Webinar Q&A Report

Electrophysiology of Human Native Receptors in Neurological and Mental Disorders

Questions in this Q&A Report were submitted during the live webinar, Electrophysiology of Human Native Receptors in Neurological and Mental Disorders.

Aside from GABA and AMPA receptors, what other kinds of receptors can you measure with this methodology?

Most ionotropic receptors and ion channels present in cellular membranes can be microtransplanted into Xenopus Oocytes. NMDA receptors, nicotinic receptors, chloride channels, voltage-gated calcium and sodium channels from diverse species have been successfully microtransplanted by different research groups. However, if the channel of interest is also expressed endogenously by the oocyte, then silencing of the endogenous protein is necessary to isolate the microtransplanted receptor, this is the case for voltage-gated calcium channels.

How do you ensure that the receptors are integrated into the oocyte membrane with the right orientation and not upside down? Or is this just by chance and you activate only the correctly inserted ones?

The receptors are inserted in both directions; because the perfusion is from the extracellular side, only the receptors with the agonist binding site facing the extracellular space are activated.

How long can this tissue be stored in brain banks and remain viable?

We have been recorded GABA and AMPA receptors that have been stored in brain banks for more than 10 years without appreciated change in amplitude responses.

How did you standardize the level of expression of proteins in oocytes?

There are at minimum two levels of normalization. First, we measure the level of total protein by Qubit in the synaptosome samples and the number of synaptosome-like particles in the preparation by flow cytometry and standardized size beads. Depending on the question we inject the same amount of protein or a similar number of synaptosomes, both values usually

correlate. The second level normalizes the response of one receptor (e.g. AMPA receptor) vs another (e.g. GABAA receptor) per oocyte. This approach helps in reducing the variability between different oocytes and experiments. We also include a well characterized rat brain cortex sample in all injections to evaluate the potential variability in the insertion capacity of oocytes from different batches.

Great talk. As there is tremendous heterogeneity in native human CNS receptors and their splice variants can you say anything about specific changes in Ca2+ permeable vs impermeable AMPA and Glu Rs?

Thank you! The involvement of Ca2+ dysregulation in neurodegenerative disorders suggests an important role for the different variants of AMPA receptors with distinct Ca2+ permeability in this process. This is a very important question that we are currently addressing right now.

Hyperexcitability with loss of dendritic spines seems counter-intuitive. Can you explain further (thinking of AD)?

Loss of dendritic spines (excitatory synapses) without changes in inhibitory synapses would mostly lead to primary neurons hypoexcitability. However, our data indicates that inhibitory synapses are being affected to a larger degree than the loss of dendritic spines, leading to inhibitory deficits and neuronal hyperexcitability, at least in the parietal cortex where we did the measurements.

Contact Information

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