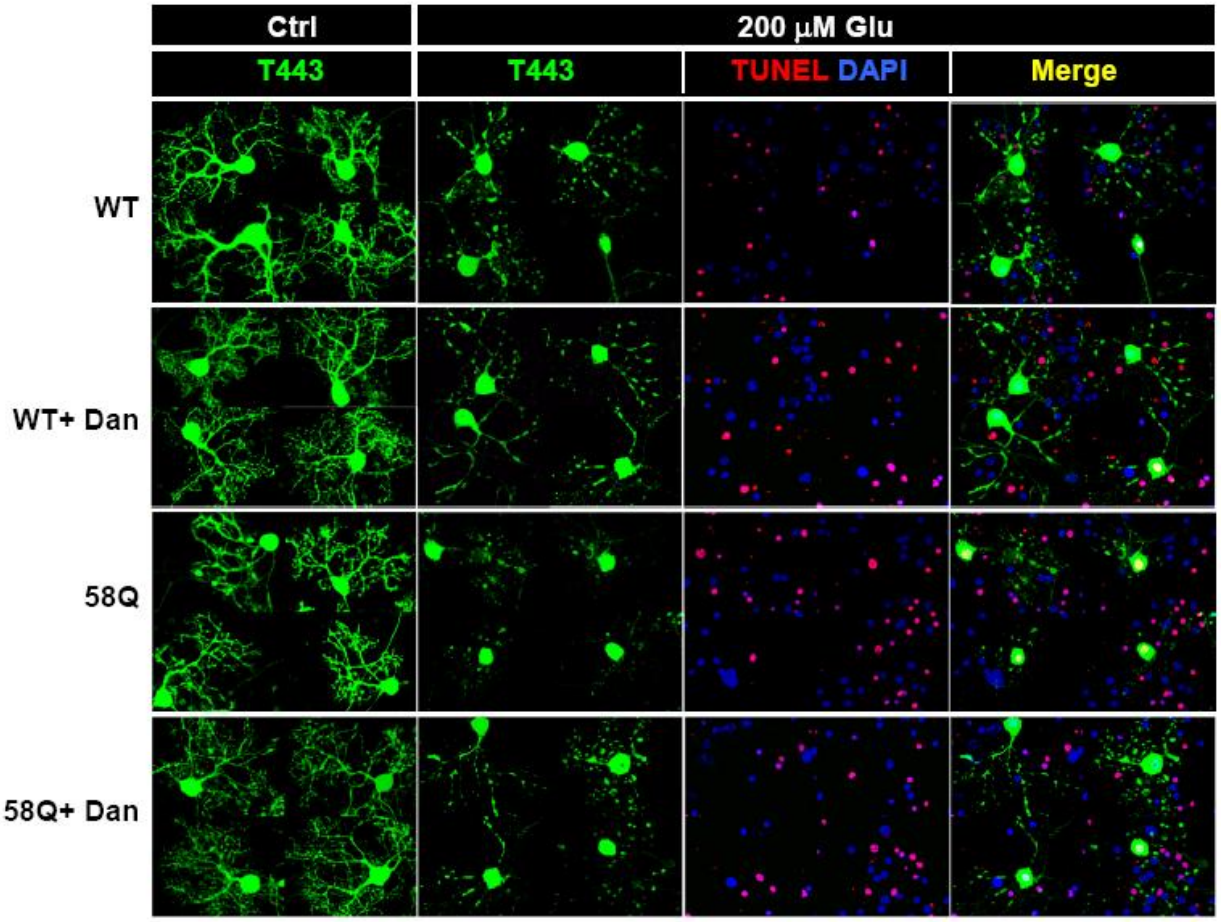



Fig 5

- GLU-induced cell death: 200 μM GLU for 7h @ 37c
- Anti-IP3R1 (T443, green), TUNEL for apoptosis (red), DAPI (blue)

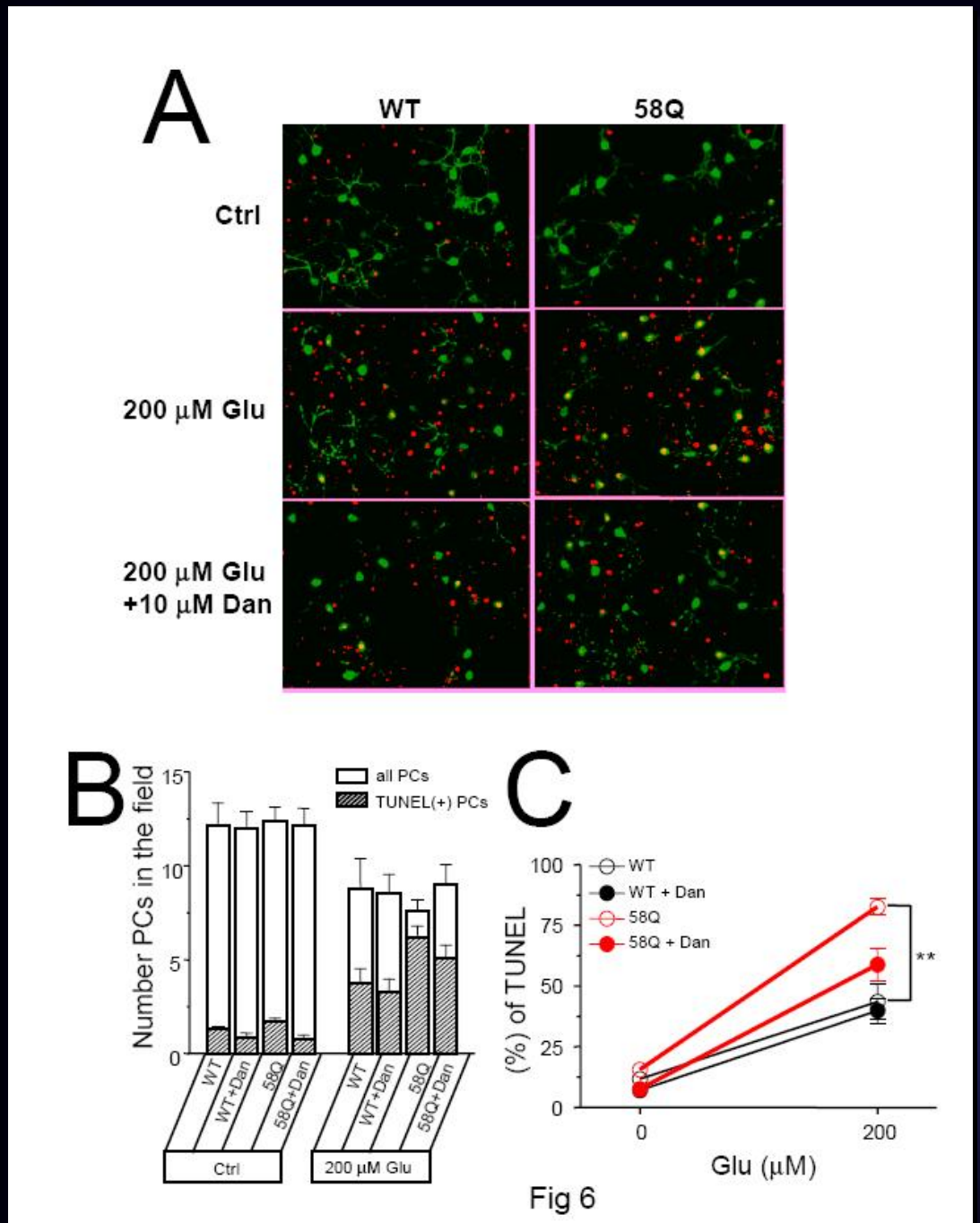



Fig 6

- PC treated with GLU and Dantrolene
- Anti-IP3R1 (T443, green) and TUNEL (red)

# Motor coordination: beam walk

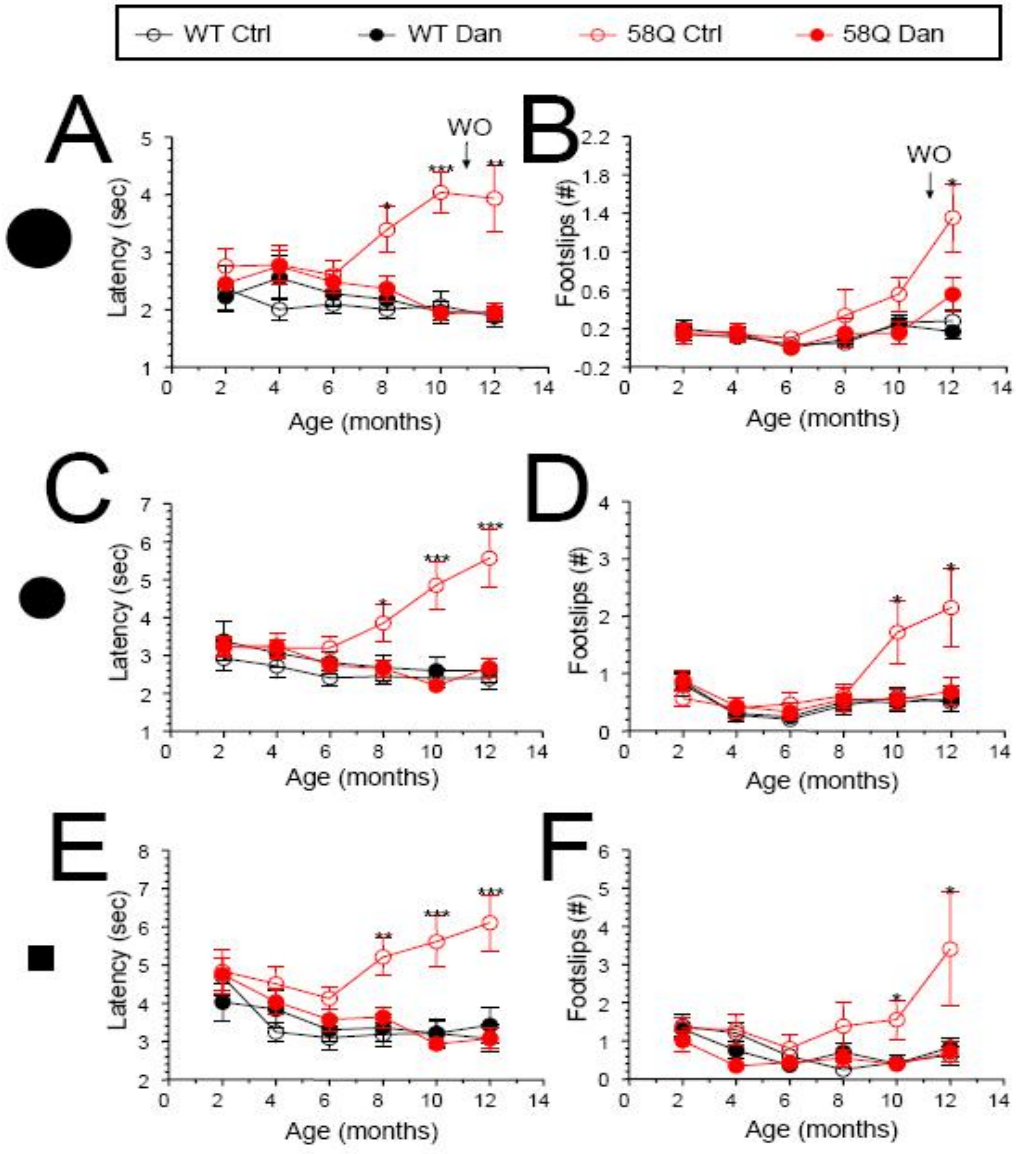
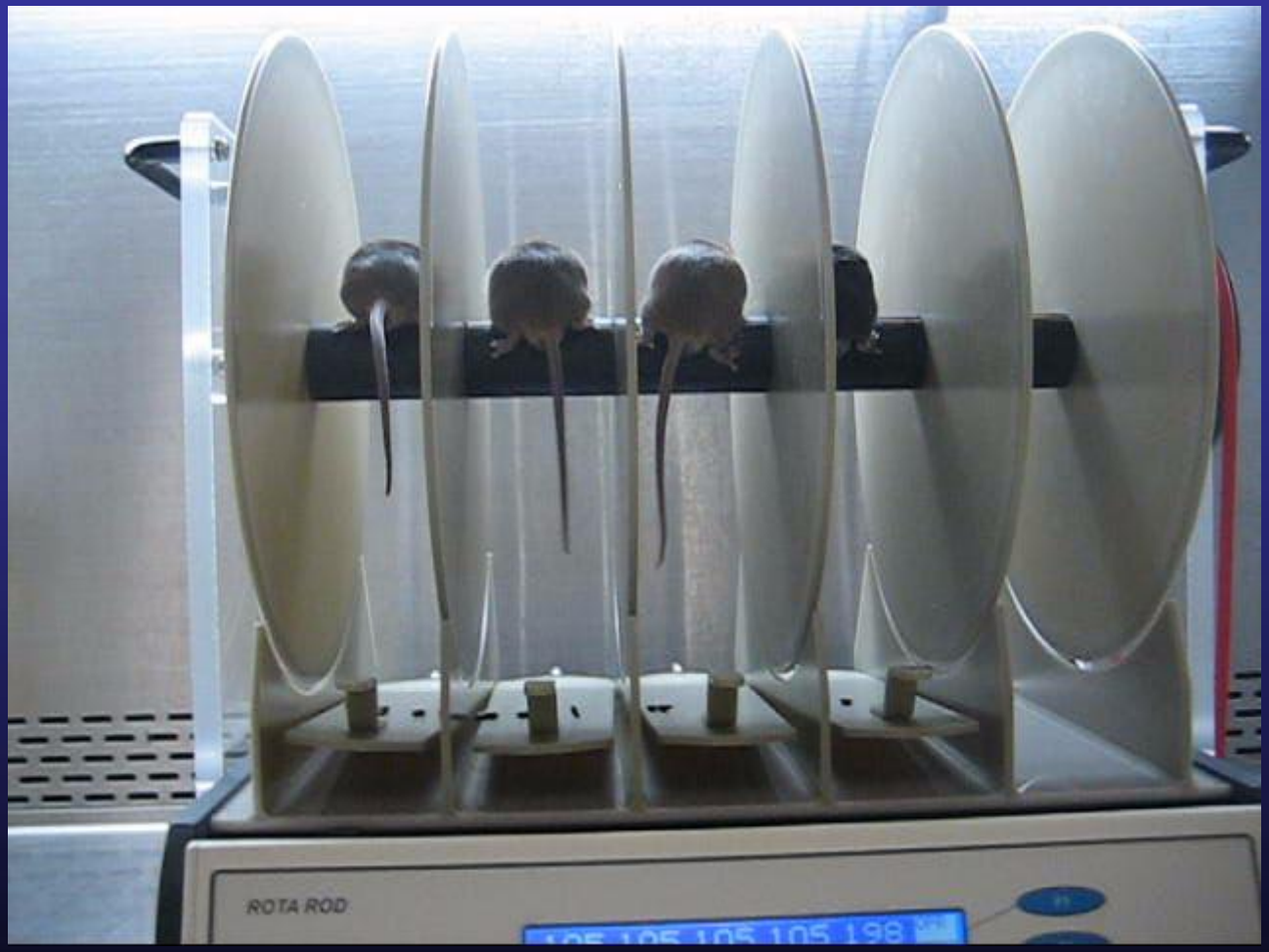


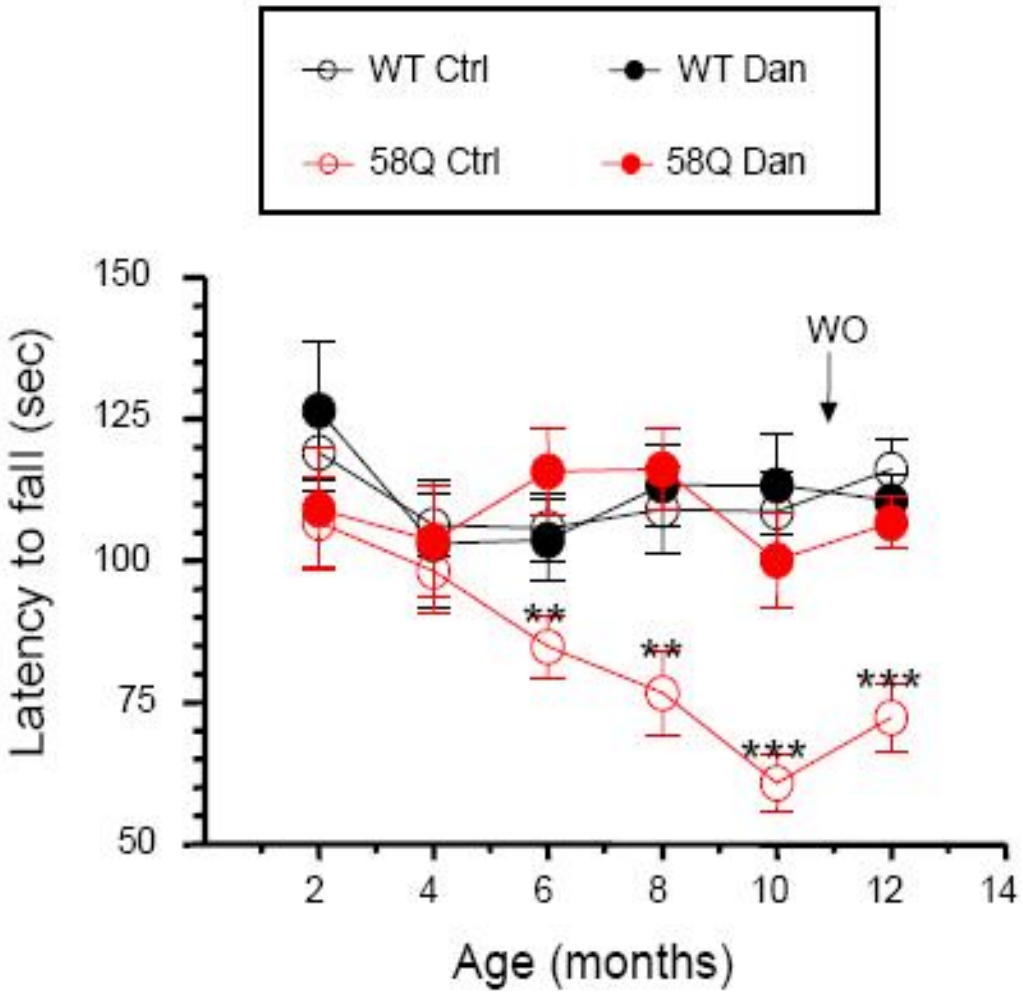

Fig 7

- 3 consecutive days (4 t/d)
- Animals treated with Dantrolene from age 2-11 months
- Tested on 3 different widths of beam
- Length of beam?

Note: cannot use Students t-test

# Rotarod






- Test of motor-coordination
- 3 trials per day for 3 days
- Data represents the avg of 3 trials on test day

Note: cannot use Students t-test

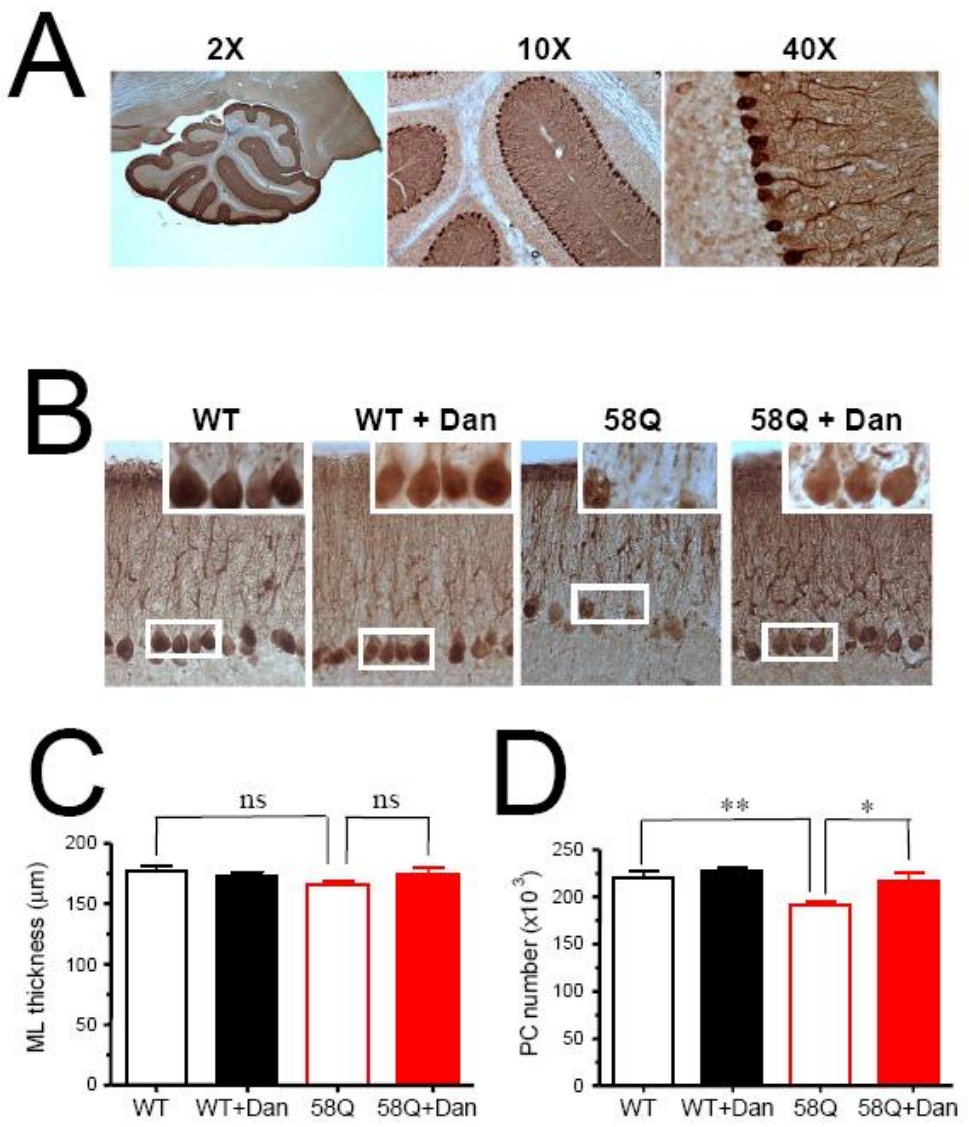



Fig 9

- Calbindin-D 28K staining of WT (A) and experimental animals (B) at 12 months age
- C) Molecular layer thickness compared across groups
- D) Number of PC cells across groups

Note: cannot use Students t-test

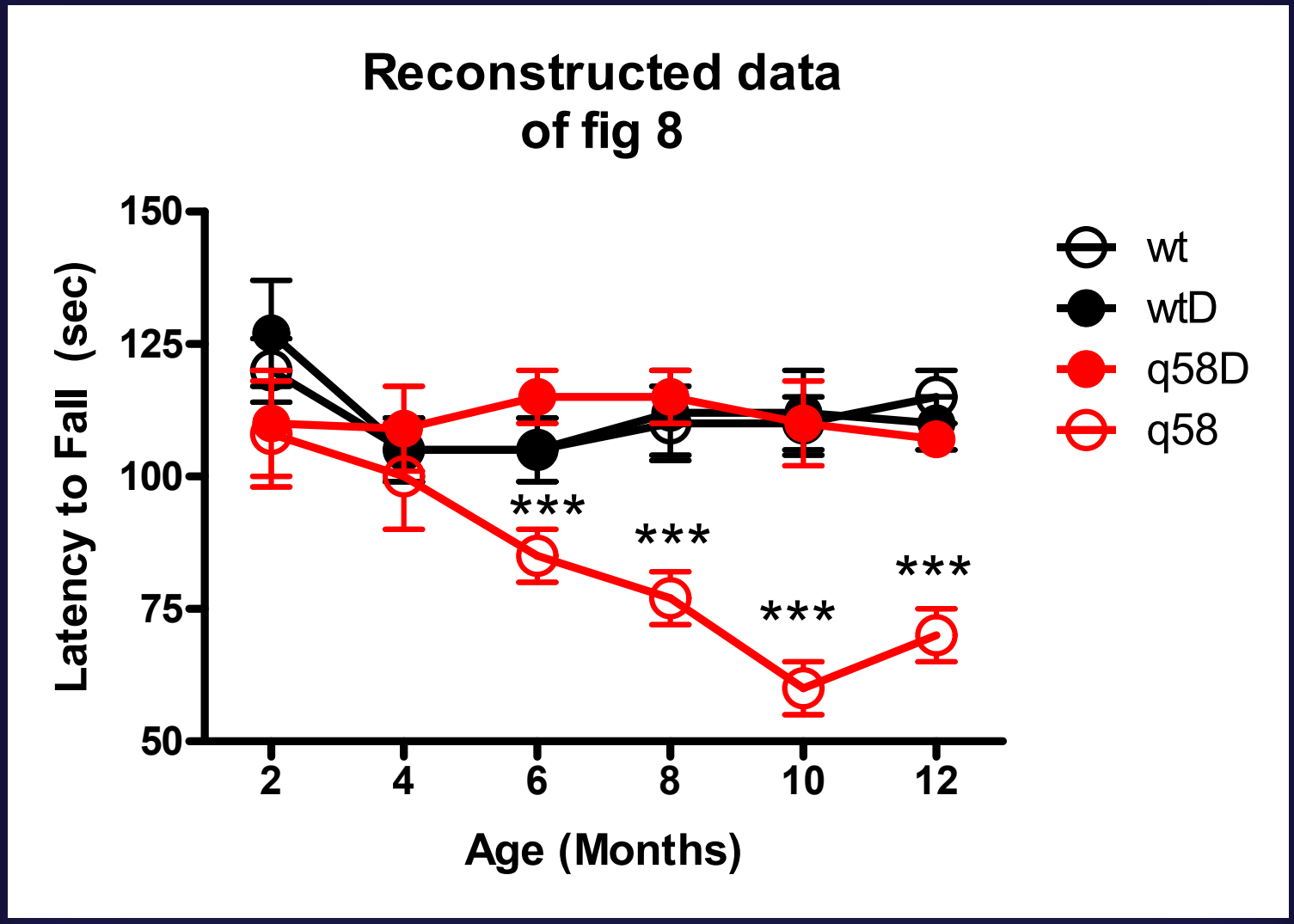
# No significant differences detected in pathological analysis

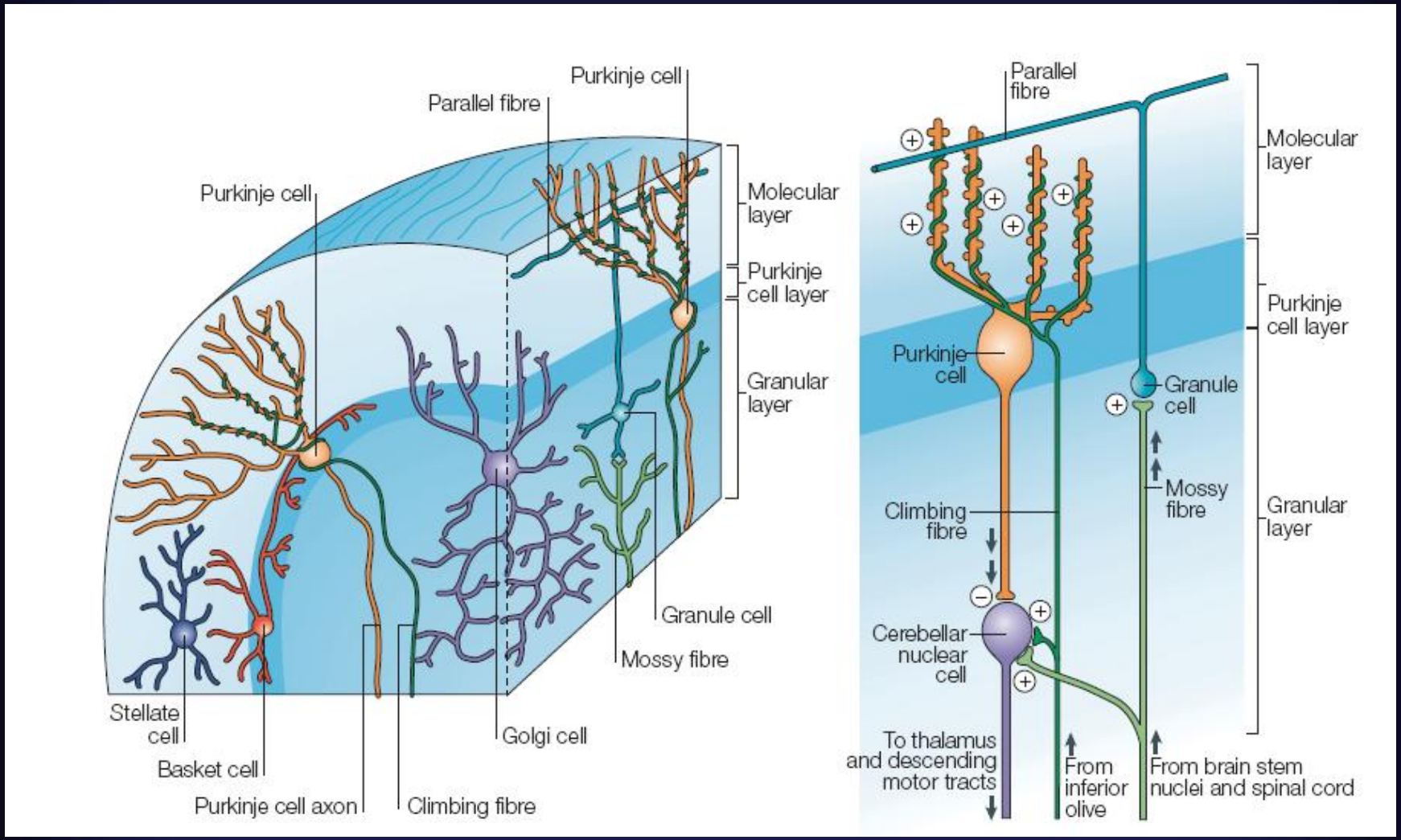
| Group name | Number of mice analyzed | No. of mice per group with any degree of inflammation | | |
|---|---|---|---|---|
| | | Sk muscle | heart | liver |
| WT Ctrl | 6 | 1 | 1 | 3 |
| 58Q Ctrl | 5 | 0 | 0 | 1 |
| WT Dan | 5 | 1 | 0 | 3 |
| 58Q Dan | 5 | 0 | 0 | 3 |

# Extra slides

# Dantrolene feeding

| Group number | Group name | Number of female mice | Mouse genotype | Single dose (2/wk) (50µl) | Drug dosage (mg/kg/3 days) | ML thickness (µm) | PC counts |
|---|---|---|---|---|---|---|---|
| 1 | WT Ctrl | 9 | WT | 50 µl PBS | PBS | 176 ± 5 | 221240 ± 6767 |
| 2 | WT Dan | 12 | WT | 100 µg Dantrolene | 5 mg Dantrolene | 173 ± 3 | 224239 ± 5171 |
| 3 | 58Q Ctrl | 10 | SCA2-58Q | 50 µl PBS | PBS | 166 ± 3 | 191305 ± 4459 |
| 4 | 58Q Dan | 9 | SCA2-58Q | 100 µg Dantrolene | 5 mg Dantrolene | 175 ± 5 | 217629 ± 8951 |


Reconstructed data
of fig 8
Latency to Fall (sec)
150
125
100
75
50
2
4
6
8
10
12
Age (Months)
***
***
***
***
wt
wtD
q58D
q58


Purkinje cell
Parallel fibre
Purkinje cell
Molecular layer
Purkinje cell layer
Granular layer
Granule cell
Mossy fibre
Golgi cell
Stellate cell
Basket cell
Purkinje cell axon
Climbing fibre
Parallel fibre
Molecular layer
Purkinje cell layer
Purkinje cell
Granule cell
Mossy fibre
Granular layer
Climbing fibre
Cerebellar nuclear cell
To thalamus and descending motor tracts
From inferior olive
From brain stem nuclei and spinal cord