

Activity-dependent plasticity in dopaminergic neurons of the olfactory bulb

Annisa N Chand and Matthew S Grubb, King's College London

Contact: annisa.chand@kcl.ac.uk or matthew.grubb@kcl.ac.uk

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Centre for
Developmental
Neurobiology

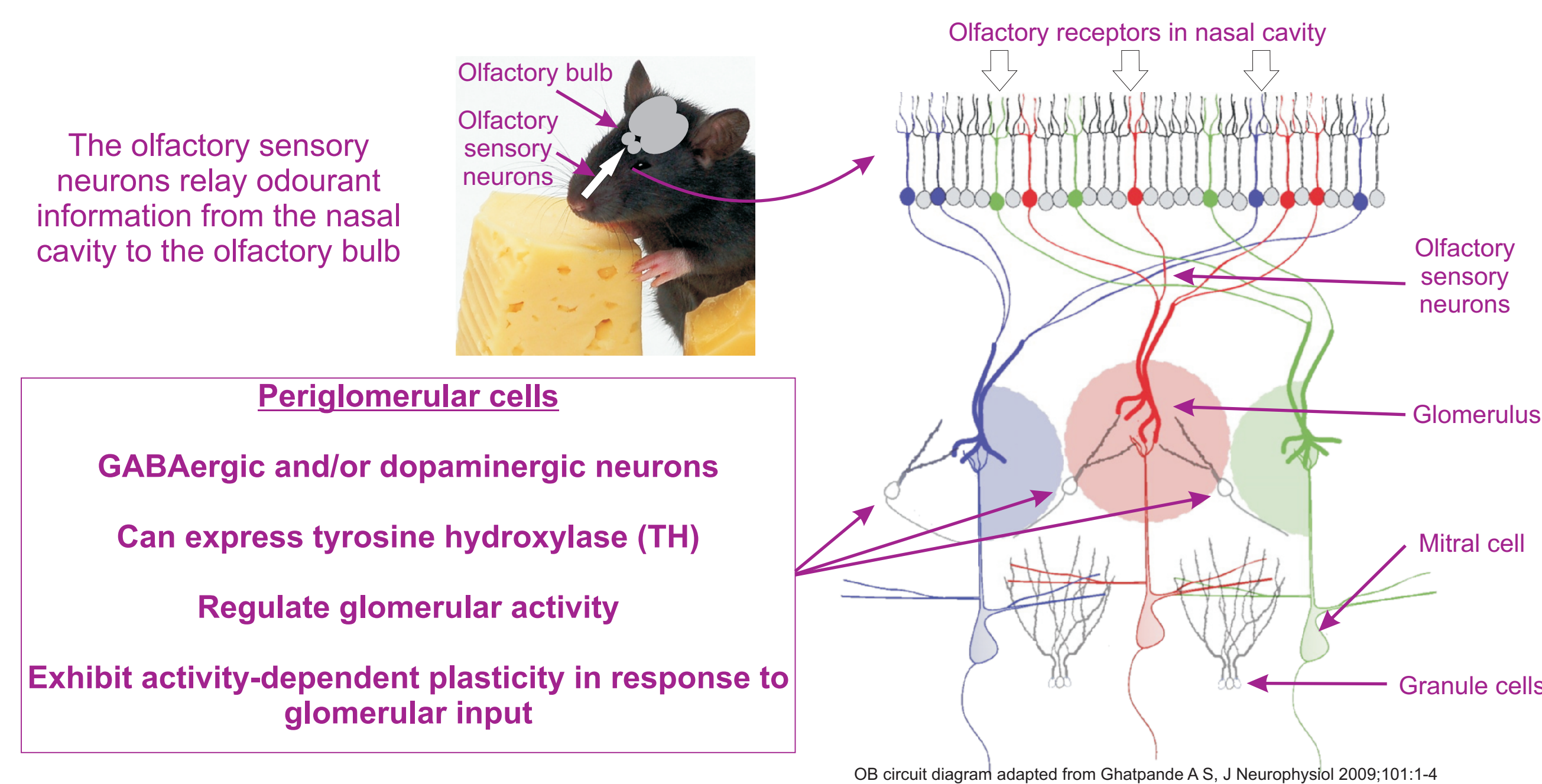
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1. Introduction

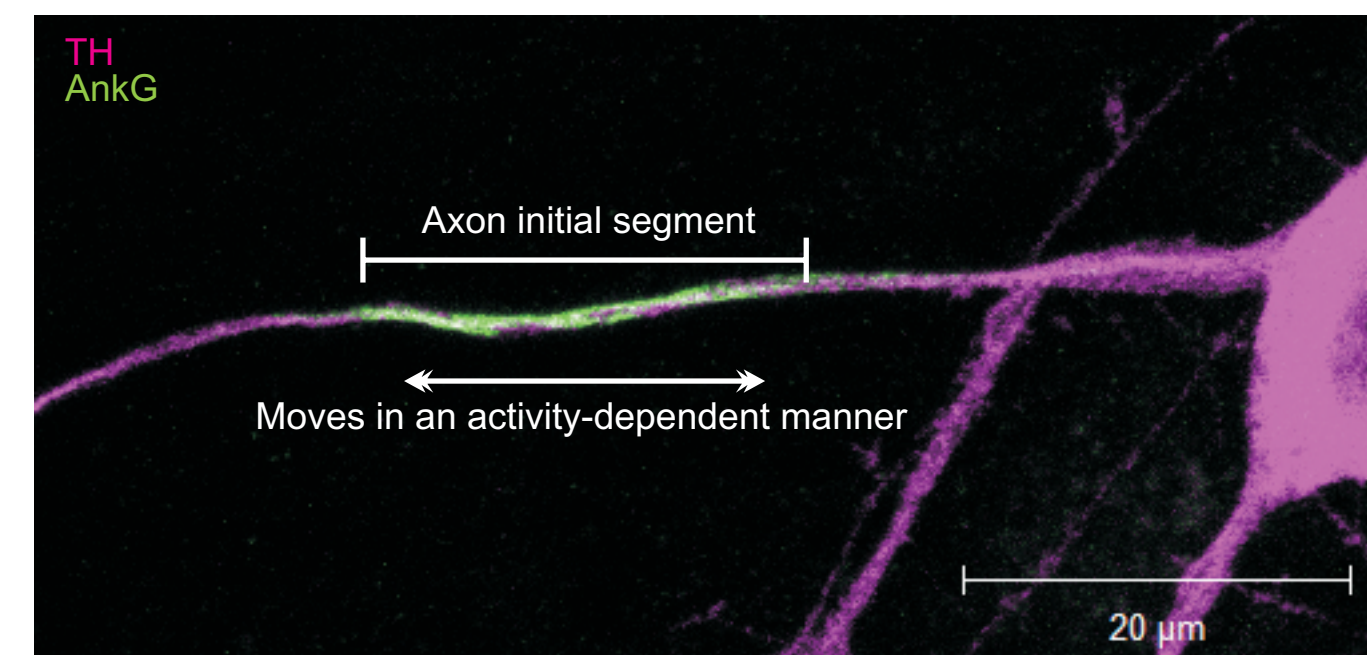
We are looking at activity-dependent plasticity in the tyrosine hydroxylase-positive (TH+) population of dopaminergic neurons in the olfactory bulb.

- We aim to determine the mechanisms of:
- Changes in the TH+ neuron population in response to high or low neuronal activity
- Axon initial segment (AIS) plasticity in response to changes in neuronal activity

The olfactory system is a highly organised network of brain areas with the **olfactory bulb (OB)** acting as the **primary regulator of sensory input** from the olfactory epithelium in the nasal cavity. The OB has a **specialised structure** consisting of highly plastic **glomerular microcircuits**. In vivo, OB dopaminergic neurons can rapidly downregulate expression of the dopamine-synthesising enzyme **tyrosine hydroxylase (TH)** in response to sensory deprivation and also undergo continual turnover throughout life via constitutive **adult neurogenesis**.



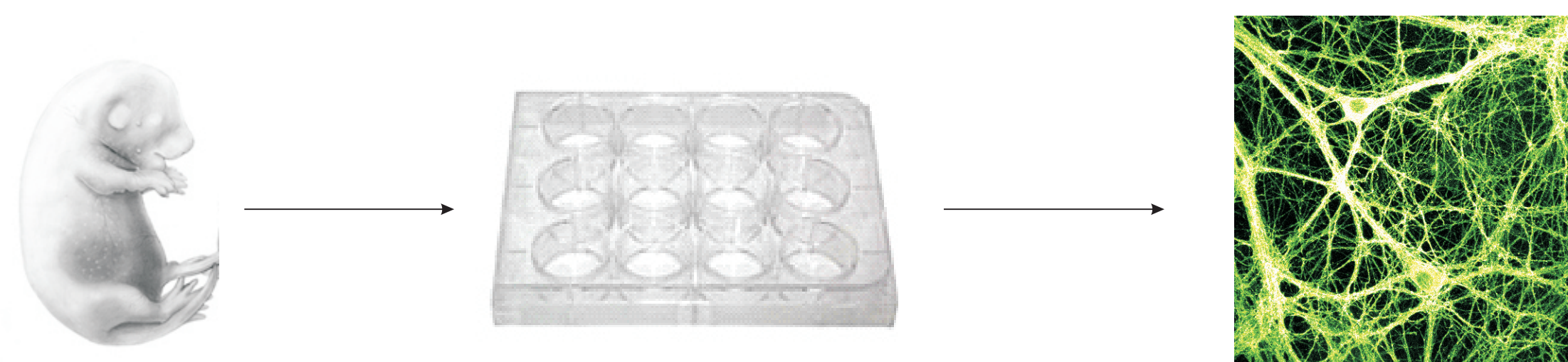
The **axon initial segment (AIS)** is a specialised region near the start of the axon which **maintains axonal polarity** and is the site of **action-potential initiation**. **Ankyrin G (AnkG)** is the main structural protein of the AIS. Our lab has recently shown that in primary cultures of rat hippocampal neurons the **AIS moves** away from the soma in **high-activity conditions** and this effect is dependent on L-type calcium channels.



References:
Grubb and Burrone (2010) Nature 465:1070-4. Baker et al. (1983) J Neurosci 3:69. Cave et al. (2010) J Neurosci 30:4717.

2. Materials and methods

In our study we have used primary cell cultures of dissociated rat or mouse olfactory bulb cells.



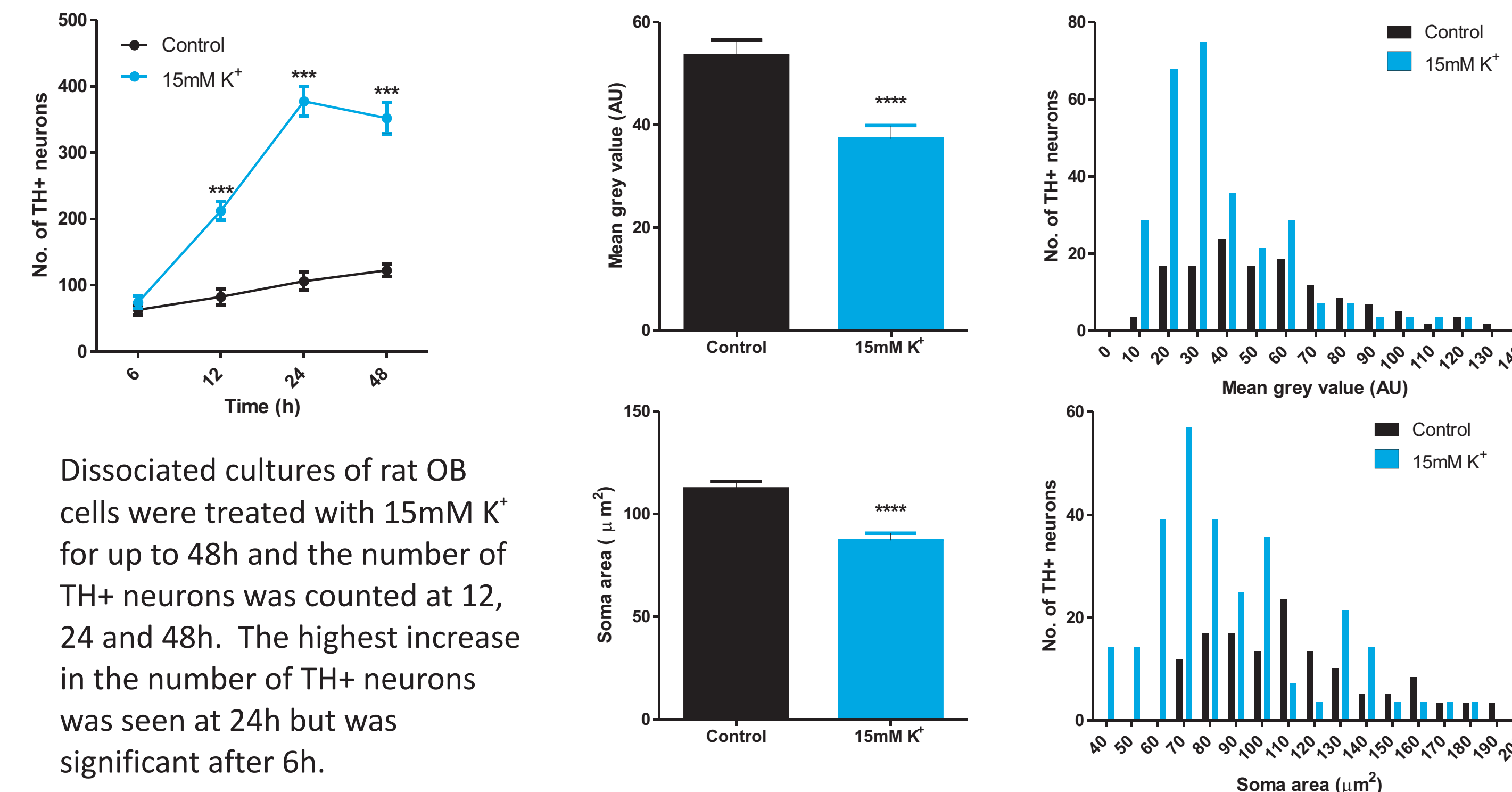
The olfactory bulb was dissected from E18 rat embryos or P3 mouse pups.

Dissociated cultures were plated on glass coverslips treated with poly-D-lysine and mouse laminin.

Rat cells were allowed to develop until 11 days in vitro (DIV) and mouse cells were allowed to develop until 6DIV prior to treatment.

Cells were treated with 15mM NaCl or 15mM KCl alongside either 1μM tetrodotoxin, 1μM nifedipine, 1μM FK506 or 10μM forskolin for 24h unless stated otherwise. Synaptic blockers used were 10μM NBQX, 50μM AP5, 100μM LY 367385, 100μM MTEP hydrochloride, 10μM SR 95531 hydrobromide and 5μM CGP 55845 hydrochloride. Anti-oxidants used were 3.2μM glutathione, 77nM superoxide dismutase, 10nM catalase, 100μM Trolox and 110μM vitamin C and are needed to counteract phototoxicity during photostimulation. Cells were fixed after treatment with PFA and immunohistochemically stained for TH and AnkG. Stained cells were imaged using a Zeiss LSM 710 confocal microscope. TH+ cells were counted per coverslip across a minimum of 4 coverslips per treatment group and two-way ANOVA was performed to test for statistical significance. TH+ cells with AnkG+ AIS staining were imaged and the AIS position and length measured using a Matlab program written by M. Grubb (available at www.grublab.org). All TH+ cells with an AIS were imaged and measured across a minimum of 4 coverslips per treatment group and statistical significance was tested using a two-way ANOVA with Bonferroni-post test.

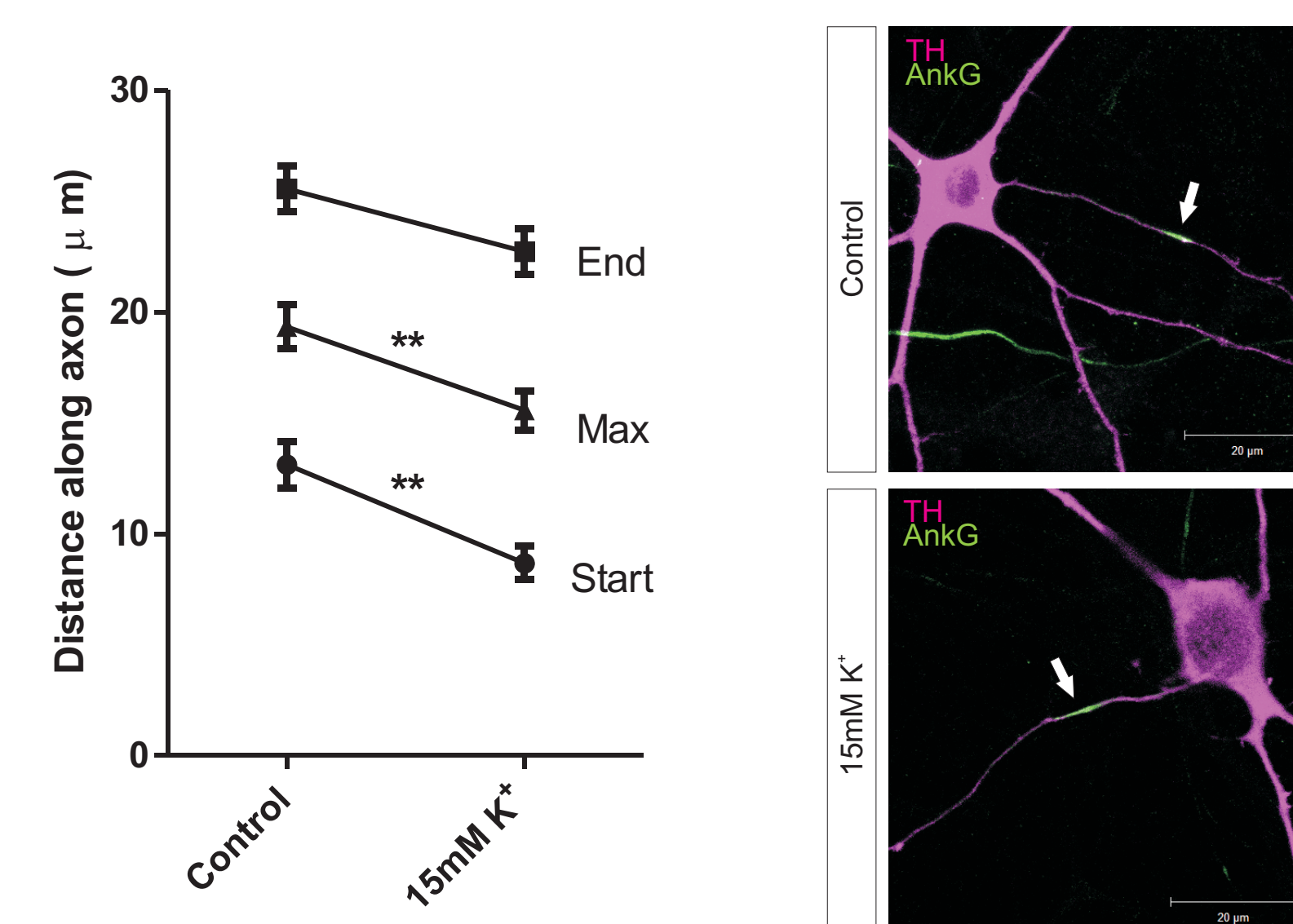
3. High activity conditions lead to an increase in TH+ neurons



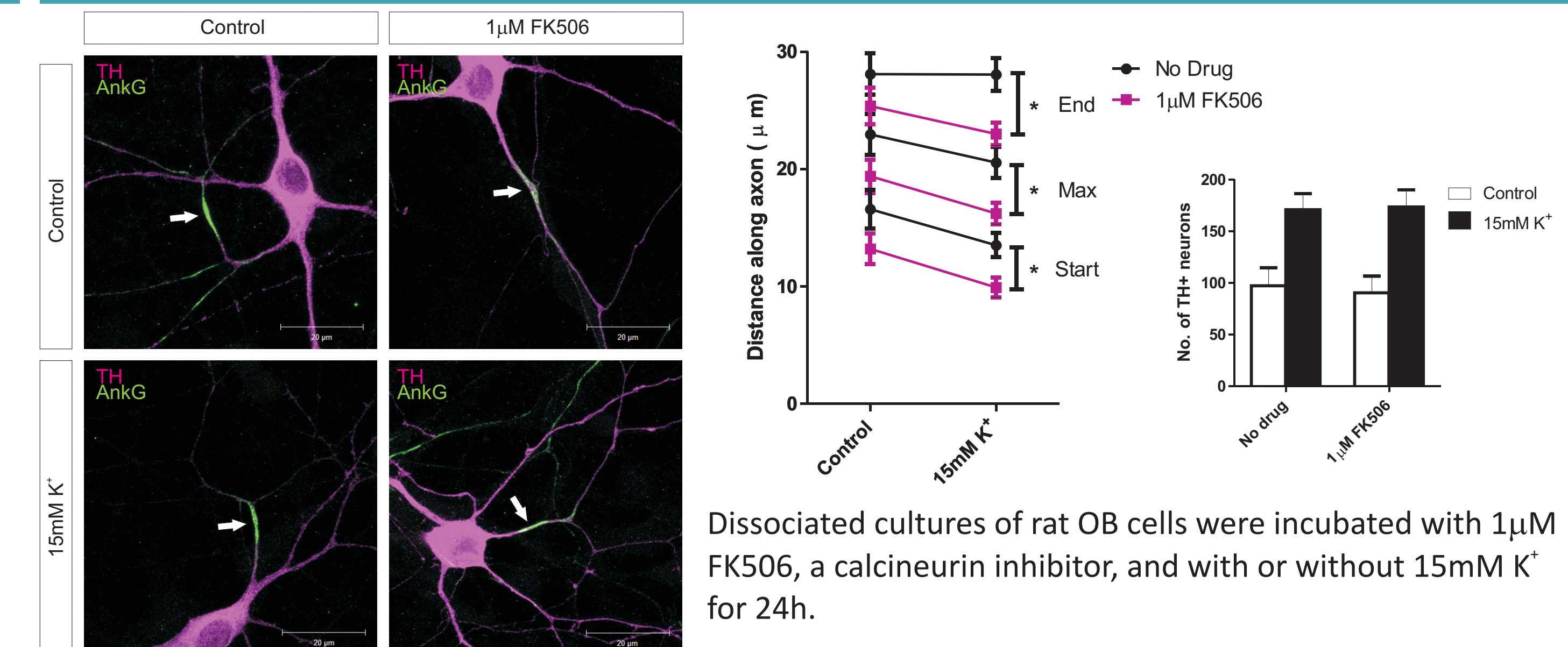
Next the soma size and staining intensity (mean grey value) of the TH+ neuron population was assessed. After treatment with 15mM K⁺ for 24h more small, weakly stained TH+ neurons were detected.

4. The AIS moves towards the soma after chronic depolarisation

Dissociated cultures of rat OB cells were treated with 15mM K⁺ for 48h and stained for TH and AnkG. The AIS was analysed for movement of the start and end positions as well as the maximum staining intensity position (max). The AIS moved towards the soma by ~4μm in 15mM K⁺ conditions. This is in contrast to previously reported data in hippocampal neurons where the AIS moved away from the soma after treatment with 15mM K⁺.



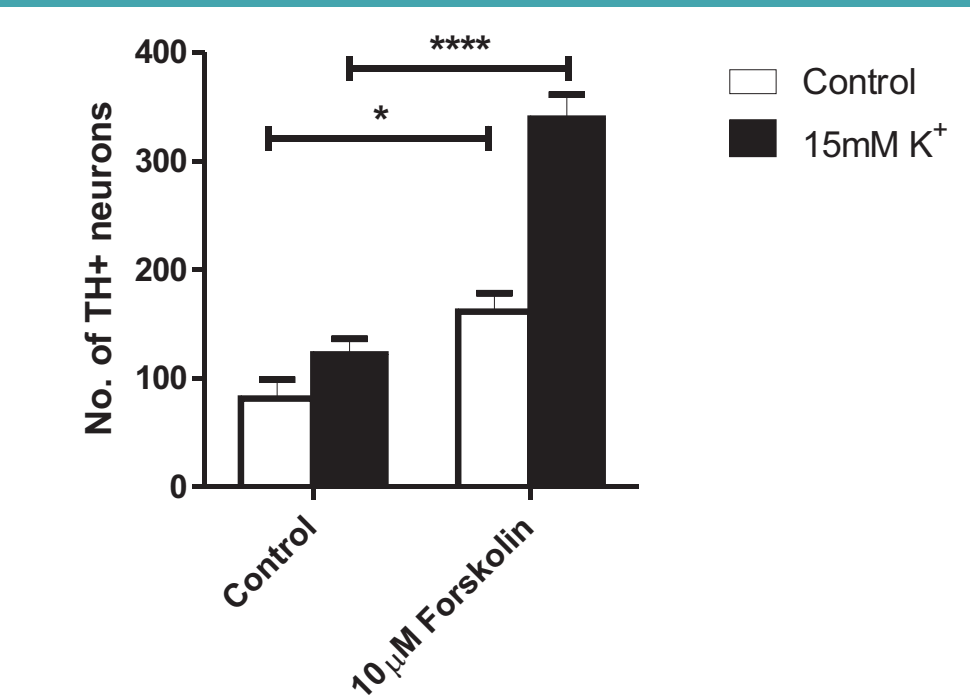
6. Calcineurin affects AIS position but not TH+ neuron numbers



Whilst FK506 had no effect on the number of TH+ neurons (above right) blocking calcineurin had an independent effect on AIS position. In the presence of FK506 the AIS was located more proximally to the soma in both control and 15mM K⁺ conditions compared to AIS position without FK506 treatment (above left and centre).

7. cAMP signalling increases the number of TH+ neurons

Forskolin was used to activate adenylyl cyclase in dissociated rat OB cultures for 24h. A significant increase in the number of TH+ neurons was observed after treatment with forskolin with an 1.5-fold increase in TH+ neurons in control conditions and 2.1-fold increase in 15mM K⁺ conditions.



8. Summary

- In our dissociated cultures of rat OB neurons the number of TH+ dopaminergic cells is regulated by chronic depolarisation, L-type calcium channels and cAMP signalling, as previously observed (e.g. Cigola *et al.* (1998) J. Neurosci. 18: 7638).
- In TH+ OB cells the AIS moves towards the soma in high activity conditions. This is in contrast to the distal AIS movement seen in hippocampal neurons in similar conditions.
- Both AIS movement and the number of TH+ neurons are regulated by L-type calcium channels.
- Calcineurin regulates proximal AIS movement independently of chronic depolarisation.

9. Questions and future work

Why does the AIS move towards the soma in TH+ dopaminergic OB cells?

What mechanisms control this proximal movement?

The TH+ population of dopaminergic cells in the OB is highly plastic in response to sensory deprivation, so how does the plasticity of these cells inter-relate with AIS plasticity?

To investigate these questions we plan to examine activity-dependent plasticity *in vivo* in the mouse OB.

We are using mouse OB cultures to look at the effects of activity on TH+ neuron and AIS plasticity. We are currently optimising the use of Channelrhodopsin-2 (ChR2), a light-gated ion channel, to control electrical activity in our neurons. This requires the cells to be treated with drugs that block synaptic activity (SynBlk) and anti-oxidants (AO). We still observe TH+ neuron plasticity in the conditions required for photostimulation (right) and treatment with 1mM Nifedipine (Nif) blocks this effect as seen previously (section 4).

We plan to generate a virus to express ChR2 in TH+ dopaminergic OB cells to examine how electrical activity regulates TH+ and AIS plasticity *in vitro*. Following on from photostimulation experiments we plan to perform sensory deprivation in mice *in vivo* and assess AIS plasticity *ex vivo* in OB slices.

5. L-type calcium channels are involved in TH & AIS plasticity

