Induced synaptic scaling in hippocampal neurons

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Abstract 1

Rett Syndrome is a mental retardation disorder that affects 1 in 10 000 females, and is caused by a mutation in the MeCP2 gene. At a cellular level, this leads to the inability of neurons to modify their synaptic communication in response to homeostatic input. There is recent evidence from animal models that Rett Syndrome might be treatable with growth factor supplementation. The goal of the project was to study synaptic scaling in neuronal cell cultures. The approach that was used was to dissect and dissociate neurons from the hippocampus of embryonic rats, and to grow the neurons in culture until electrophysiological activity appeared. Cells were treated at 1 and 2 weeks with either: bicuculline, a competitive antagonist of GABA to augment network activity or tetrodotoxin, a voltage-gated sodium channel inhibitor to inhibit neuronal firing. Immunohistochemistry was then used to assess the changes in presynaptic neurotransmitter transport protein levels following treatment. The long term goal of these studies was to determine the role of growth factors and intracellular pathways through co-administering antibodies or inhibitors to neutralize or inhibit known or suspected modulators of synaptic scaling. This study will improve comprehension into the mechanisms and players at hand in synaptic scaling and will hopefully provide new targets for the restoration of normal synaptic dynamics in Rett Syndrome which will in time lead to the possibility of improving mental function in Rett Syndrome.

Results 4

- Cell cultures treated at 7 days in vitro with tetrodotoxin showed positive changes indicative of synaptic scaling, but cell cultures treated with bicuculine did not show signs of synaptic scaling
- vGAT and vGLUT stains were shown to regulate changes in opposite directions
- Tetrodotoxin treated cultures showed synaptic scaling effects indicative of inhibition of neuronal firing

References 7

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Introduction 2

The basis of Rett Syndrome is a mutation in the MeCP2 gene leading to the inability of neurons to properly modify their communication in response to learning or homeostatic input. In mouse models of Rett Syndrome, synaptic scaling is known to be deficient. Synaptic scaling is the homeostatic adjustment of synapses following too much or too little neuron network activity. This form of homeostatic plasticity allows neurons to regulate their overall action potential firing rate. This study will focus on whether synaptic scaling can be induced in hippocampal neurons, as demonstrated through an immunohistochemical approach. The same mechanisms seen in the central nervous system should be mirrored in the enteric nervous system.

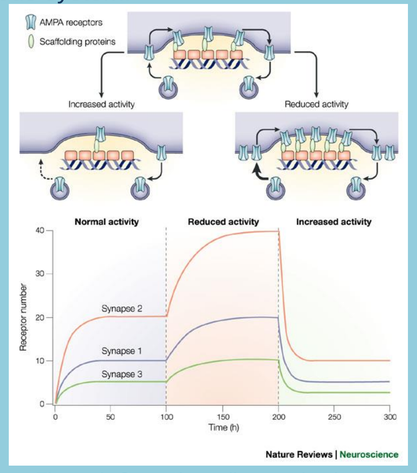

Figure 1: Synaptic scaling mechanism

Immunoflourescence Results 5

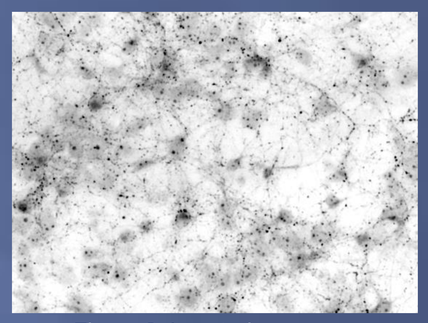

Figure 3: Inverted immunofluorescence vGAT on control culture

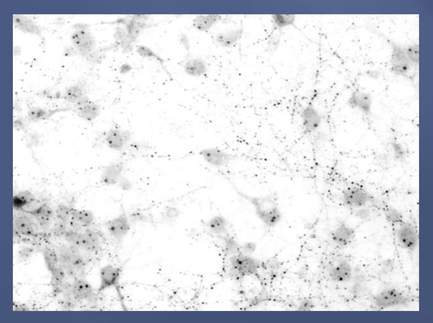

Figure 4: Inverted immunofluorescence vGAT on TTX treated culture

Figure 5: Inverted immunofluorescence vGLUT on control culture

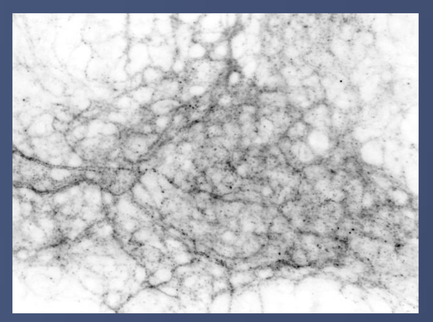

Figure 6: Inverted immunofluorescence vGLUT on TTX treated culture

Methodology 3

- Embryonic rat hippocampal neurons were dissected and dissociated
- Cells were grown in neurobasal medium and plated at a density of 80,000 cells per well
- Cells were treated at 7, 10, and 14 days in vitro with tetrodotoxin, bicuculine, or neurobasal medium for 48 hours
- Cells were fixed and stained with vGAT or vGLUT primary antibody for 24 hours
- Cells were stained with a guinea pig or rabbit secondary antibody for 30 minutes.
- Cells were viewed under a fluorescent microscope

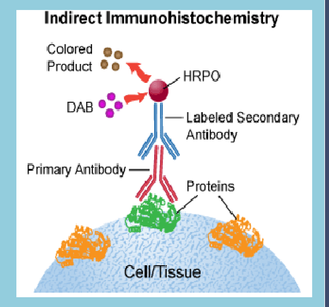

Figure 2: Immunohistochemistry process –a primary antibody, and a fluorescently labeled secondary antibody.

Conclusion 6

This study proved the following conclusions:

- It was demonstrated that synaptic scaling can be induced in hippocampal neurons and shown through immunohistochemical techniques
- Inhibition of hippocampal cultures with TTX results in scaling up as shown by downregulation of the inhibitory neurotransmitter marker GABA transporter in the membrane of synaptic vesicles (ie. downregulation of inhibition is excitatory)
- Inhibition of hippocampal cultures with TTX results in scaling up as shown by upregulation of the excitatory neurotransmitter marker glutamate transporter in the membrane of synaptic vesicles

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