Structural and functional subdivision of the axon initial segment following activity-dependent plasticity

Matt Grubb

MRC Centre for Developmental Neurobiology, King's College London, UK
matthew.grubb@kcl.ac.uk

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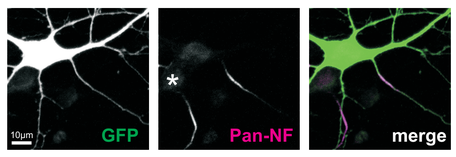
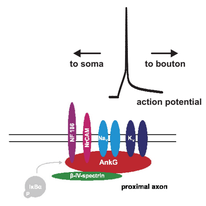
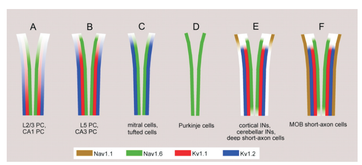
Background

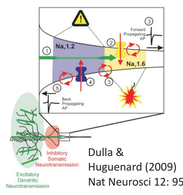
In neurons, the axon initial segment (AIS) is a specialised structure which marks the boundary between the somatodendritic and axonal compartments, and which is the site of action potential initiation.







Lorincz & Nusser (2008) J Neurosci 28:14329

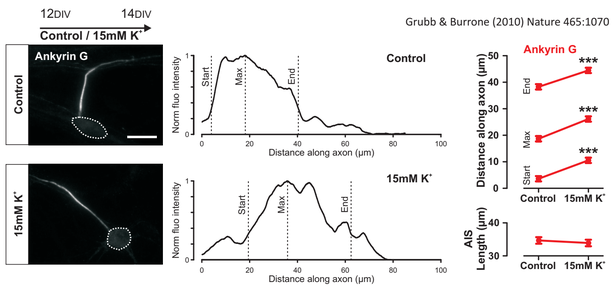


Dulla & Huguenard (2009) Nat Neurosci 12: 959

The AIS is subdivided into distinct proximal and distal domains. This subdivision varies according to neuronal type, but a common feature is the aggregation of $Na_v1.6$, $K_v1.1$ and $K_v1.2$ channels in the distal portion of the structure. It is this precise sub-region of the AIS where action potentials are believed to initiate.

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 moved distally along the axon up to 17μm away from the soma.

So, given that the AIS is a subdivided structure, and that it relocates in response to changes in electrical activity...



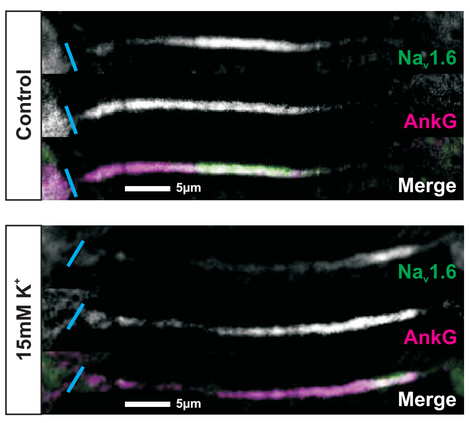
Grubb & Burrone (2010) Nature 465:1070

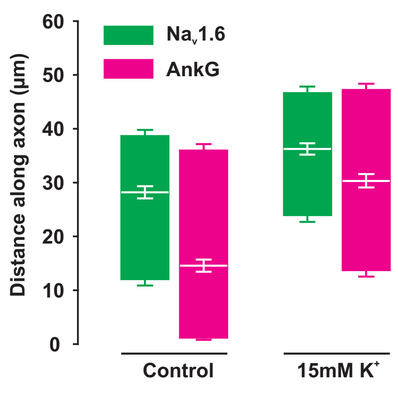
What happens to AIS subdivision after activity-dependent relocation?

① Structural subdivision

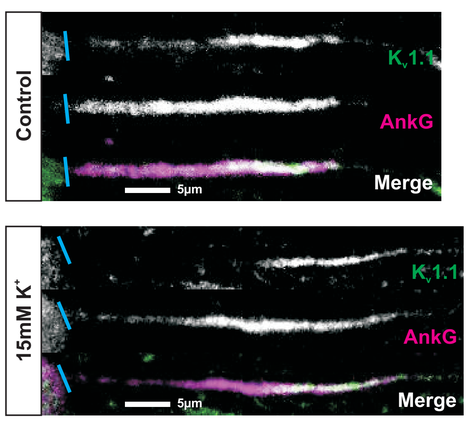
Immunohistochemical labelling for voltage-gated ion channel subunits $Na_v1.6$, $K_v1.1$, or $K_v1.2$ in conjunction with label for ankyrin-G (ankG) revealed clear structural subdivision of the AIS. In control dissociated hippocampal neurons at 14 days in vitro (DIV), $Na_v1.6$, $K_v1.1$ and $K_v1.2$ label all occupied the distal-most portion of the ankG-defined AIS. This structural subdivision was maintained following activity-dependent AIS relocation: 48h treatment with 15mM K^+ caused a distal shift in overall AIS position, but no change in the distal localisation of $Na_v1.6$, $K_v1.1$ and $K_v1.2$ channel subunits.

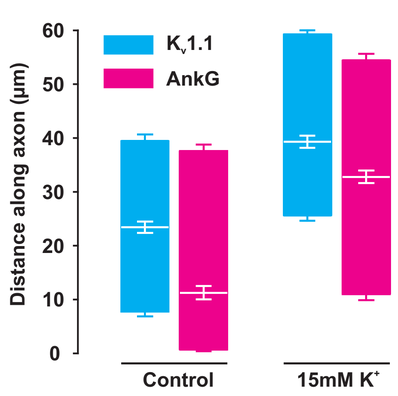
$Na_v1.6$



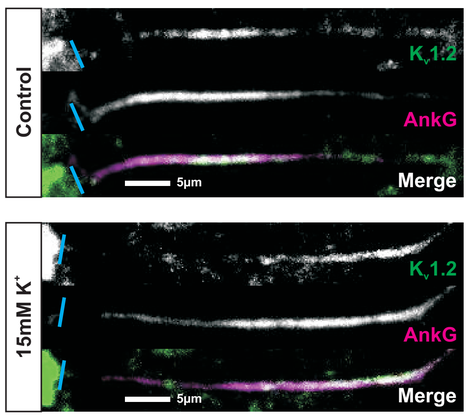


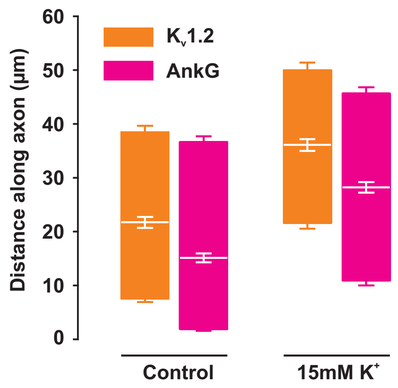
$K_v1.1$



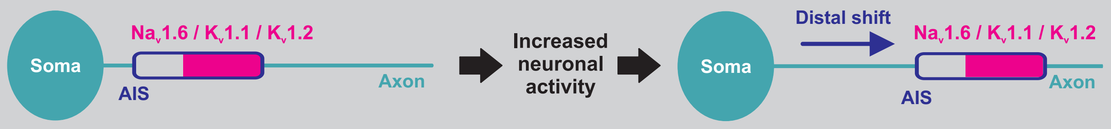


$K_v1.2$





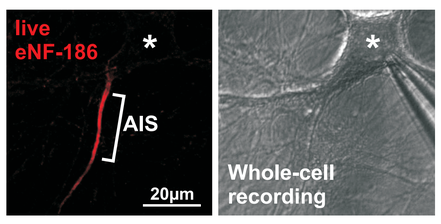
Summary

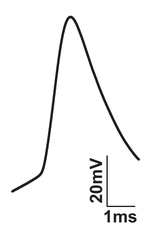


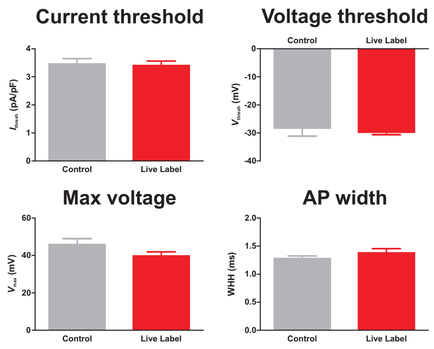
Are these structural effects mirrored by functional subdivision of the AIS?

② Integrating AIS structure and function

The precise position of the AIS can be revealed in live neurons using an antibody raised against an extracellular domain of neurofascin-186 (eNF-186). These cells can then be targeted for whole-cell patch clamp recordings, without any effect of the live AIS label on fundamental action potential properties.

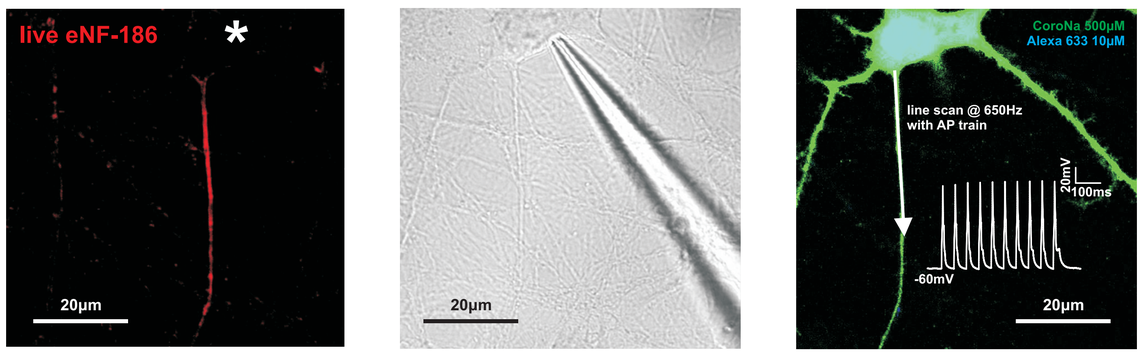


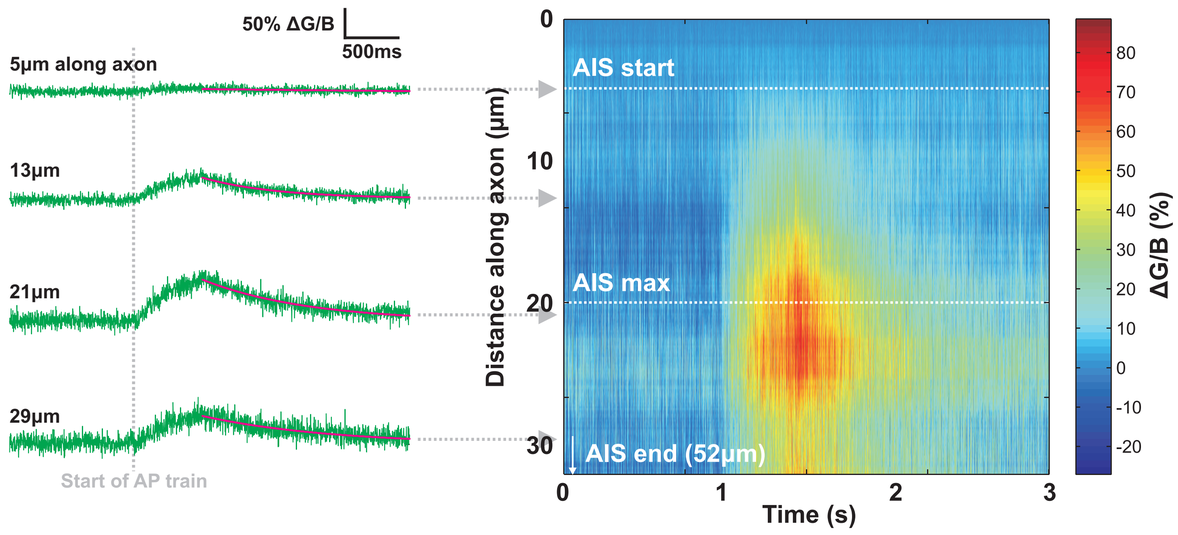




Functional subdivision I: Na^+ imaging

Loading live-AIS-labelled neurons with the sodium indicator dye CoroNa Green enables precise localisation of the axonal Na^+ transients associated with action potential firing. These can then be directly compared with the structurally-defined position of the AIS.



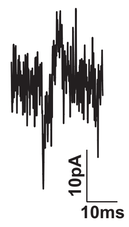


Functional subdivision II: extracellular recording

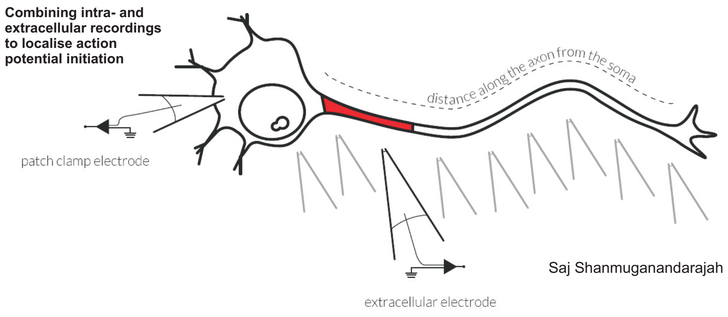
Live AIS structural label can also be combined with an electrophysiological estimate of the site of action potential initiation, based on the relative timing of somatic whole-cell action potentials and axonal extracellular spikes.

Extracellular recording

Extracellular action potential



Combining intra- and extracellular recordings to localise action potential initiation



Saj Shanmuganandarajah

Supported by wellcome trust