Temporal expression of BMP4 in the hippocampus following spatial learning

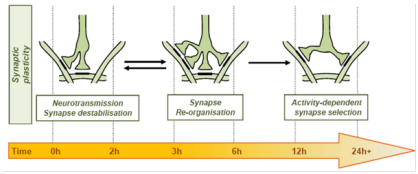
Olivia Gutgsell, Sean O' Shea, and Keith Murphy

Neurotherapeutics Research Group, UCD School of Biomolecular and Biomedical Science,
UCD Conway Institute of Biomolecular and Biomolecular Research, University College Dublin, Dublin 4.

Introduction

Bone morphogenetic proteins belong to the transforming growth factor-beta superfamily, and have many different functions in cell differentiation and proliferation, and bone formation. BMPs are also able to promote dendritic growth and stabilize synapses in the CNS.

Memory formation and consolidation, and learning take place largely in the hippocampus. The dentate gyrus in particular is thought to contribute to formation of new memories. The mechanism by which biological changes in the brain occur due to learning is termed synaptic plasticity.



BMP4 has been implicated in memory-associated synaptic plasticity. It transduces changes through two pathways. The canonical pathway affects genes associated with plasticity through phosphorylated signalling molecules Smad 1/5/8. The non-canonical pathway affects cytoskeleton stability and organization.

A decrease in pSmad is known to increase differentiation of neural stem cells in the dentate gyrus. An increase in BMP inhibition through the non-canonical pathway leads to synaptic instability.

Methodology

•Adult rats trained in Morris water maze, a spatial learning paradigm. Naïve animals were not placed in the maze or trained. Passive animals were allowed to swim in the maze with the hidden platform removed, and therefore did not learn. Trained animals were allowed to find the platform.

•Animals were sacrificed 0.5 hours post-training. Dentate gyrus was dissected out and protein was extracted.

•Western blot for BMP4.

•Immunohistochemical staining for BMP2/4 was performed on tissue sections from one naïve adult rat brain.

Aims

- To investigate BMP4 regulation in the dentate gyrus at 0.5 hours post-training
- To localize BMP4 expression in the hippocampus

Results

1. Expression of BMP4 in 0.5 h samples

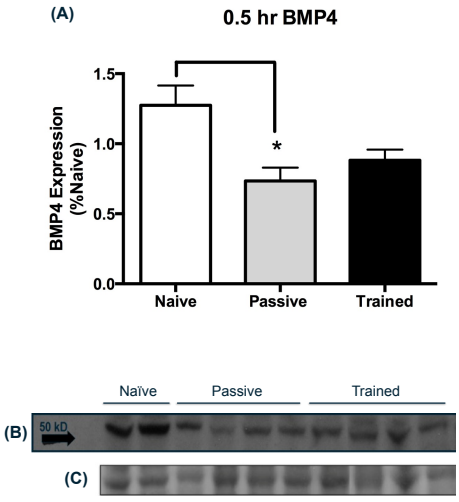


Figure 1: (A) Comparison of BMP4 expression between the three sample groups. A one-way ANOVA was used to carry out statistical analysis. * = p<.05, n=4. (B) Western blotting of hippocampal proteins from rats culled 0.5 hours post-training (20 µg/lane). 1:200 primary α-BMP4 in 5% BSA was used. 1:2000 secondary α-mouse HRP in 5% BSA was used. BMP4 bands were visualized at 55 kD. Bands were normalized to a naphthol stain to act as a loading control. (C) Transfer to membrane was visualized using naphthol staining.

2. Immunohistochemical staining of the dentate gyrus

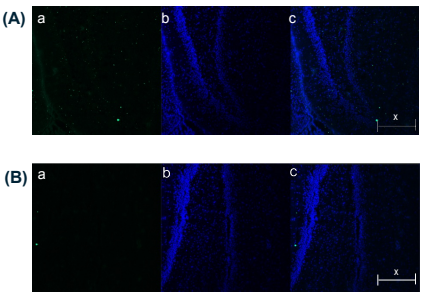


Figure 2A: Tissue sections for immunohistochemical staining were 12 µm in thickness. X= 30 µm. (a) 1:50 primary α-BMP2/4 in 5% BSA was used. 1:500 secondary Alexa 488-mouse α-goat in 5% BSA was used. (b) DAPI staining of the nuclei of the dentate gyrus. (c) Merged image of the immunohistochemical and DAPI stains.

Figure 2B: Immunohistochemical staining, negative control. (a) Tissue was blocked in 5% BSA. 1:500 secondary Alexa 488-mouse α-goat in 5% BSA was used. (b) DAPI staining of the nuclei of the dentate gyrus. (c) Merged image of the immunohistochemical and DAPI stains

Discussion

- Western blotting showed no significant difference between the passive and the trained groups (Fig. 1A). No learning-induced regulation of BMP4 occurs at 0.5 hours post-training.
- The passive samples showed a significant decrease in BMP4 expression compared to the naïve samples (Fig. 1A), suggesting that there is an change in BMP4 expression due to the spatial learning paradigm itself.
- Immunohistochemical staining of the hippocampus did not detect BMP2-4 expression in the dentate gyrus (Fig. 2). More investigation into optimizing the BMP4 antibody for immunohistochemistry is needed.

Conclusion

- No learning-induced regulation of BMP4 occurs 0.5 hours post-learning.
- The spatial learning paradigm itself causes a change in BMP4 expression.

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