A Novel Role for Neuronal Hyaluronan Production in Early Neurite Development

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Background

In peripheral tissues, there is strong evidence for a role of the extracellular matrix molecule hyaluronan (HA) in cellular differentiation and process extension[1,2,3]. In neurons, HA is expressed in a pericellular pattern, where it is thought to be involved in neuronal signalling. However, a specific role for HA in neuronal process development is unknown.

Hyaluronan and process growth

- High molecular weight glycosaminoglycan
- Produced by hyaluronan synthase enzymes (HAS1-3) that are expressed in cortical neurons
- In non-neuronal cells, HA signals through CD44 and RHAMM receptors to control process growth[2,4]
- Further, blocking HA synthesis impairs process growth and lamellipodia/filopodia development[1]

Aims

1) Determine whether neurons synthesize HA in their developing processes.

2) Determine whether inhibition of HA synthesis in neurons inhibits neurite development, including lamellipodia and filopodia.

Methods

- Primary cortical neuronal culture (E16 Sprague Dawley rats)
- HA synthesis inhibited with 0.1, 0.3, 0.6 and 1 mM 4-methylumbelliferone (4-MU) treatment at DIV0 (4h) and DIV1.
- Immunocytochemistry at DIV1 and DIV4 for HA expression (biotinylated hyaluronic acid binding protein (bHABP)) on neuronal processes (MAP2) and filopodia/lamellipodia (actin).
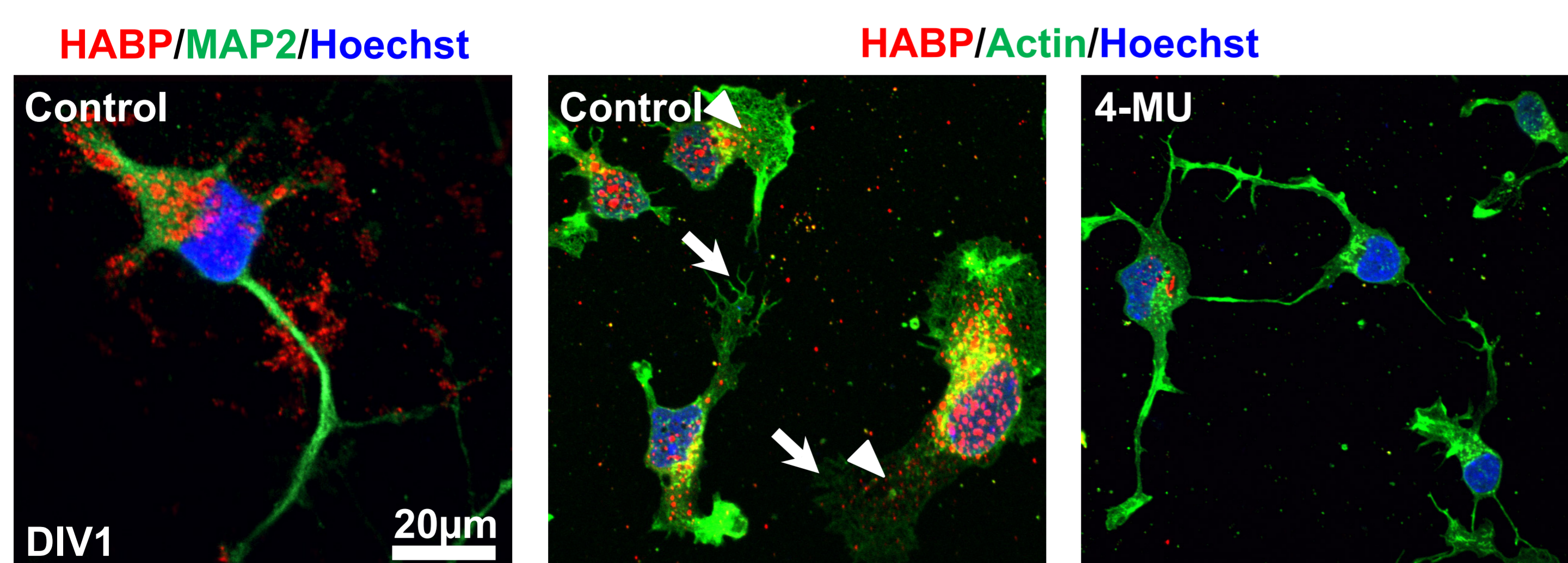
- Imaging and neurite outgrowth analysis: ImageXpress/ MetaXpress system (Molecular Devices)
- Sampling: 16 sites per well, area per site: ~0.5mm^2

Example of a result image for neurite outgrowth analysis produced by MetaXpress software.

Results

### 4-MU treatment reduces neuronal HA expression

Left: Example of neuronal HA localisation. **Centre, right:** Reduction in HABP staining after 4-MU treatment (0.3 mM). Note also the diversity in HA expression patterns on cell bodies and processes. Arrows: filopodia; arrowheads: lamellipodia.

References
1. Twarock S, Markku I, et al. (2010) J Biol Chem 285(30):23276-23284.
2. Oliferenko S, Kaverina I, et al. (2000) J Cell Biol 148(6):1159–1164.
3. Hunt LC, Gorman C, et al. (2013) J Biol Chem 288(18):13006-21
4. Nagy JI, Hacking J , et al. (1995) J Neurosci 15(I):41-252.

4-MU is assocated with a trend for decreased actin-stained area in neurons at DIV1 and DIV4
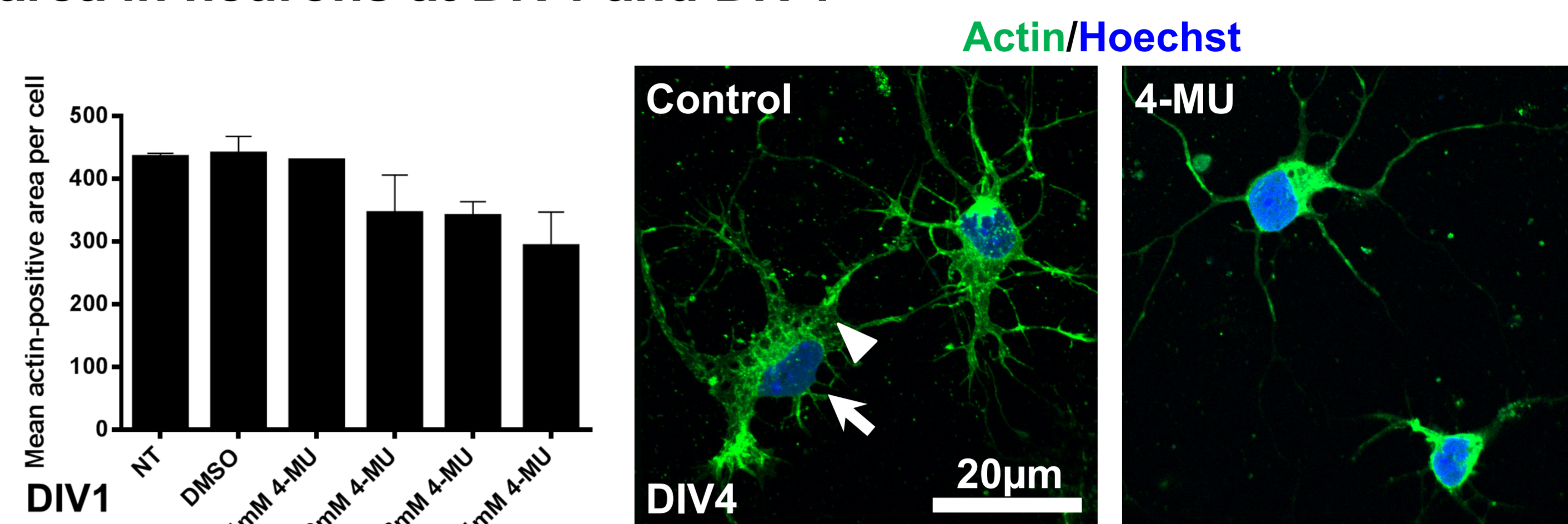
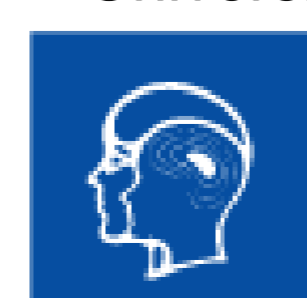### 4-MU impairs neuronal process growth at DIV4
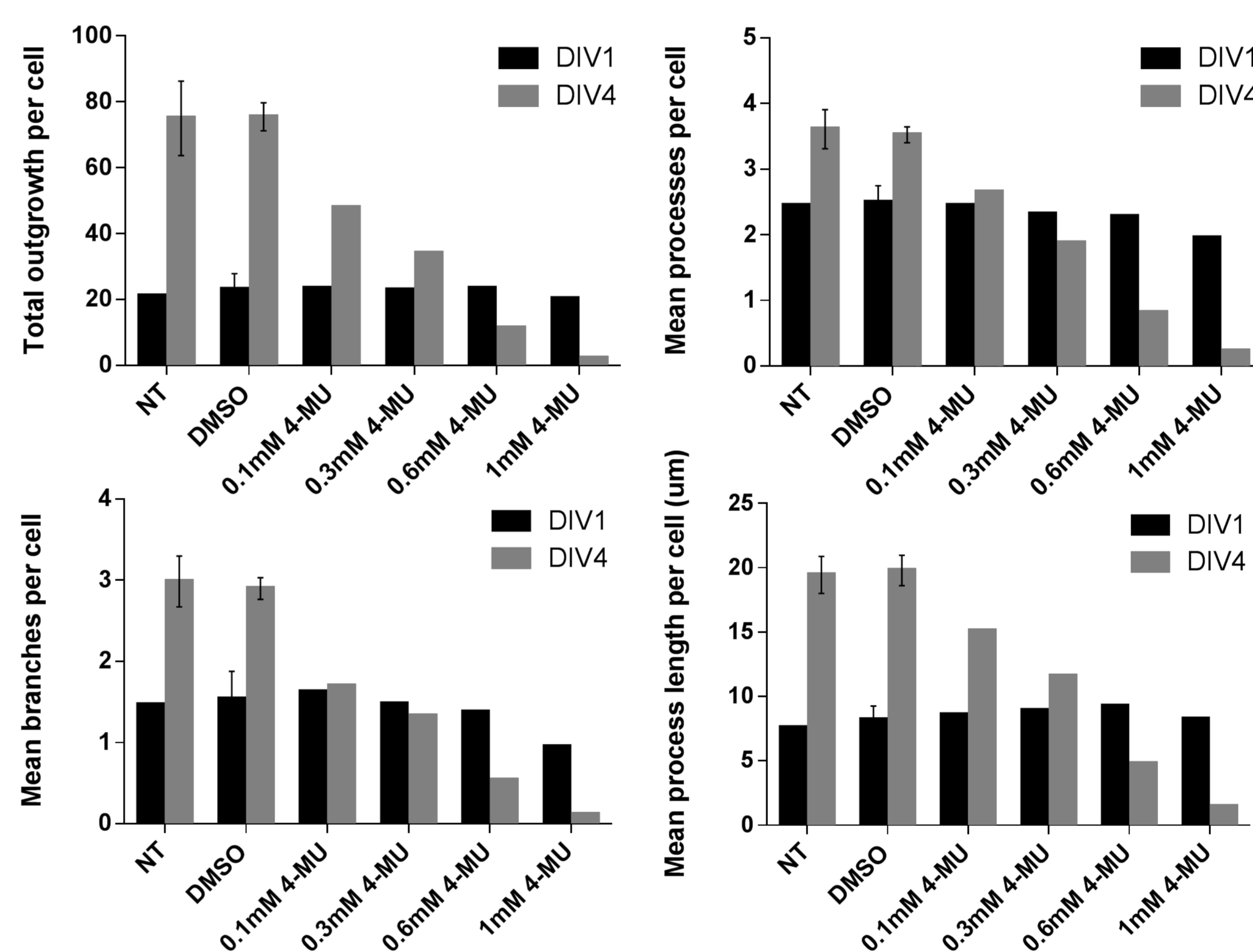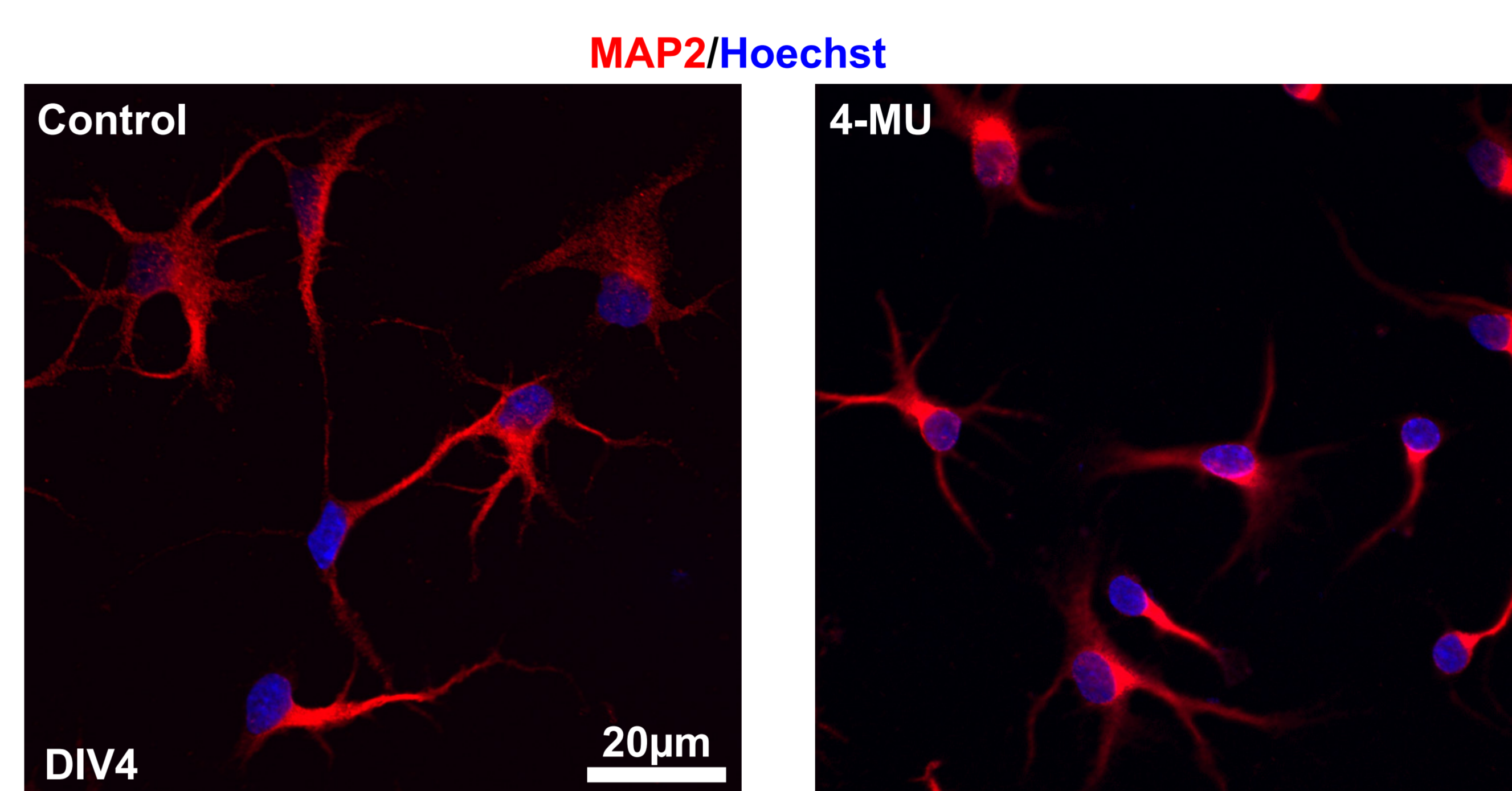
Discussion

- 4-MU reduces neuronal HA production
- HAS inhibition reduces neuronal actin staining, suggestive of a decrease in lamellipodia and filopodia complexity
- HAS inhibition has no apparent effect on processes at DIV1, but reduces multiple aspects of neuronal process development at DIV4 including:
    - Total outgrowth
    - Number of processes
    - Process length
    - Branching
- Further studies will examine the role of individual HAS enzymes in neurite development and specific receptor signaling pathways