GluA2-lacking AMPA receptor expression in homeostatic synaptic plasticity

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Introduction

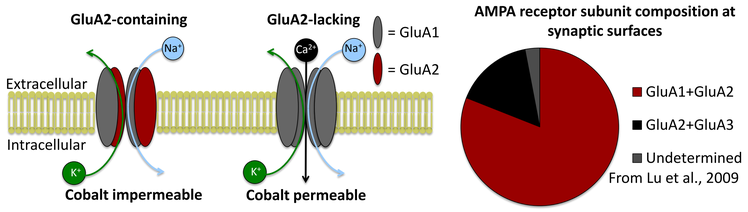
Numerous studies conducted in the last half century have shown that the mammalian brain is intrinsically plastic and is not composed of static networks.

The strength of synapses, the points of connection between neurons, can be modulated by experience through several different mechanisms. Collectively, the effects of these activity-dependent mechanisms have been termed **synaptic plasticity**. Long-term potentiation (LTP) and long-term depression (LTD) are two notable examples of synaptic plasticity (Malenka, 2004).

Some of these activity-dependent phenomena are regulated by **homeostatic synaptic plasticity** mechanisms, which maintain neural excitability levels within an optimal range by means of several processes including changes in the number and types of **ionotropic postsynaptic receptors** (Turrigiano, 2008).

Recently, homeostatic synaptic plasticity has emerged as a plausible model for functional recovery of the brain following traumatic brain injury, such as **ischemic stroke** (Murphy and Corbett, 2009).

GluA2-lacking AMPA receptors are a class of ionotropic glutamate receptors that exhibit permeability to Ca^{2+} ions. In normal conditions, expression of GluA2-lacking AMPA receptors is very limited in pyramidal neurons but they are believed to play a role during homeostatic synaptic plasticity.



Previous studies in the lab using a variety of biochemical and electrophysiological techniques have shown a **homeostatic upregulation** of GluA2-lacking AMPA receptors in CA1 pyramidal neurons in response to **long term network silencing by treatment with tetrodotoxin (TTX)**. TTX blocks voltage-gated sodium channels and therefore ceases action potential firing (Soares et al., 2013).

In this study, a cobalt uptake staining technique was used to investigate, from a histological point of view, the number and distribution of hippocampal cells expressing GluA2-lacking AMPA receptors in response to long-term TTX treatment. The cobalt staining protocol utilizes the cobalt permeability of these channels to allow cobalt to flow specifically into cells that contain GluA2-lacking AMPA receptors.

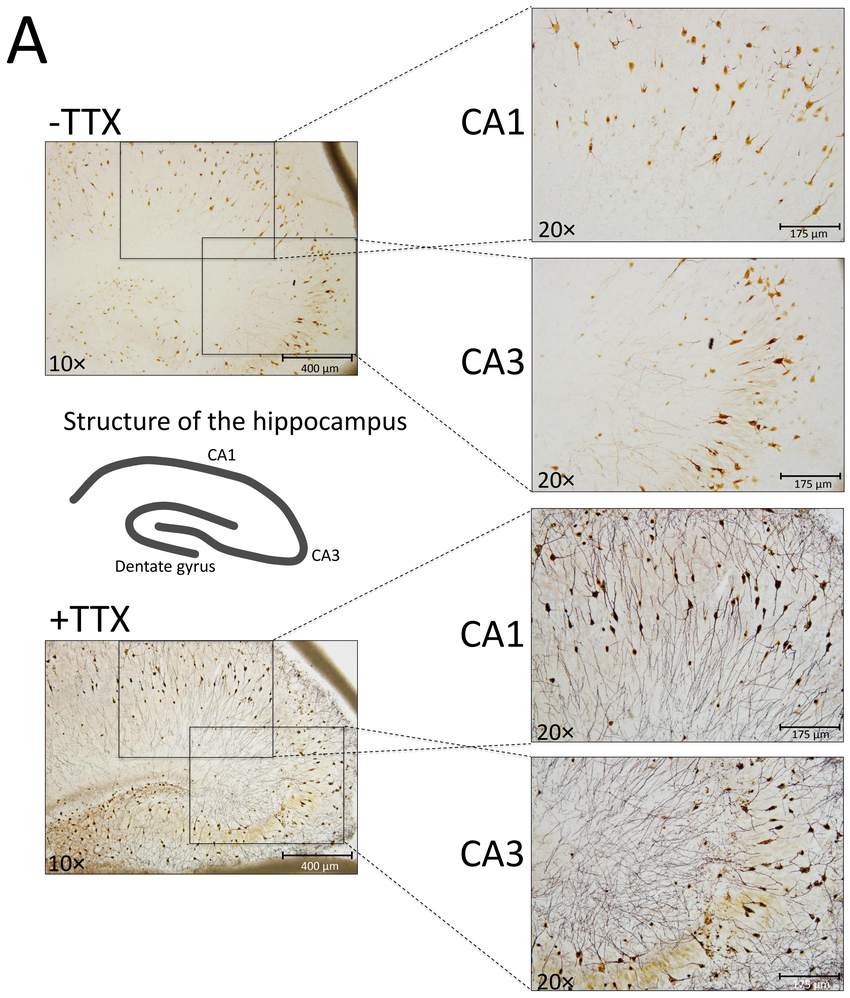
Materials and Methods

Organotypic slices: The hippocampi were dissected from 1 week-old rats and cut at 400 µm using a tissue slicer. The slices were cultured on porous membrane inserts for 7 days, then treated with or without TTX (1 µM) for an additional 72 hours.

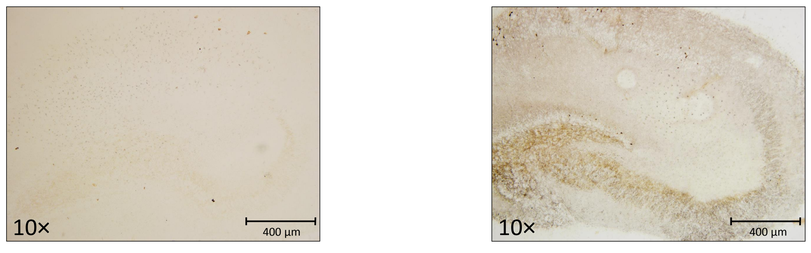
Cobalt staining: The slices were then stained using a protocol that I adapted from Arousseau *et al.* (2012), in which they were treated with a solution containing 10 µM Kainic Acid (AMPA receptor agonist) and 5 mM $CoCl_2$ for 15 min. The negative control slices were preincubated in 10 µM CNQX (AMPA receptor antagonist) for 30 min prior to the kainic acid treatment. After chelating excess cobalt with 2 mM EDTA, the slices were treated with 0.24% $(NH_4)_2S$ which precipitates cobalt. Slices were then fixed with PFA and cryosectioned at 25 µm and mounted on microscope slides.
The slices were then developed using a silver enhancement protocol that I adapted from Davis (1982) and images were taken.

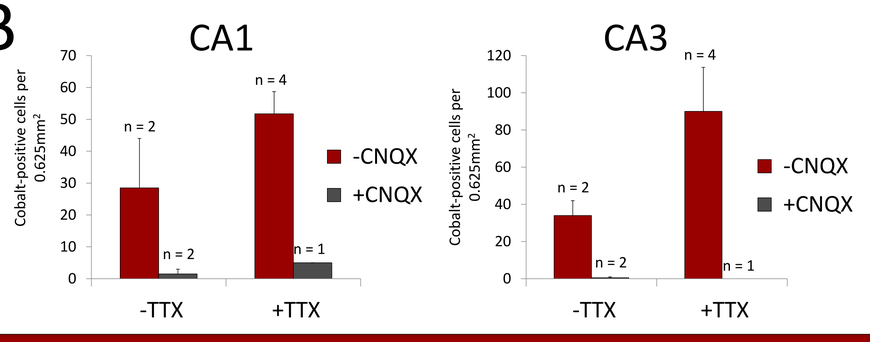
Results

GluA2-lacking AMPA receptors are expressed in a higher number of cells after long-term network silencing with TTX. A) Images at 10× (left) and 20× (right) of cobalt stained hippocampi, as well as negative controls with AMPA receptor antagonist CNQX. B) Number of cobalt positive cells in a 0.625 mm^2 area (size of the microscope field at 20×) in CA1 and CA3 regions of the hippocampus.



Negative controls with CNQX





"Somehow the unstable stuff of which we are composed has learned the trick of maintaining stability"
- Walter Cannon, The Wisdom of the Body

Conclusion

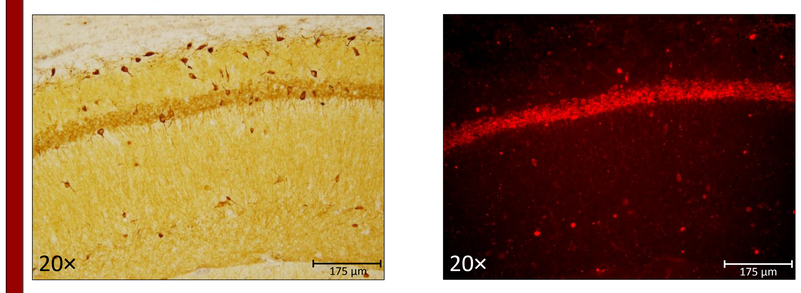
There seems to be a higher number of cells expressing GluA2-lacking AMPA receptors in TTX-treated slices than in non TTX-treated slices. The number of samples needs to be increased to verify this.

Cobalt staining in the TTX-treated slices seems inherently more intense than in non-TTX treated slices. A method will be developed to better quantify variations in staining intensity between cells.

Future Experiments

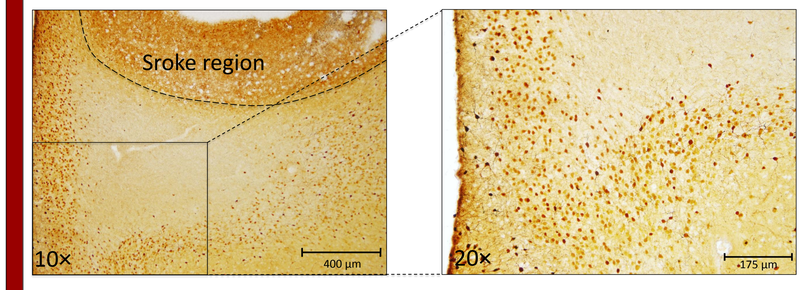
1. To use an immunohistochemical staining procedure to allow for a more rigorous analysis of the cobalt staining results

- Staining for **NeuN** can be used to determine the total number of neurons in a given field.
- Staining for **GAD67** to identify GABA-ergic neurons expressing GluA2-lacking AMPA receptors under normal conditions.



2. To study the expression of GluA2-lacking AMPA receptors in regions adjacent to the stroke area. Given that the role of homeostatic synaptic plasticity in functional recovery from a stroke is still poorly understood, cobalt staining experiments could help us identify neural networks that are expressing GluA2-lacking AMPA receptors following a stroke.

Cortex 3 days after photothrombotic stroke



Acknowledgements

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