Decreases in the precision of Purkinje cell pacemaking cause cerebellar dysfunction and ataxia

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Nature Neuroscience 2006;9:389-397

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Background

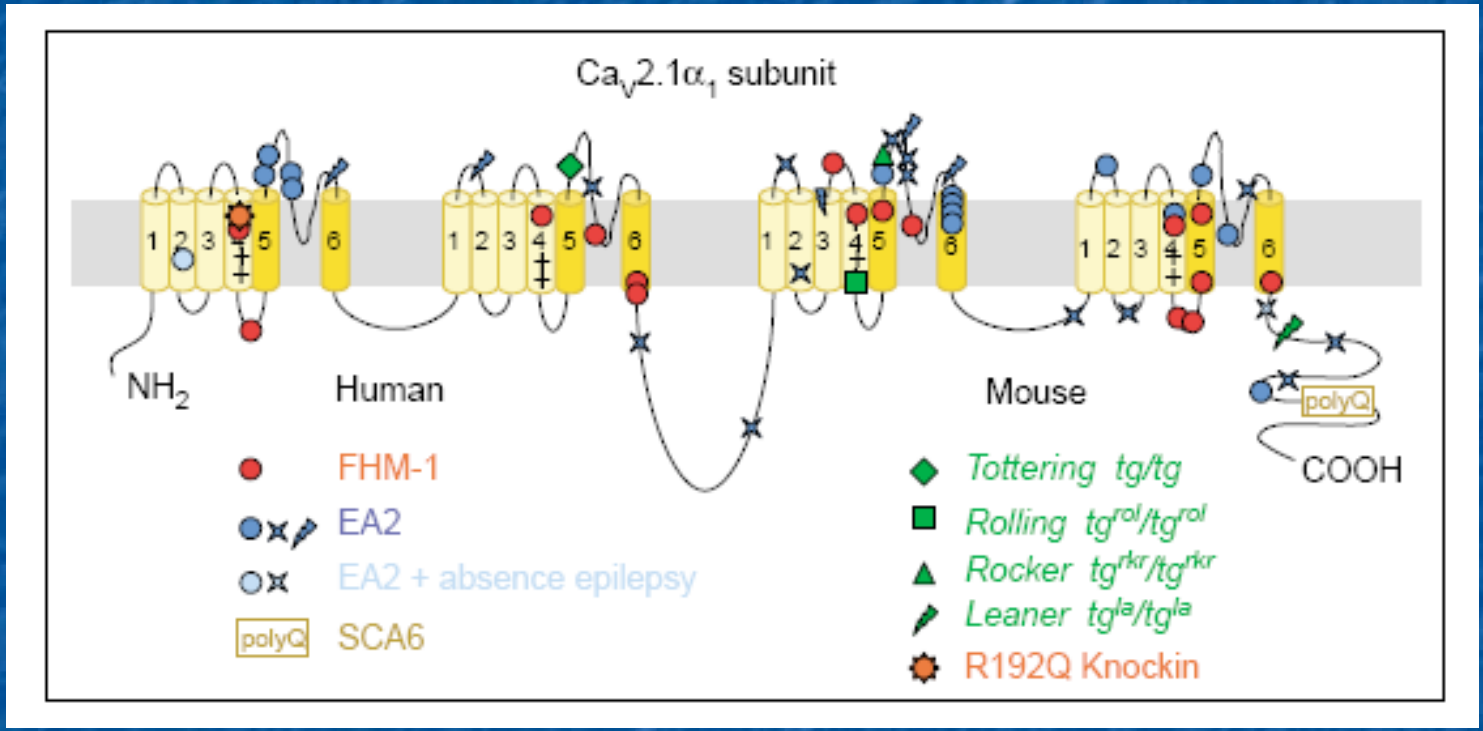
	EA 1	EA 2	EA 3	EA 4	EA 5	EA 6
Age of onset	2-15	2-20	1-42	23-60	3-teen	5
Duration of attacks	sec-min	hours	min-hours	hours	hours	hours-days
Interictal abnormalities	myokymia	nystagmus	myokymia, nystagmus	nystagmus	nystagmus	cognitive impairment
Progressive ataxia	no	yes	no	no	yes	yes
Vertigo	no	yes	yes	yes	yes	no
Associated symptoms	muscle spasms, tinnitus	dysarthria, headaches	tinnitus	tinnitus	juvenile myoclonic epilepsy	alternating hemiplegia
Seizures	yes	no	yes	yes	yes	yes
Response to ACTZ	occasional	yes	yes	no	transient	no
Genetic locus	12p13	19p13	1q42	unknown	2q22-23	5p13
Gene	KCNA1	CACNA1A	unknown	unknown	CACNB4	SLC1A3

ACTZ= acetazolamide

Background

- CACNA1A gene encodes: Voltage gated P/Q calcium channel protein
- Abundant throughout the CNS, especially cerebellar Purkinje neurons
- Allow presynaptic Ca^{++} influx to trigger neurotransmitter release
- Associated with:
  - EA2
  - Familial Hemiplegic Migraine
  - SCA6
  - Absence Epilepsy
  - Lambert-Eaton Syndrome

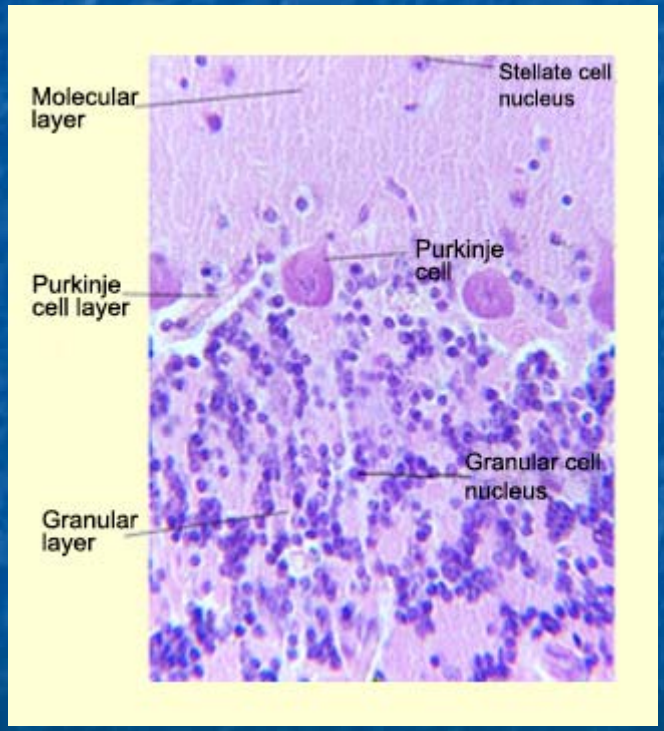
Background



Pietrobon. Current Opin Neurobiology 2005;15:257-265

Background

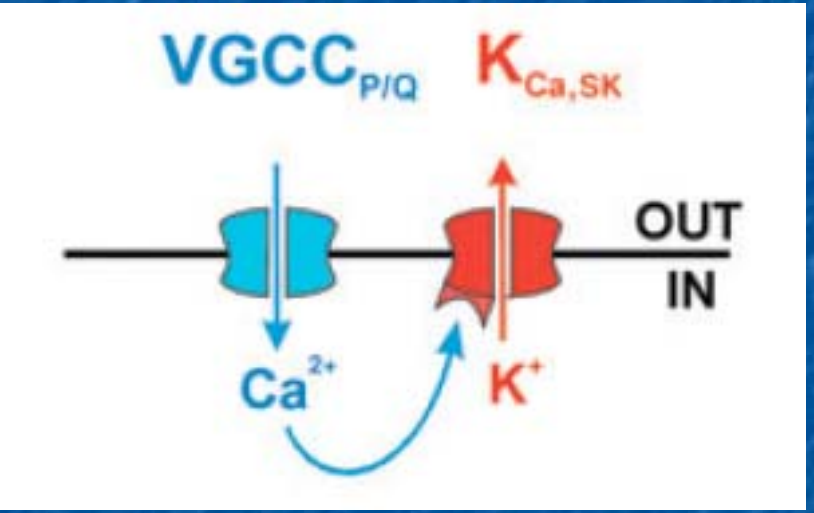
- Purkinje neurons
  - GABAergic (inhibitory) output neurons of cerebellar cortex
  - Inhibits dentate, globose, embolliform, and fastigial nuclei
  - Able to fire continuously at high rates (~40Hz) without synaptic inputs



www.thebrain.mcgill.ca/.../a_06_cl_mou.html

Background

P/Q channel mediated depolarizing spikes recruit SK channels in between spikes to cause hyperpolarization, which allows continuing spiking



Otis, Jen. Nat Neurosci 2006; 9:297-8

Hypothesis

- 1. Synaptic information encoding relies on stability of intrinsic neuronal activity. Ataxia results from loss of precision of intrinsic pacemaking activity of Purkinje neurons
- 2. Precision of intrinsic Purkinje firing is dependent on K_{Ca}(SK) activity

Methods

- Record spike potentials from Purkinje neurons of brain slices of transgenic mouse models of EA2 with external synaptic input blocked
- Block wildtype P/Q activity with agatoxin and observe spike variation
- Treat brain slices with genetic P/Q defect or pharmacologically treated to block P/Q channel with EBIO, a potassium channel enhancer, to restore intrinsic pacemaking activity
- Allow intrinsic synaptic input to the Purkinje neurons and observe spike variability
- Treat transgenic mice with EBIO and observe effect on ataxia
- Treat another transgenic mouse model, *tottering*, with EBIO to see if the effect can be generalized

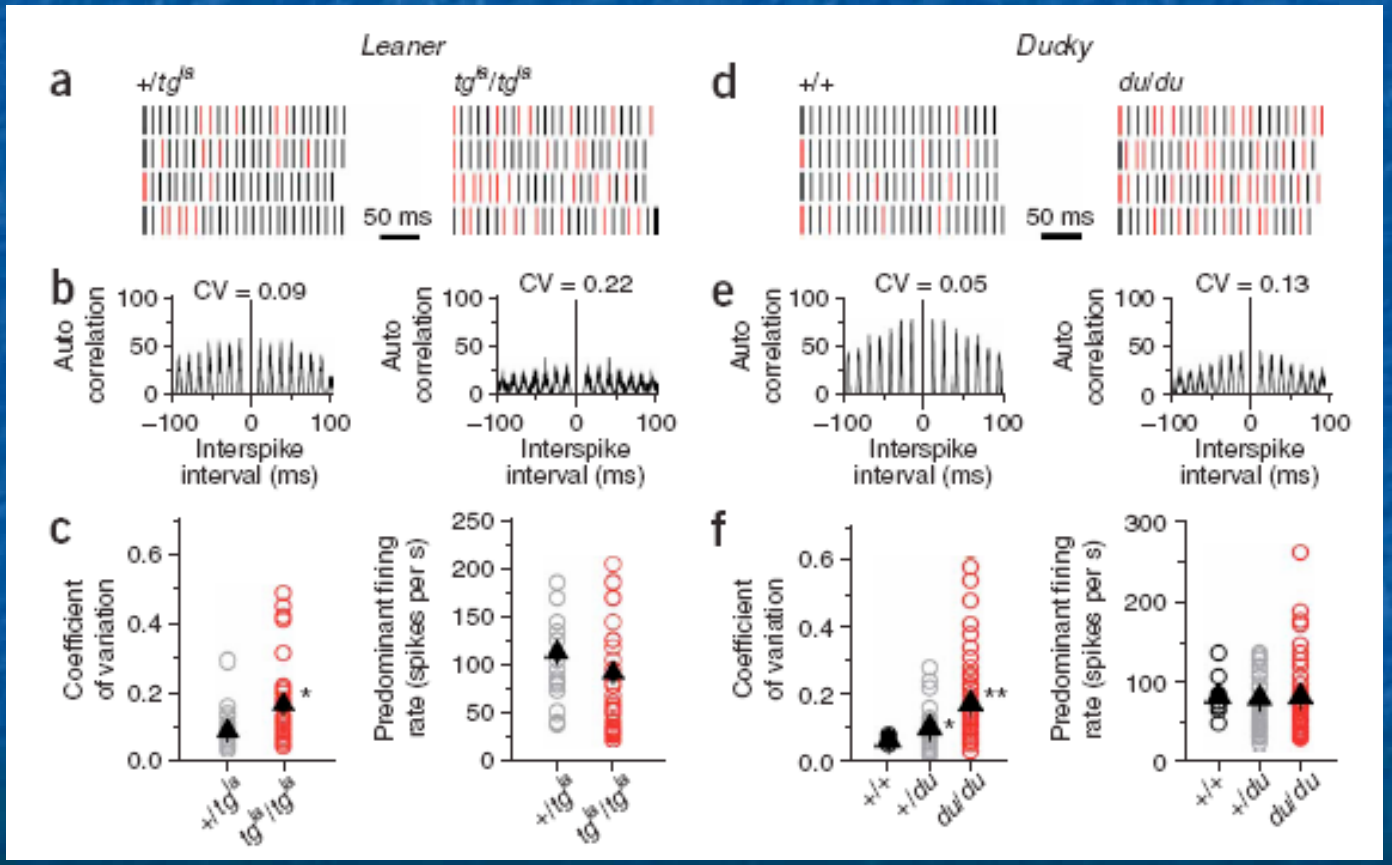
Background-Mouse models

- *leaner*
  - Autosomal recessive mouse model, absence seizures and ataxia
  - Splice donor site mutation in C-terminal regulatory region of CACNA1A
- *tottering*
  - Autosomal recessive mouse model with absence and motor seizures and ataxia
  - Missense mutation in CACNA1A
- *ducky*
  - Autosomal recessive mouse model with seizures and ataxia
  - Truncation mutation in auxiliary subunit, CACNA2d2
  - The encoded protein, $\alpha 2\delta 2$ is only expressed on Purkinje cells

- Is there variability in the interspike intervals between wildtype, *leaner* and *ducky* mice?

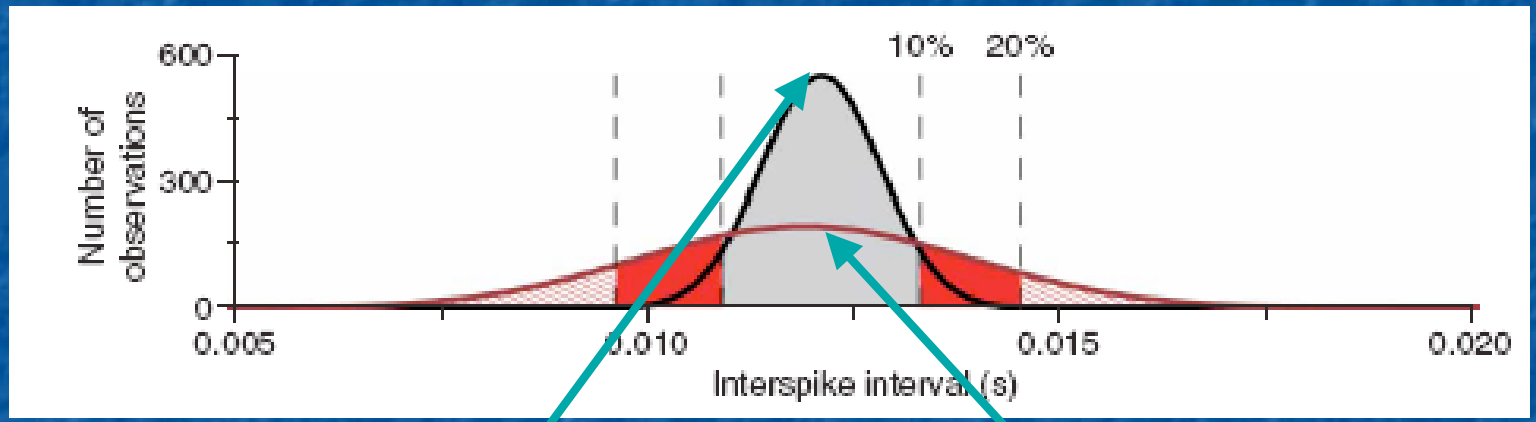
Results-Recording spike potentials

Figure 1



Results-Recording spike potentials

Figure 1g



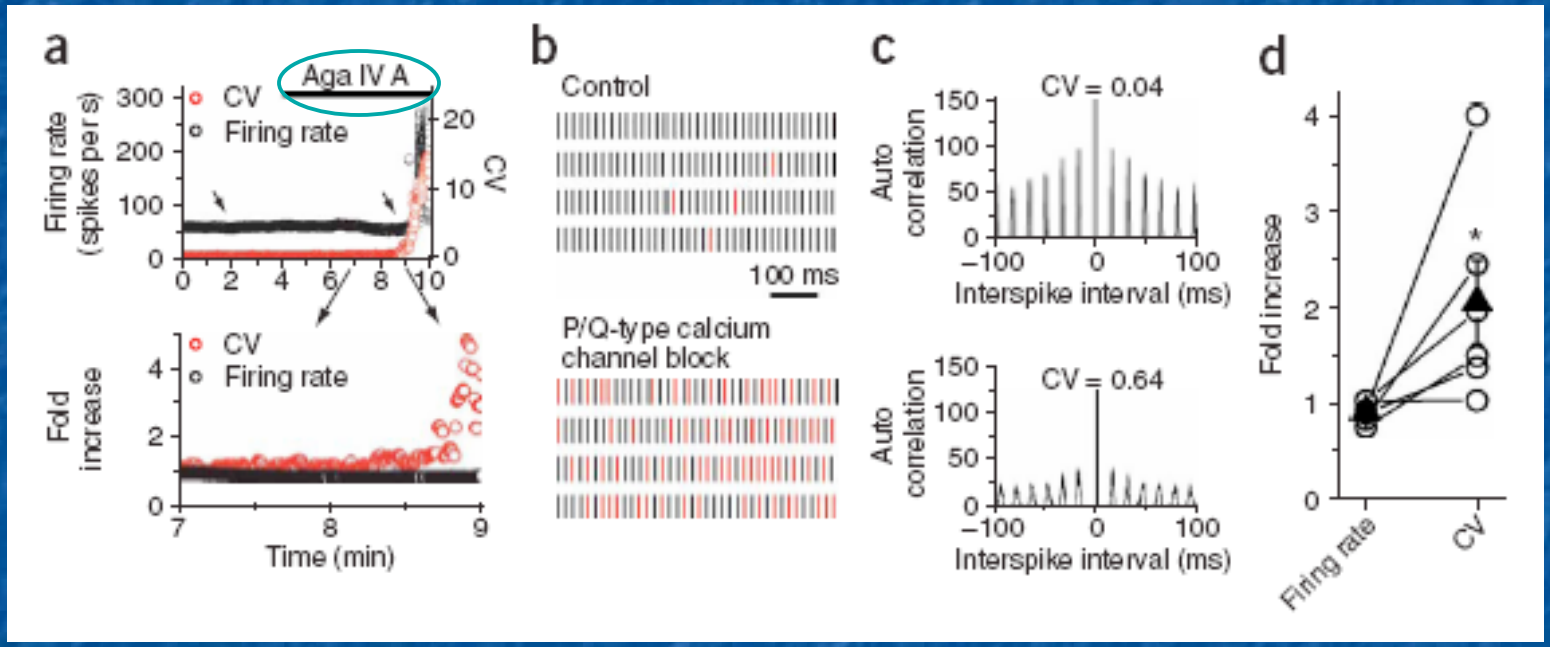
Wildtype

ducky

- How do you know if this irregular firing is due to a problem with the P/Q channel?
- Experiment: pharmacologically block the P/Q channel and observe the variability

Results

Figure 2

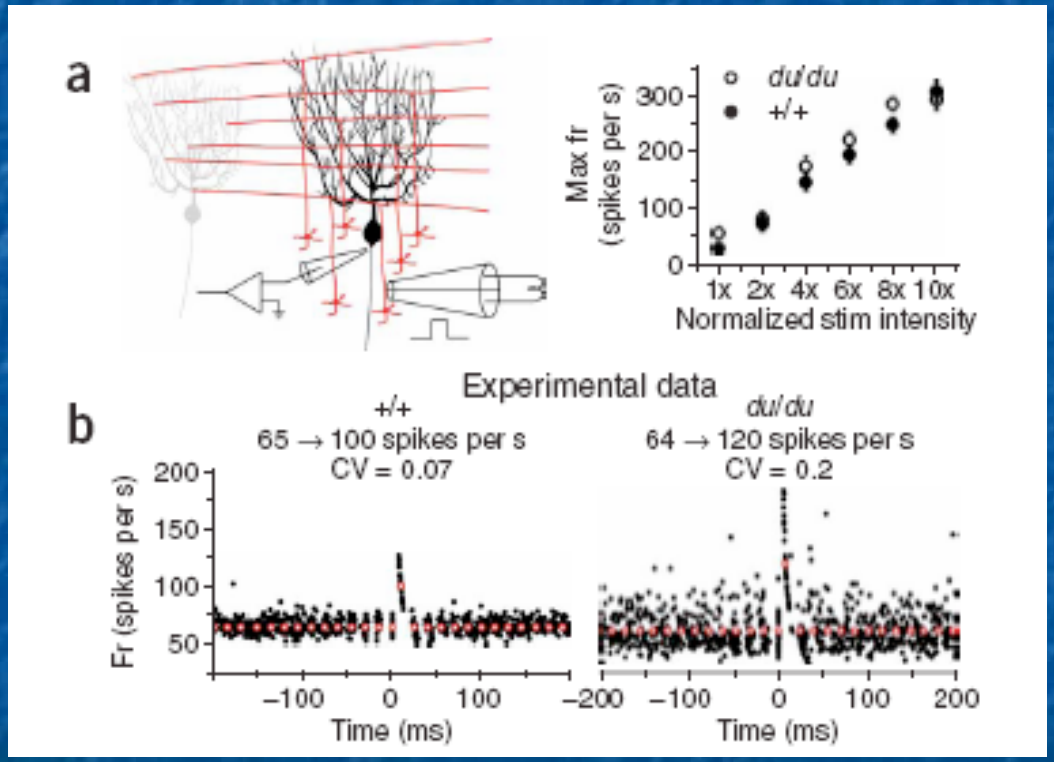


- What impact does this variability have on encoding synaptic inputs?

- Experiment: Stimulate parallel fibers, which synapse on Purkinje neurons and record from Purkinje neuron, i.e. correlate input and output

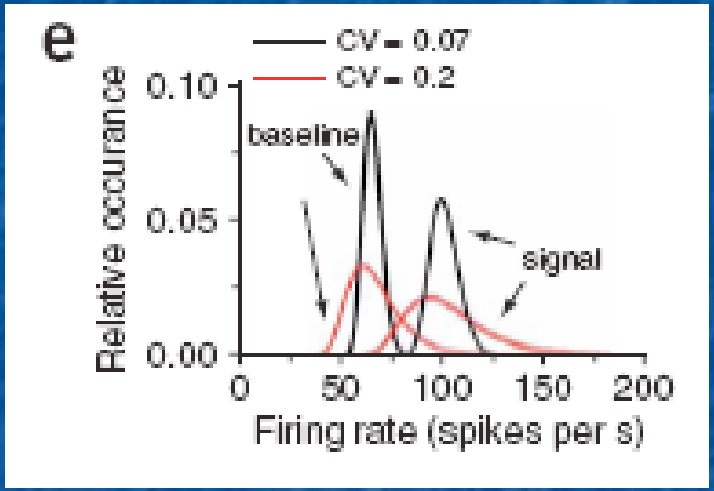
Results

Figure 3



Results

Figure 3 c-e



- Can this variability be pharmacologically improved?

- Experiment: Expose two groups of brain slices which have impaired P/Q function to EBIO. Group 1: pharmacologically treat with a P/Q blocker. Group 2: mouse with genetic defect in P/Q.

Background

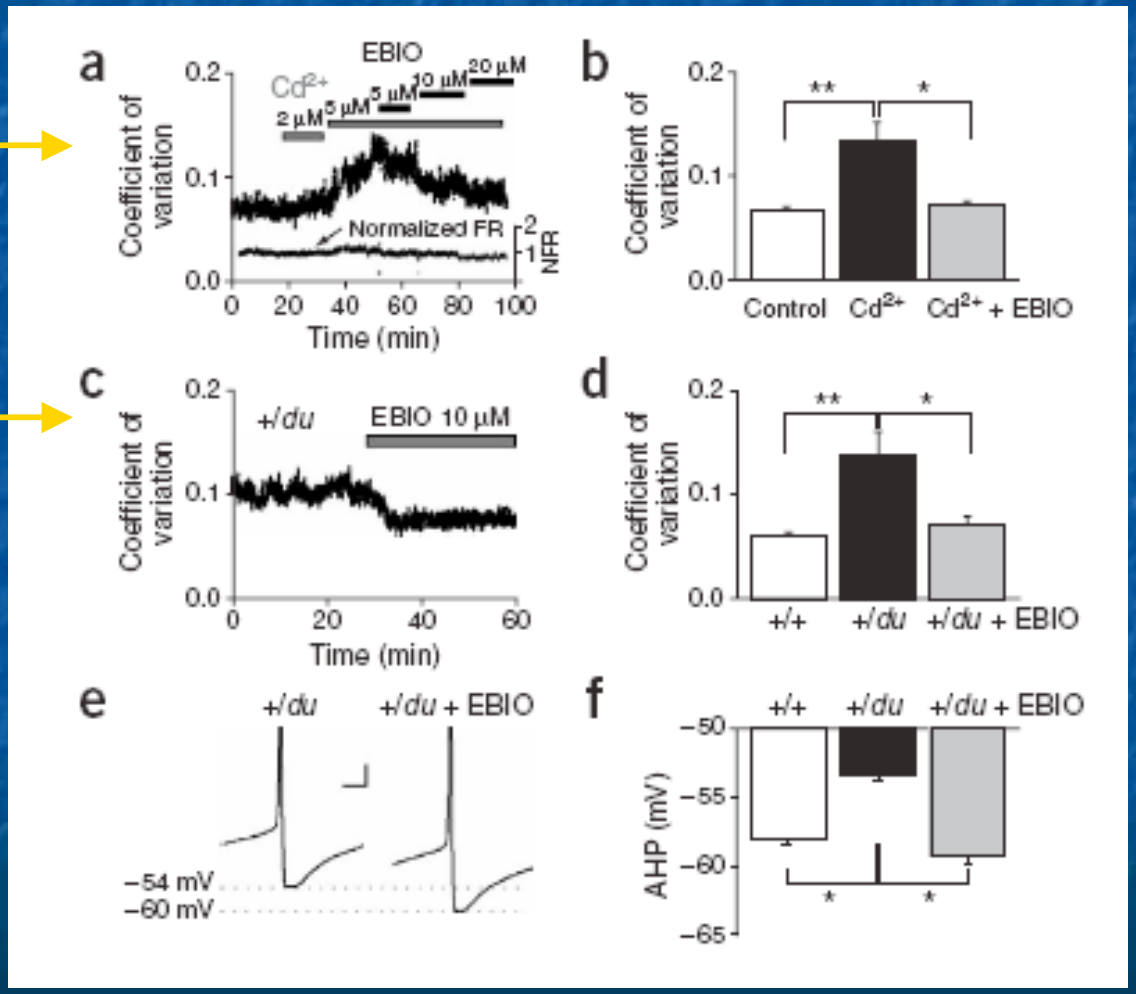
- EBIO: 1-ethyl-2benzimidazolinone, positively modulates SK channels which increases the affinity for Ca^{++}
- Increases SK activity

Results

Figure 4

Pharmacological P/Q blockade

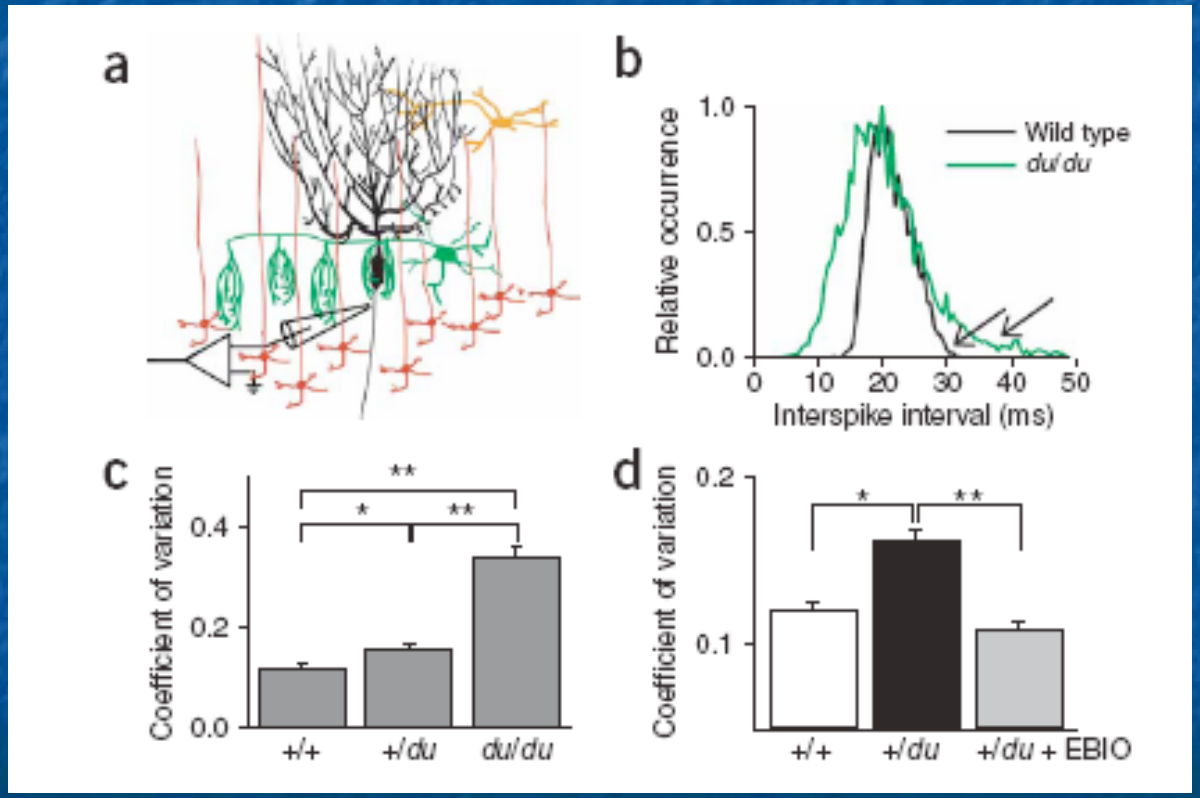
Genetic defect: *ducky*



- What happens to intrinsic activity if other synaptic inputs are allowed to contribute?
- Experiment: Repeat the experiments without blocking the inhibitory inputs from interneurons

Results

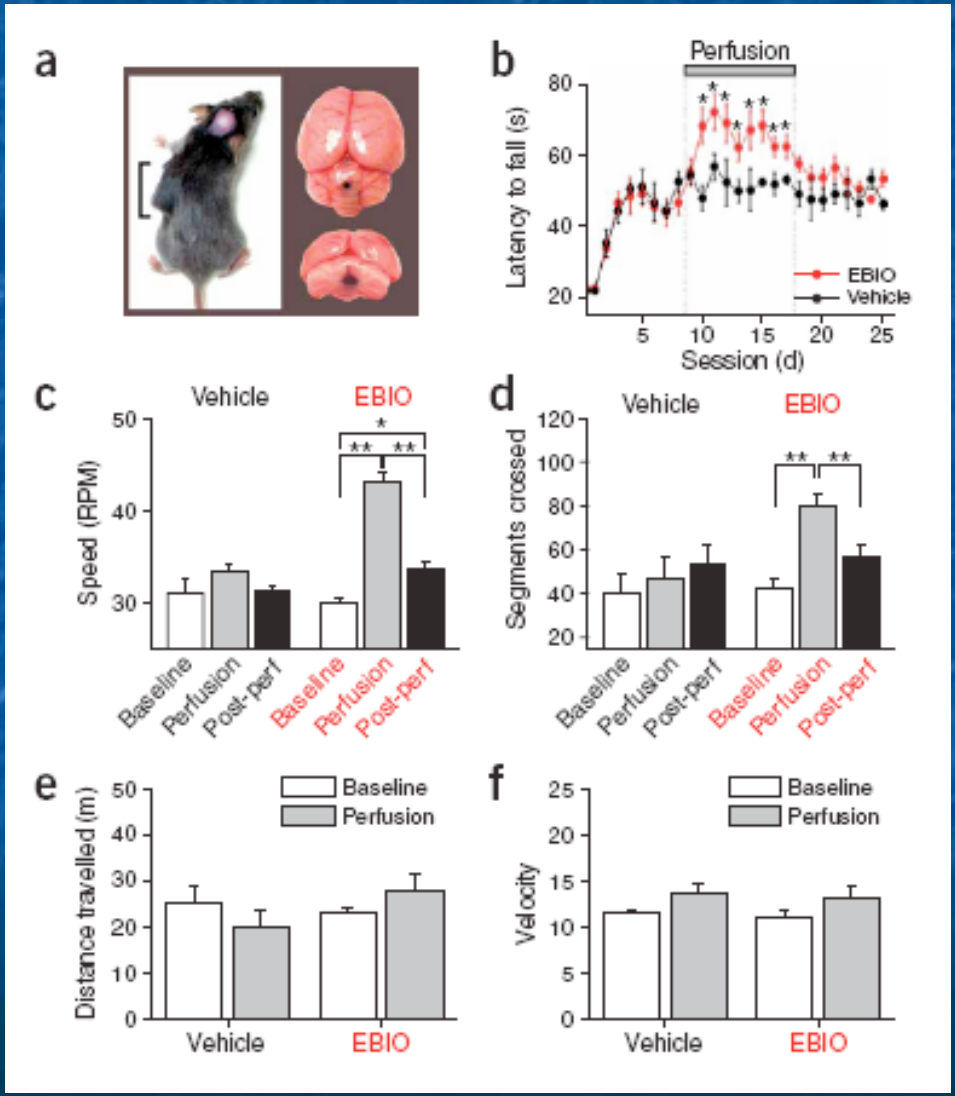
Figure 5



- Does EBIO affect ataxia *in vivo*?
- Experiment: Infuse EBIO into the cerebellar vermis of *ducky* mouse and observe behavior

Results-Treating with EBIO

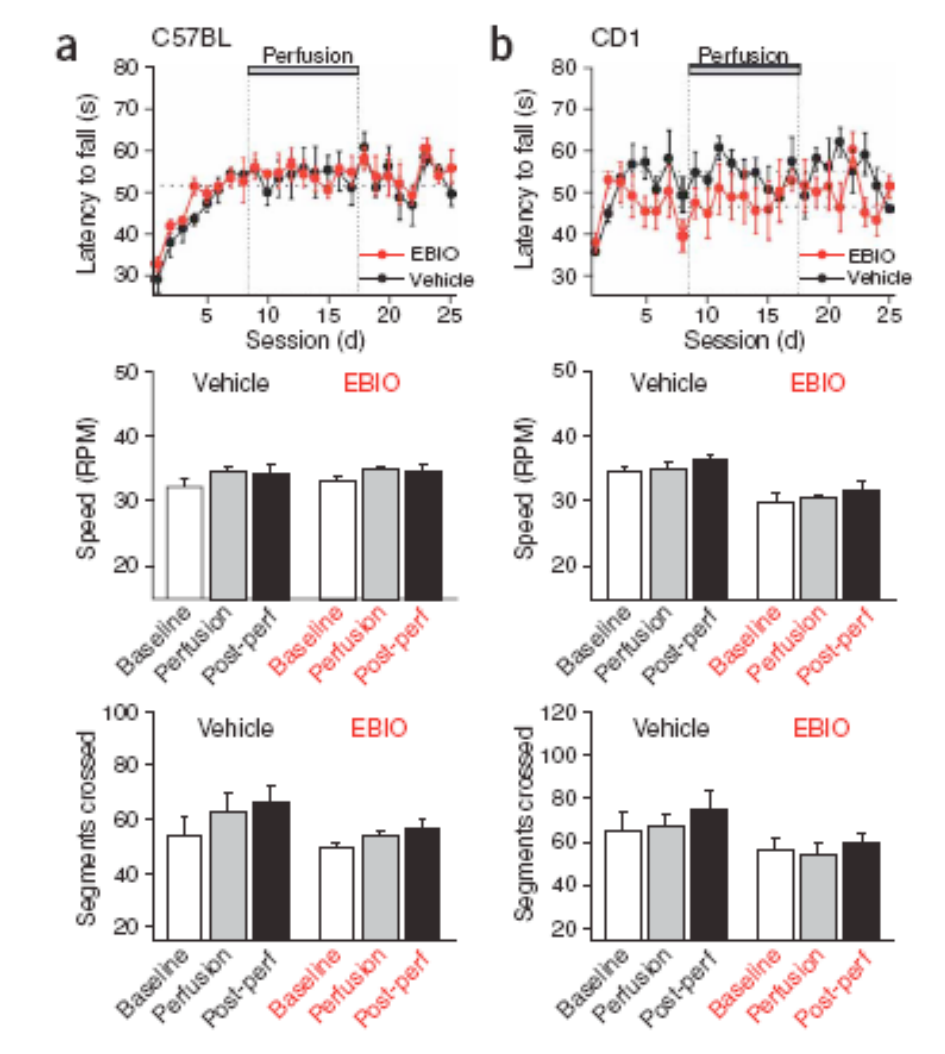
Figure 6



- Does EBIO affect coordination if P/Q is not defective?
- Experiment: Try EBIO infusion in wildtype mice

Results-Treating with EBIO

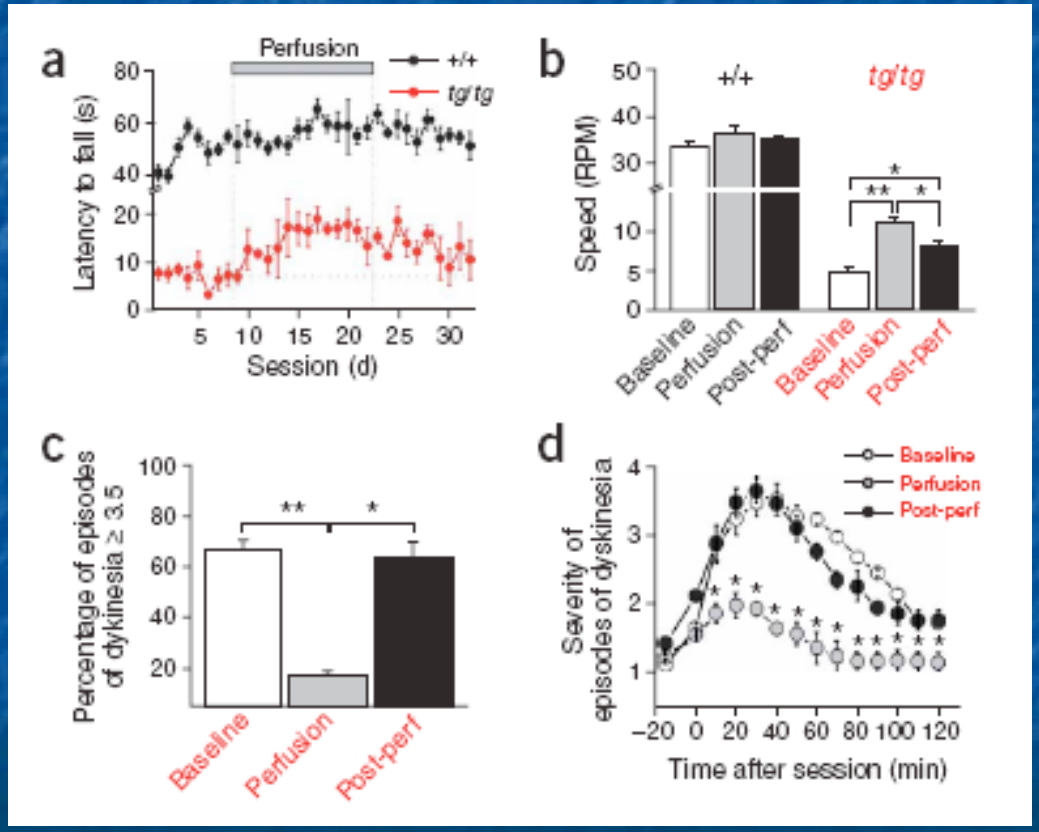
Figure 7



- Can the effect of EBIO be generalized to a different defect in P/Q?
- Experiment: Try the EBIO infusion in a different mouse model

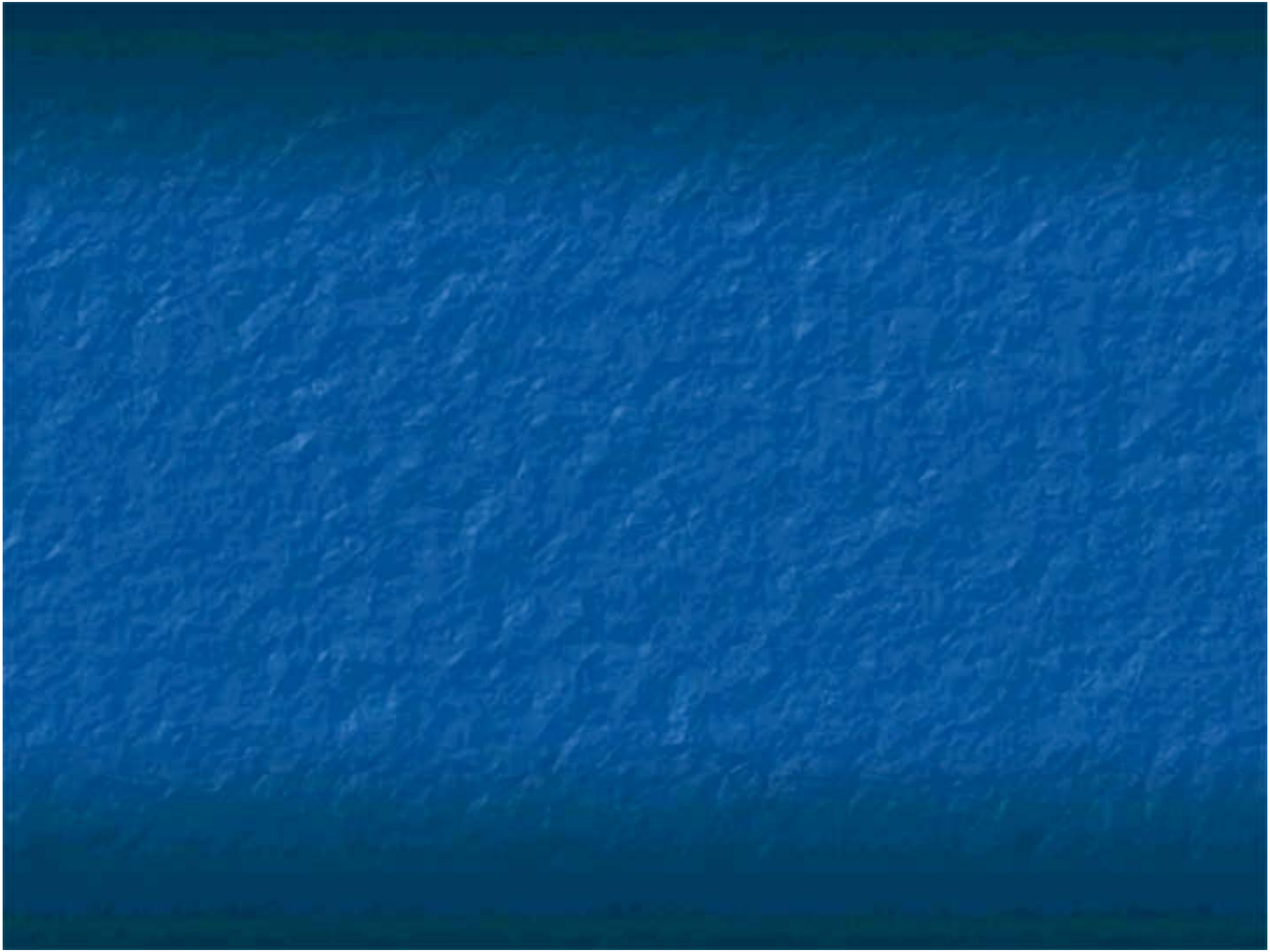
Results

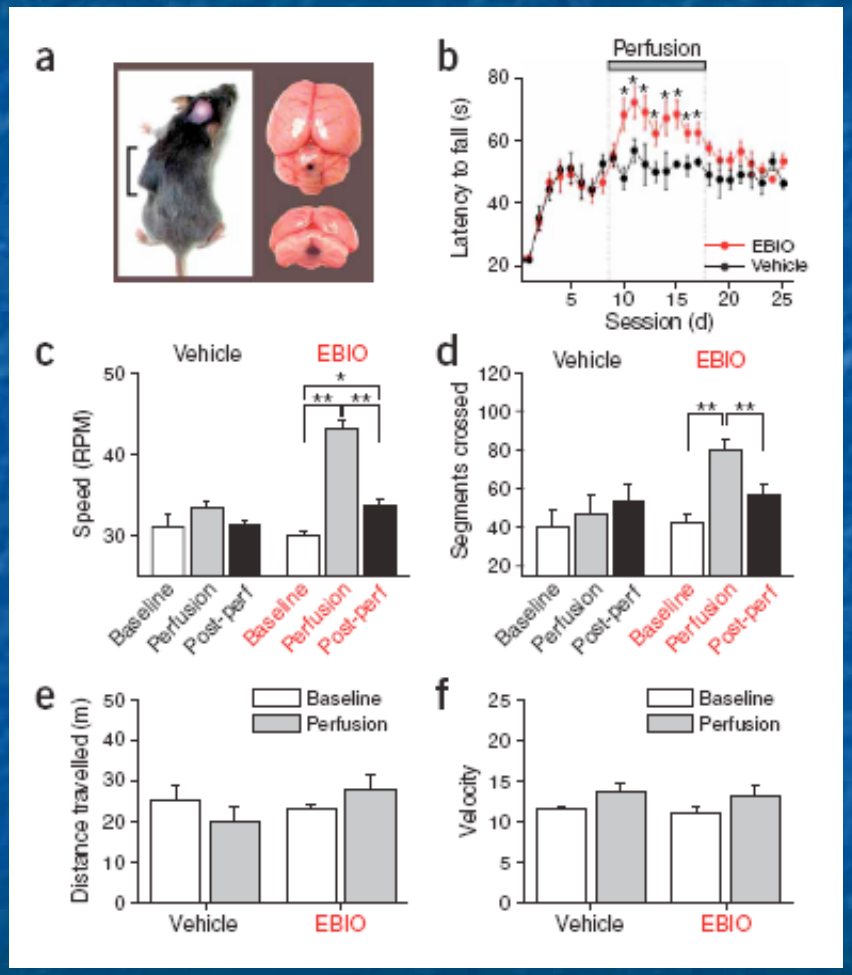
Figure 8

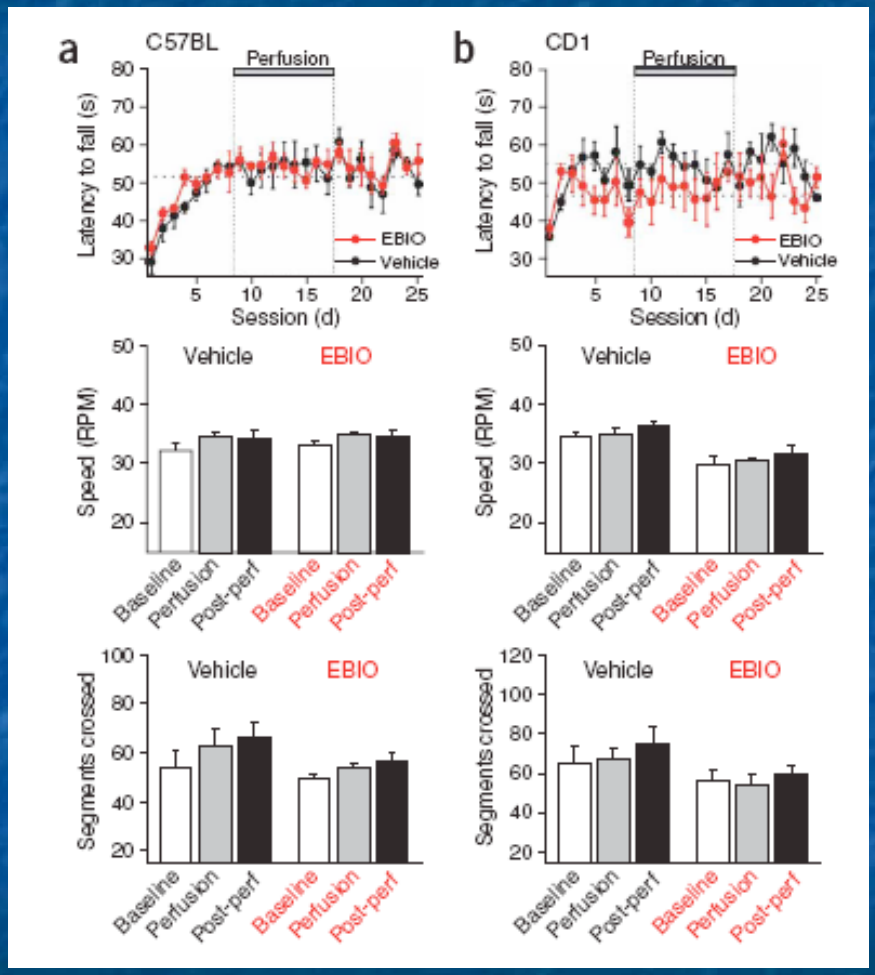


Conclusions

- Intrinsic pacemaking activity of Purkinje neurons is variable in EA2 transgenic mice
- Increased spike variability causes lower signal/noise and less synaptic information transmitted
- This variability is also dependent on potassium channel function and can be reduced by increasing SK activity
- This variability is independent of other synaptic input—i.e. this is an intrinsic property of the Purkinje neurons
- This effect is seen in both brain slices where P/Q function is defective and in vivo in two different transgenic mouse models
- The effect of increasing SK activity does not affect wildtype function




a
b
Perfusion
Latency to fall (s)
Session (d)
EBIO
Vehicle
c
Vehicle
EBIO
Speed (RPM)
d
Segments crossed
Baseline
Perfusion
Post-perf
e
Distance travelled (m)
f
Velocity


a
C57BL
b
CD1
Perfusion
Latency to fall (s)
Session (d)
EBIO
Vehicle
Speed (RPM)
Segments crossed
Baseline
Perfusion
Post-perf