Mechanisms of activity-dependent plasticity at the Axon Initial Segment

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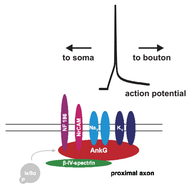
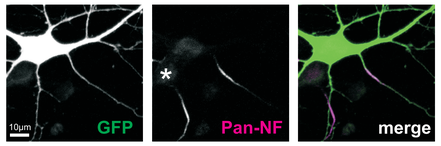
Mark D. Evans and Matthew S. Grubb
MRC Centre for Developmental Neurobiology, King's College London
Contact: mark.m.evans@kcl.ac.uk

KING'S College LONDON | MRC Centre for Developmental Neurobiology

Background

In neurons, the **axon initial segment** (AIS) is a specialised structure which marks the boundary between the somatodendritic and axonal compartments, and which is the site of action potential initiation.

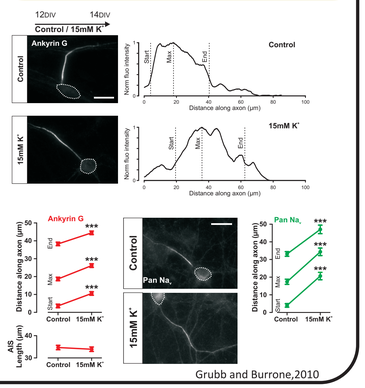




In order to perform these crucial neuronal roles the AIS recruits both voltage-gated sodium and potassium channels through interactions with a number of important scaffolding proteins.

In dissociated hippocampal cultures, we recently found that a prolonged period of depolarisation can produce relocation of the entire AIS. Components including the scaffolding protein ankyrin-G and voltage-gated sodium channels moved distally along the axon up to 17µm away from the soma.

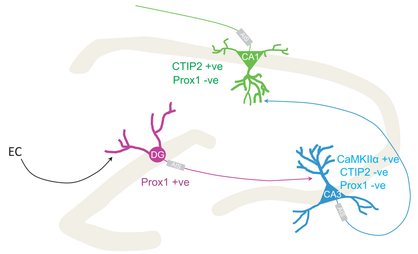
The relocation of the AIS along the axon is dependent upon calcium entry through voltage gated L-type Ca^{2+} channels.



Grubb and Burrone,2010

Results 1 - Cell type plasticity

1. AIS plasticity in excitatory neurons of the hippocampus

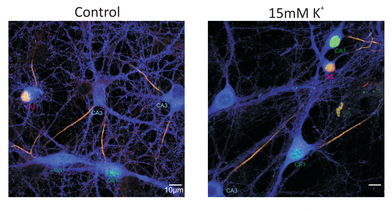


Dentate granule cells (DGCs) can be reliably identified by the expression of the Prox1 transcription factor.

CTIP2 is expressed in CA1 neurons and some DGCs. CA1 cells were identified by the presence of CTIP2 but absence of Prox1.

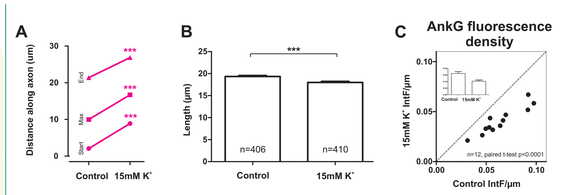
Excitatory neurons can be identified by the somatodendritic expression of CAMKIIα. CA3 neurons were identified by the presence of CAMKIIα staining and lack of both Prox1 and CTIP2 (Williams *et al.*, Neuron, 2011).

In dissociated hippocampal cultures all excitatory neurons show AIS movement plasticity. Conversely in GABAergic neurons, although the AIS start position shows a small distal shift, its max and end points do not relocate.



Sabrina Lebron

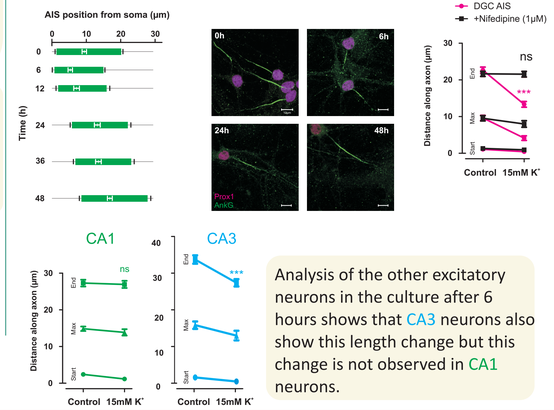
2. DGCs show reliable AIS plasticity over 48hours of 15mM KCl



Combining data from 12 individual experiments analysing DGCs shows:

A) AIS start, max and end positions reliably relocate along the axon after 48hours depolarisation.
B) The AIS becomes slightly shorter
C) Ankyrin-G fluorescence density always decreases

3. DGCs show a remarkable plasticity within just 6 hours



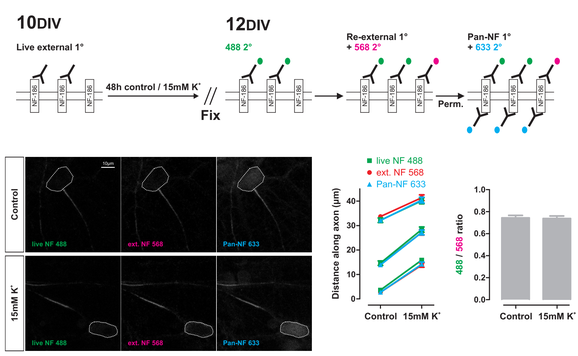
Studying AIS movement over in more detail over the 48 hour treatment revealed that in DGCs after only 6 hours the AIS shortens considerably. After this initial period of shortening the AIS starts to move and begins to grow until it reaches its final position at 48hours.

The shortening of the AIS after 6 hours is blocked by the L-type channel antagonist Nifedipine.

Analysis of the other excitatory neurons in the culture after 6 hours shows that CA3 neurons also show this length change but this change is not observed in CA1 neurons.

Results 2 - Mechanisms of movement

1. The AIS is not entirely re-built

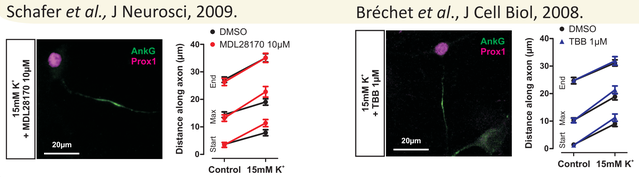


Live antibody labelling for NF-186 shows that a) the AIS is not entirely rebuilt upon relocation, b) no part of the relocated AIS is either entirely 'old' or entirely 'new', and c) turnover rates of NF-186 are unaffected by AIS movement.

2. Effector mechanisms

Calpain - is responsible for AIS destruction in response to neuronal injury but its inhibition does not affect AIS movement... Schafer *et al.*, J Neurosci, 2009.

CK2 - is needed to build an AIS in neuronal development but is not required for its relocation... Bréchet *et al.*, J Cell Biol, 2008.



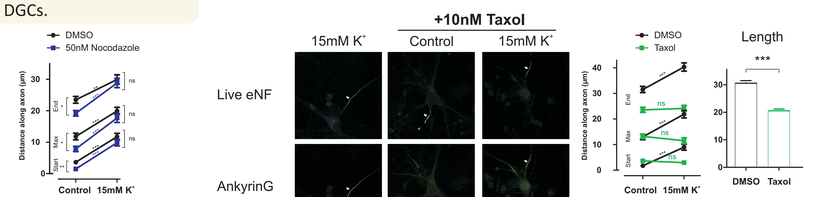
3. Microtubules

i) Destabilisation

Microtubule destabilisation with 50nM Nocodazole does not prevent AIS movement in DGCs.

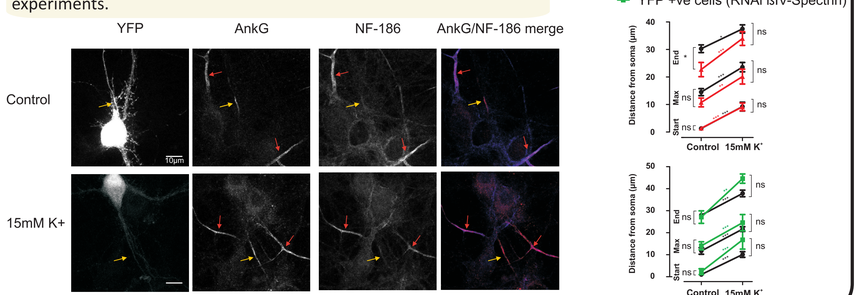
ii) Stabilisation

Microtubule stabilisation using 10nM Taxol leads to multiple axons with multiple AISs. We determined that the original AIS shortened and did not move in Taxol by employing a similar 'live label' protocol as shown earlier.



4. RNAi Knockdown

AIS relocation is unaffected by the knockdown of either NF-186 or ßIV spectrin. The figure below shows images from the NF-186 knockdown experiments.



Summary

Cell type plasticity

- All excitatory hippocampal neurons show AIS movement plasticity.
- Before moving, the AIS of both DGC and CA3 neurons shortens substantially within 6 hours.
- AIS shortening, like movement plasticity, is dependent on Voltage Gated L-type Ca^{2+} channels.

Mechanisms of movement

- The majority of extracellular NF-186 antibody applied before relocation remains at the surface of the AIS suggesting that the AIS is not entirely broken down as it moves.
- CK2 and Calpain inhibition support these data by suggesting that the AIS is not degraded and re-built.
- 10nM Taxol treatment prevented the original AIS from moving suggesting that microtubule re-arrangement may be important for AIS movement plasticity.

Current questions

- Which part of the AIS is lost after 6 hours during shortening?
- How does this length change effect the electrophysiological properties of the neuron?

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