Rapid structural plasticity at the axon initial segment

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Background

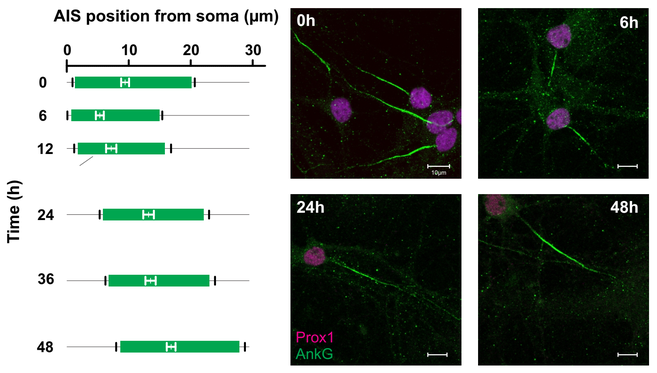
The axon initial segment (AIS) is a specialised region of the proximal axon defined by an aggregation of scaffolding proteins and a high density of voltage gated channels. Ankyrin-G (AnkG) is the AIS master organiser scaffolding protein.

Functionally the AIS plays two roles:
- Site of action potential generation
- Maintains neuronal polarity

Using dissociated hippocampal cultures it was shown that the AIS is a dynamic structure that undergoes plastic morphological changes in response to long-term 48 h alterations in activity.

Interestingly, during 48 h AIS relocation in dentate granule cells (DGCs), we noticed changes at the AIS over much shorter time scales.

At 6h post +10mM KCl treatment DGCs displayed a 20% AIS length shortening. As such we decided to investigate this further.



Questions: Exploring AIS plasticity within hours - not days

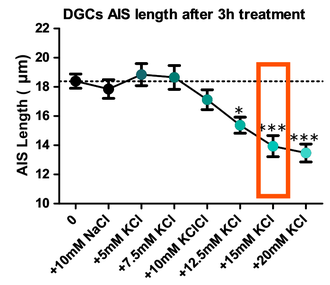
1. Physically: What stimulation shortens the AIS? Do all AIS components shorten? Do all cell types exhibit the phenotype?
2. Pharmacologically:Are the mechanisms responsible for AIS shortening similar to long term (48h) AIS relocation?
3. Functionally: What are the consequences of this plasticity for neuronal excitability?
4. Is it possible to capture the shortening through live imaging?

Characterising rapid AIS shortening

1 Physically

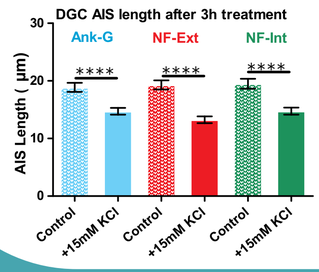
A:Stimulus characterisation

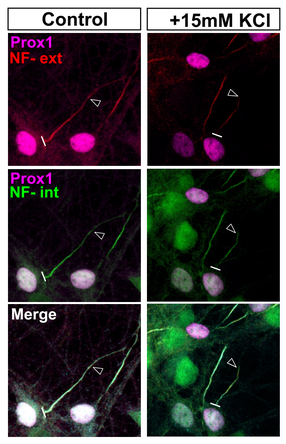
Looking at shorter time-frames of plasticity we focused on a 3h treatment. Trying different concentrations of KCl we found that a +15mM treatment reliably shortens DGCs AISs labeled with AnkG.



B:Structural characterisation

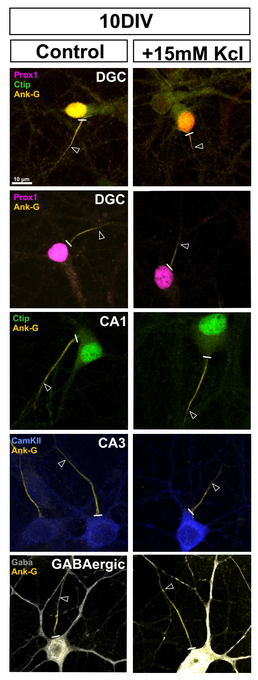
The 3h +15mM KCl treatment shortens not only the AnkG AIS label , but also the cell adhesion molecule Neurofascin- visualised with antibodies against internal and external epitopes.

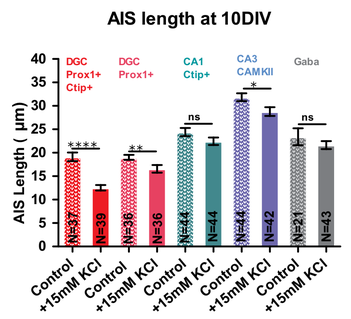


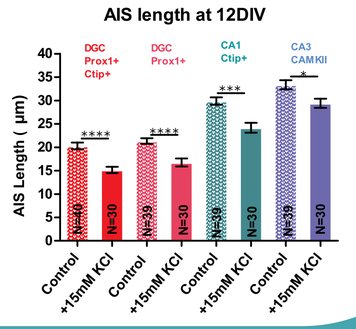


C: Cell-type characterisation

Immunolabeling with a combination of several antibodies makes it possible to identify hippocampal neuron sub-types in dissociated cultures. After a 3h +15mM KCl treatment:
- DGCs and CA3 neurons show AIS shortening at 10 DIV.
- GABAergic neurons do not display this type of plasticity.
- All excitatory cell types (DGC, CA1 and CA3 neurons) display truncated AISs at 12DIV.



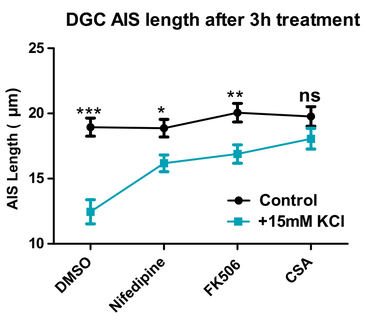


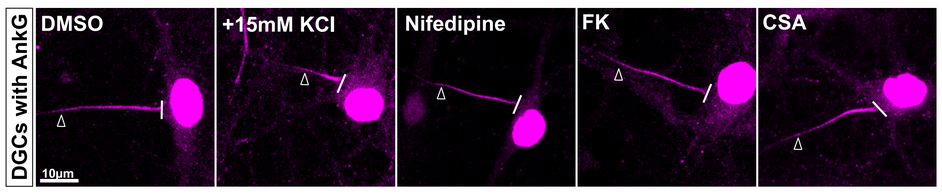


2 Pharmacologically

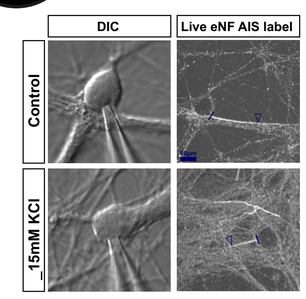
Similar to the 48h AIS relocation phenotype, we discovered that signaling through L-type voltage gated calcium channels (VGCCs) underlies AIS shortening after a 3h depolarisation treatment.

Blocking all L-type VGCCs with nifedipine or inhibiting its downstream effector - calcineurin- with either FK506 or cyclosporin(CSA) prevented the majority of AIS shortening.



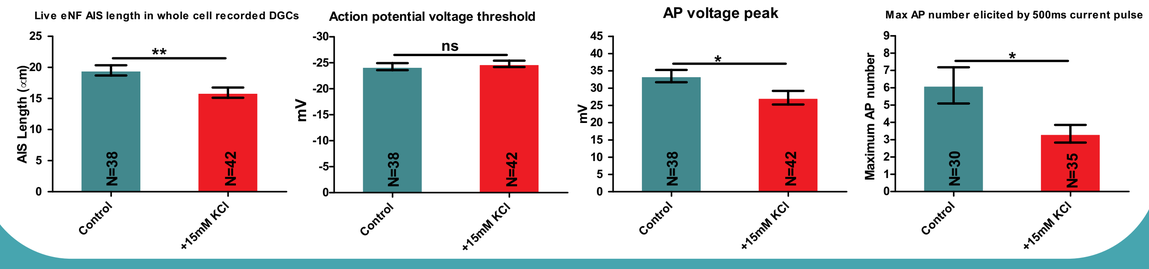


3 Functionally



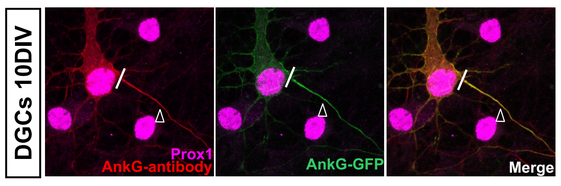
We did whole cell patch clamp recordings in 10-12DIV hippocampal cultures subjected to a 3h +15mM KCl treatment. We targeted DGCs morphologically and used a live NF antibody staining to measure AIS length in all recorded cells.

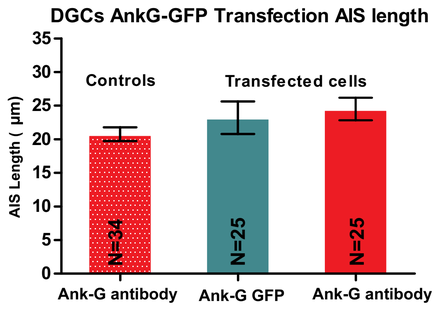
Morphologically AIS length was shorter in KCl treated cells. This was correlated with a small decrease in excitability levels as maximum AP voltage peak and number of spikes elicited by a 500ms current injection pulse were lower in potassium treated cells.



4 Live imaging the AIS:

We are currently working on developing an AIS live imaging assay using hippocampal dissociated cultures. For this purpose we are trialing a construct in which the CMV promoter drives expression of AnkG fused to GFP. Our initial experiments show that DGCs transfected with AnkG-GFP show a slight AIS length increase when labeled with AnkG, compared with control untransfected cells. More work is needed to check whether this construct will provide a reliable method of capturing the 3h AIS shortening through live imaging.





Summary

Looking at rapid AIS plasticity we found that:

Physically:
- An AIS length shortening is visible in hippocampal dissociated cultures treated with +15mM KCl for 3h.
- All excitatory cell types (DGCs, CA1, CA3 neurons) exhibit this form of AIS plasticity.
- GABAergic neuron AISs did not shorten within the 3h treatment time frame.

Pharmacologically:
- We found that signaling through L-Type calcium channels and more specifically calcineurin, is responsible for most of this rapid form of AIS plasticity.

Functionally:
- Using whole cell patch clamp recording in DGCs with an AIS live label, it became apparent that cells treated with +15mM for 3h also displayed reduced excitability levels. Hence an AIS length shortening alters a cell's intrinsic excitability.

- We are currently working on creating a live imaging assay that will allow us to capture this rapid 3h AIS length shortening.

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