Mechanisms of activity-dependant plasticity at the Axon Initial Segment

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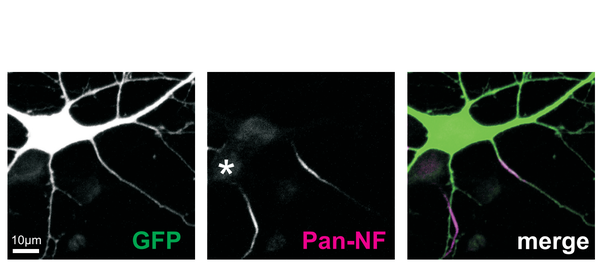
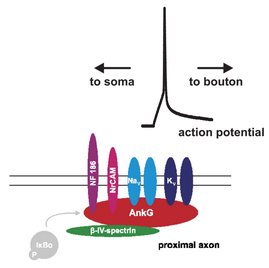
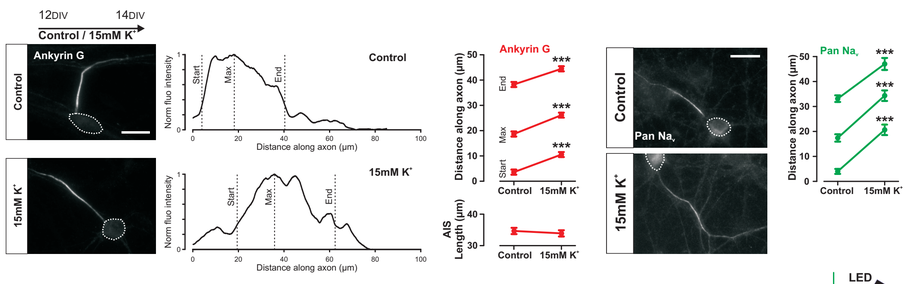
Background

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.

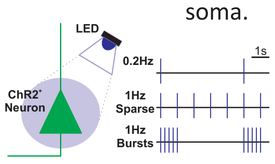


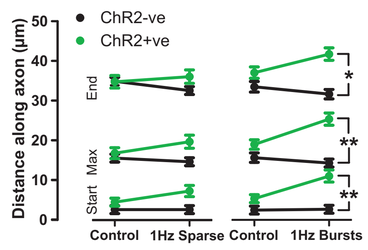




In dissociated hippocampal cultures, we recently found that a prolonged period of depolarisation can produce relocation of the entire AIS. Components including the scaffolding protein ankyrin-G and voltage-gated sodium channels moved distally along the axon up to 17µm away from the soma.

Using the light-gated ion channel channelrhodopsin-2 (ChR2) coupled with LED-based photostimulation, we investigated the activity patterns required for AIS relocation. Steady stimulation at 1Hz was not enough to move the AIS, but burst stimulation at the same overall frequency produced significant distal AIS relocation (Grubb & Burrone 2010 Nature 465:1070).





How does the AIS move?

Potential mechanisms

Breakdown and rebuild?

Membrane insertion?

'Sliding' en masse?

Cycling of AIS constituents?

Neuronal Depolarisation → **Mechanism** → **AIS Relocalisation**

Experimental Strategy

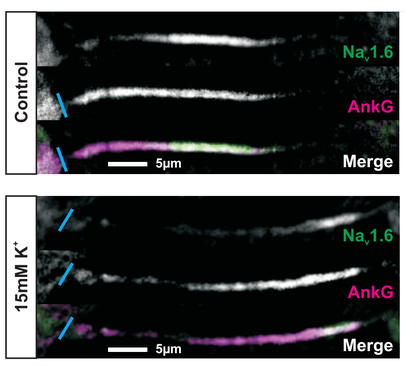
1. **Immunohistochemistry -** to test the maintenance of subdivision structure after AIS relocation.
2. **Live antibody labelling** - for pulse-chase analysis of AIS proteins before and after movement.
3. **Pharmacology** - to probe signalling pathways involved in AIS relocation.
4. **RNAi knockdown** - to investigate AIS proteins required for movement.

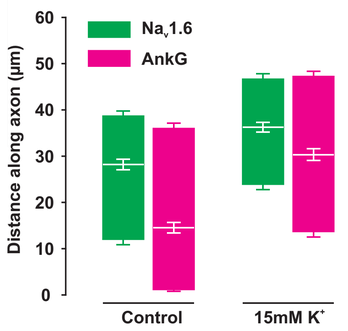
Results

1 Subdivision structure is maintained

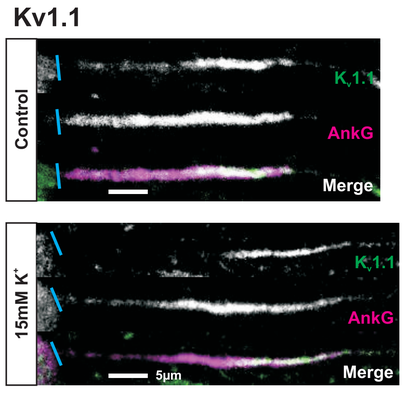
Important subdivisions of the AIS, especially the distal concentration of Nav1.6 channels that is vital for action potential initiation, remain in place following activity-dependent relocation. This is also true for both Kv1.1 and Kv1.2 channels (data not shown).

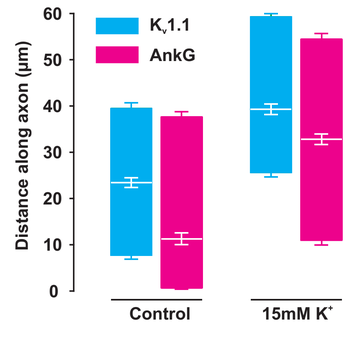
Nav1.6





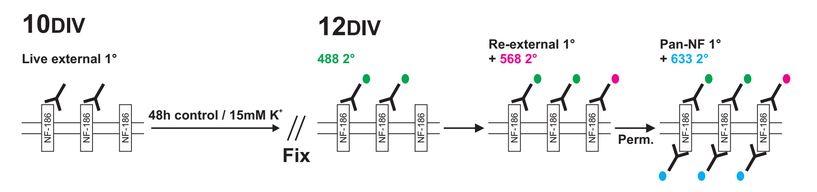
Kv1.1

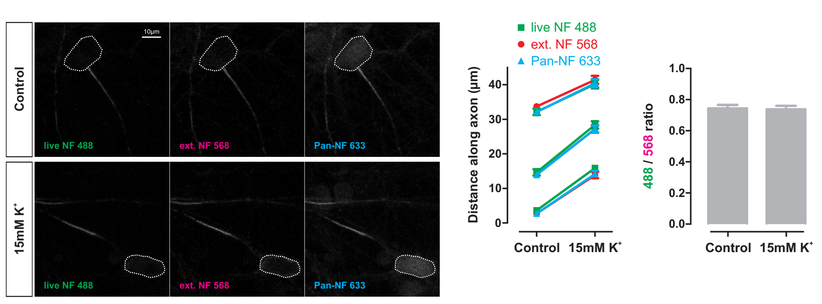




2 The AIS is not entirely re-built

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.



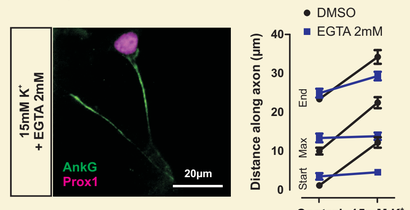


3 Pharmacology

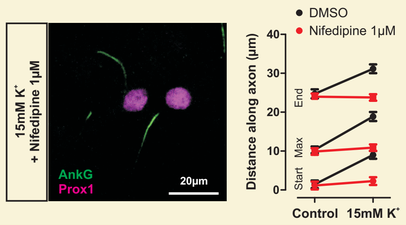
In each drug tested we have measured AIS position in Prox1-positive dentate granule cells. The drugs have been segregated into to three categories targeting different aspects of AIS movement:

A) Trigger

Ca^{2+} - Quenching extracellular Ca^{2+} with EGTA showed that AIS movement requires a Ca^{2+} dependent mechanism...

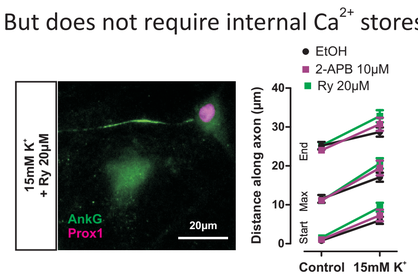


L-type VGCa^{2+}Cs -This was narrowed down to Ca^{2+} entry through L-Type Ca^{2+} channels...



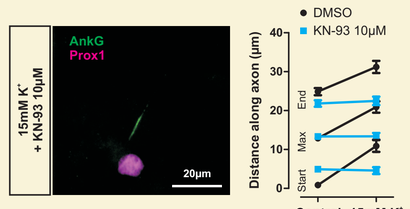
Ca^{2+} stores - IP_3Rs/RyRs

But does not require internal Ca^{2+} stores...

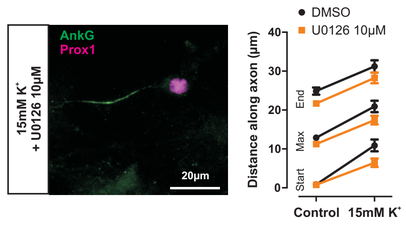


B) Signalling

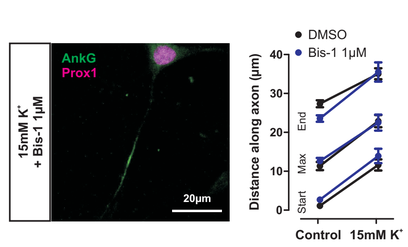
CaMK - Blocking CaMK signalling with KN-93 totally blocked AIS movement...



MAPK - However, inhibiting MEK with U0126 did not prevent AIS relocation...

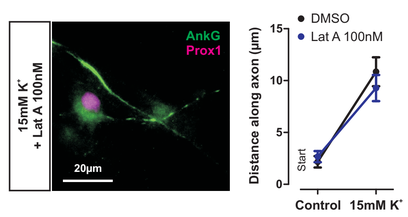


PKC - And neither did blocking PKC with bisindolylmaleimide-1...

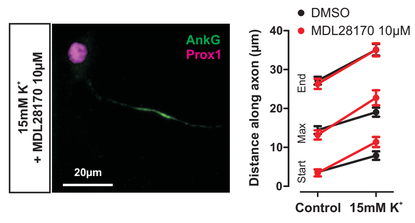


C) Movement

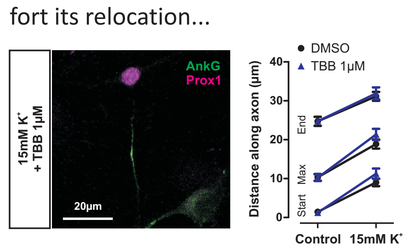
Actin - Inhibiting actin polymerisation with latrunculin-A can break apart the AIS but does not affect its start position...



Calpain - is responsible for AIS destruction in response to neuronal injury but its inhibition does not affect AIS movement...

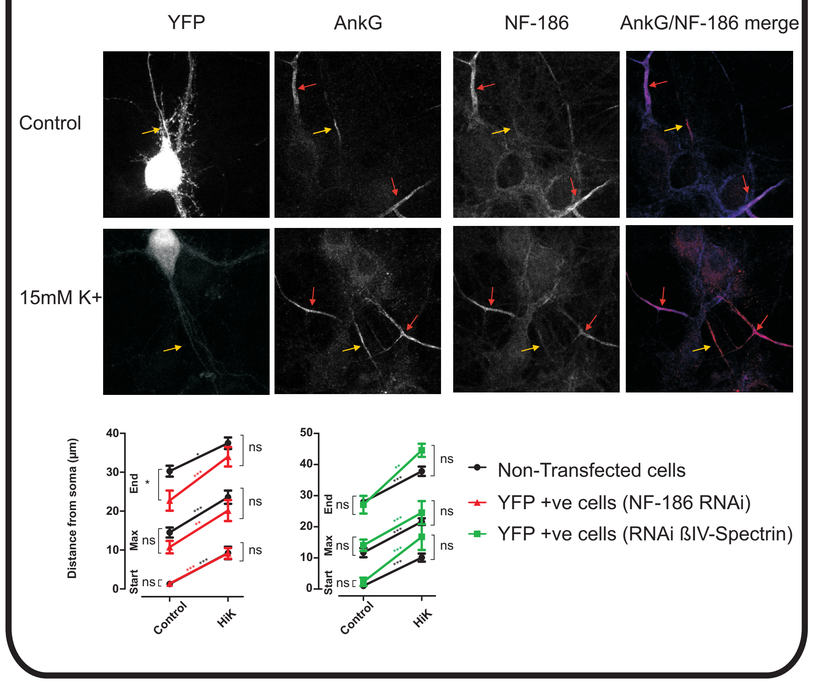


Ck2 - is needed to build an AIS in neuronal developmental but is not required fort its relocation...



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

Trigger and preliminary pharmacology

- AIS movement is triggered by Ca^{2+} entry through L-Type VGCa^{2+}Cs.
- Downstream events then activate a CamK-based signalling pathway. (3)

Movement mechanism

- The AIS sub-structure of VGCs is maintained. (1)
- The majority of extracellular NF-186 antibody applied before relocation remains at the surface of the AIS. (2)
- Ck2 and Calpain inhibition support this data by suggesting that the AIS is not degraded and re-built. (3)

These data are consistent with a model of the AIS 'sliding' en masse along the axon.

- Finally, total knockdown of ßIV-spectrin does not prevent the movement of the AIS. This, along with latrunculin-A pharmacology studies, suggests that actin cytoskeleton re-arrangements are not essential for AIS relocation. (4)

Work supported by:

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