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Cell type-dependent axon initial segment plasticity in the olfactory bulb

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Introduction

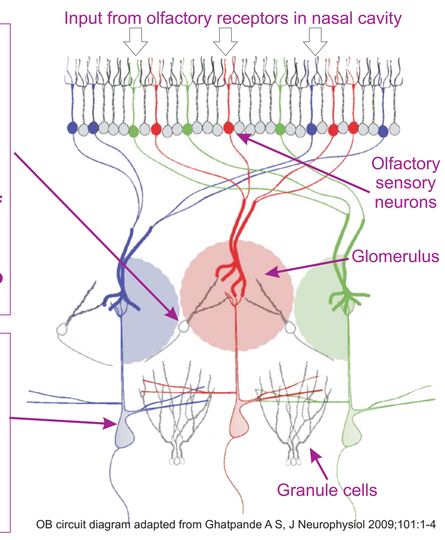
We are interested in activity-dependent plasticity at the axon initial segment in inhibitory periglomerular cells and in excitatory mitral/tufted cells in the rodent olfactory bulb.

Periglomerular cells

- GABAergic and/or dopaminergic neurons
- Undergo constituitive turnover throughout life through adult neurogenesis
- Regulate glomerular activity at the first synapse of olfaction
- Exhibit activity-dependent plasticity in response to glomerular input

Mitral / tufted cells

- Glutamatergic excitatory output neurons
- Do not express TH or GABA
- Can receive input directly from olfactory sensory neurons

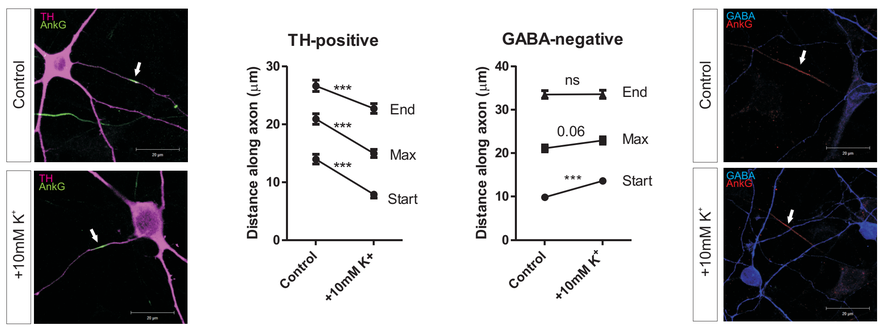


OB circuit diagram adapted from Ghatpande A S, J Neurophysiol 2009;101:1-4

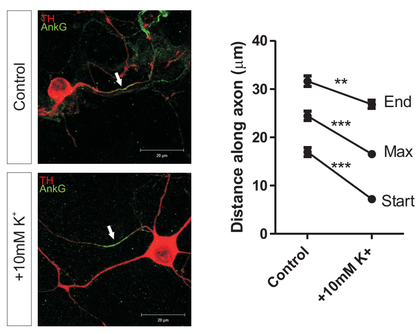
The **axon initial segment (AIS)** is a specialised protein-rich sub-compartment of the neuron found at the beginning of the axon. The AIS is important for **action potential-generation** and **regulating neuronal polarity**.

The AIS is a recently-discovered site of neuronal plasticity but is the AIS plastic in olfactory bulb neurons?

1: AIS plasticity differs in dopaminergic vs. excitatory neurons

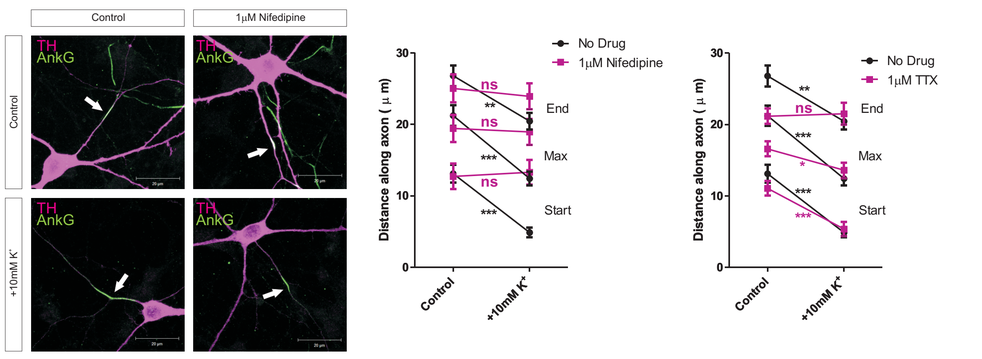


Dissociated cultures of embryonic rat olfactory bulb (OB) cells were treated 11-12 days in vitro (DIV) with 10mM K^+ for 24h and immunolabelled for tyrosine hydroxylase (TH) to label dopaminergic (DA) neurons, GABA to label inhibitory neurons and Ankyin-G (AnkG), the main scaffolding protein of the AIS. The start, end and maximum staining intensity positions (max) were measured. The AIS moved towards the soma by ~6μm in dopaminergic neurons in 10mM K^+ conditions. Excitatory projection neurons were identified as being negative for both GABA and TH staining. In contrast to TH+ neurons, the start position of the AIS in these cells moved away from the soma by ~4μm (*** P<0.001; ns, not significant).



AIS movement was consistent in mouse OB DA cells. OB cells from P3 wild-type mouse pups were cultured then treated with +10mM K^+ 13-14 DIV. The AIS moved towards the soma by ~9μm (*** P<0.001; ** P<0.01; * P<0.05)

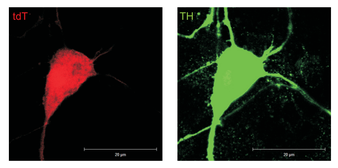
2: L-type calcium channels are required for AIS relocation



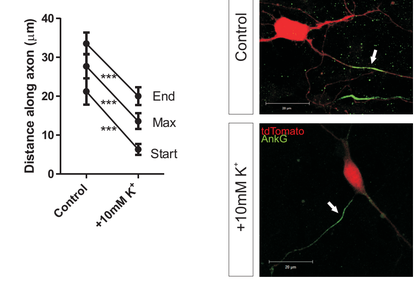
In similar experiments, rat OB cells were treated 11-12 days in vitro (DIV) with +10mM K^+ in the presence of 1μM tetrodotoxin (TTX) to block sodium channels or 1μM nifedipine to block L-type calcium channels. Nifedipine completely blocked activity-induced AIS movement, implying that L-type calcium channel activation is required for AIS movement in dopaminergic OB cells. TTX shortened the AIS in control conditions but had no effect on AIS position in +10mM K^+ conditions, suggesting that action potentials are not required for AIS relocation (*** P<0.001; ** P<0.01; ns, not significant).

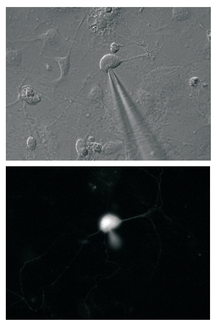
3: Studying the electrophysiological properties of OB DA neurons in high activity conditions

Activity-dependent AIS plasticity is associated with altered electrophysiological properties in hippocampal neurons. To investigate the physiological effects of the opposing AIS changes seen in OB neurons we used TH:Cre x Rosa:tdTomato mice to enable us to record from fluorescently-labelled DA cells. tdTomato-positive neurons in these cultures also express TH (right).

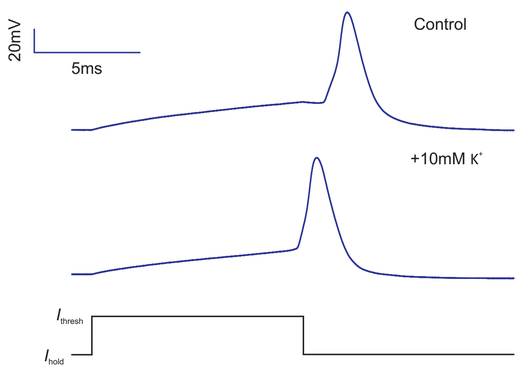


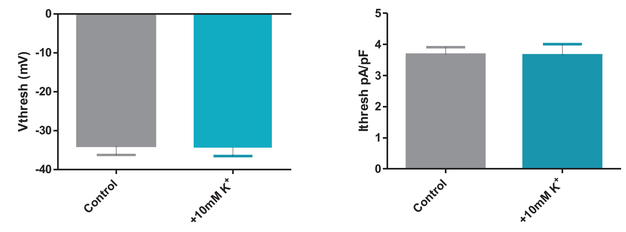
OB cells cultured from P3 TH-tdTomato mice were treated with +10mM K^+ 13-14 DIV and immunolabelled for AnkG. Neurons expressing tdTomato had AISs and there was a strong inward AIS movement in +10mM K^+ conditions (right) (*** P<0.001).





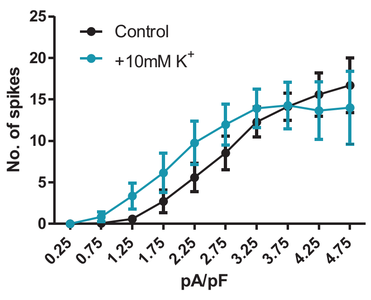
We treated these mouse OB cultures with +10mM K^+ 13-14 DIV then targeted tdTomato+ neurons for whole-cell electrophysiological recording (left).
Both tdTomato-positive and -negative cells were depolarised identically by the +10mM K^+ stimulus (left).

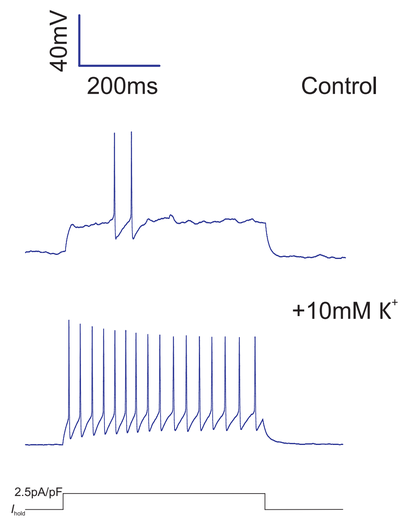




Preliminary data suggested there was no difference in the voltage threshold (above, left) and the current threshold (above, right) at which tdTomato+ neurons fire action potentials in control vs. +10mM K^+ conditions (left).

However, a 500ms injection of current resulted in tdTomato+ cells firing multiple spikes with a trend towards neurons treated with +10mM K^+ firing more spikes at a lower current threshold compared to control. (Control n = 5; +10mM K^+ n = 6).

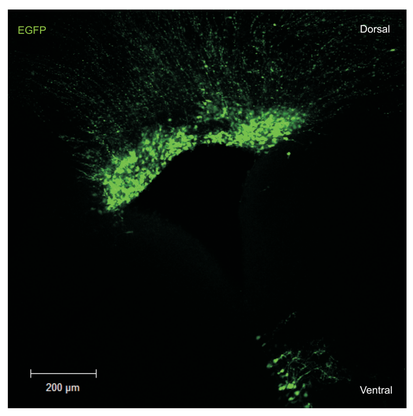


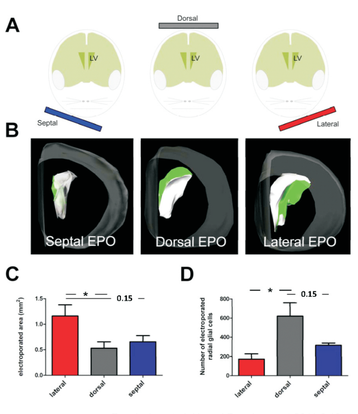


4: Identifying activity-dependent AIS plasticity in the OB *in vivo*

We are employing postnatal electroporation to label newborn OB DA cells in developing mice allowing us to assess the characteristics and consequences of AIS plasticity in different OB neuronal populations *in vivo*.

Using targeted electroporation of the dorsal wall of the lateral ventrical, P1 WT mouse pups were injected with pCX-EGFP (right, 2 days post electroporation (DPE)).





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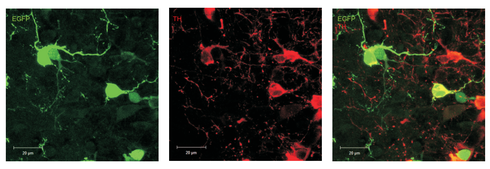
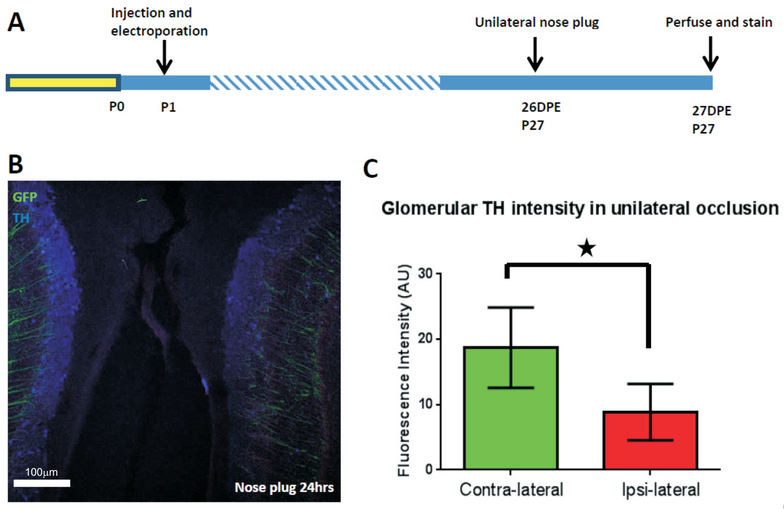
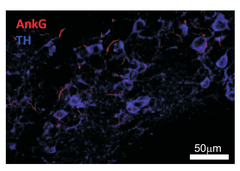


At 15DPE cells electroporated with pCX-EGFP have migrated along the rostral migratory stream to the OB, differentiated into neurons and integrated into the OB circuitry. Brains were fixed, sliced and immunostained for TH. We found neurons that were positive for both EGFP and TH stain (left).

We are able to modulate OB activity *in vivo* using naris occlusion. At 26DPE unilateral naris occlusion was performed using nose plugs constructed from PE tubing, suture thread and dental floss. After 24h of occlusion brains were fixed, sliced and immunostained for TH (A).

A decrease in TH staining intensity was observed (B, C) in the OB with deprived sensory input (B, right) compared to the non-occluded OB (B, left).





We can also identify neurons within the glomerular layer that have AISs. We plan to identify and trace AISs in DA neurons to measure AIS plasticity (left).

Conclusions

- **There are opposing forms of plasticity at the axon initial segment in excitatory vs. inhibitory neurons in rat OB. A similar form of plasticity is seen in both rat and mouse DA neuron AISs *in vitro* (1).**
- **The inward AIS movement seen in dopaminergic OB neurons is triggered by L-type calcium channel activation (2).**
- **OB DA neurons show a trend towards increased excitability in high activity conditions (3).**
- **We are able to label OB DA neurons *in vivo* and modulate OB activity using naris occlusion (4).**

Acknowledgements

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