October 16th, 2018

Dr. Aaron Berard, Editor

JoVE

1 Alewife Center, Suite 200

Cambridge, MA 02140, USA

Dear Aaron,

Accompanying this letter, please find a version of our revised manuscript entitled “**A high-content assay for monitoring AMPA receptor trafficking**” which was recently submitted to JoVE. Postsynaptic trafficking of receptors to and from the cell surface is an important method by which neurons modulate their responsiveness to different stimuli. The α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, which are largely responsible for fast excitatory synaptic transmission in neurons, are trafficked to and from the postsynaptic surface to dynamically alter neuronal excitability. Moreover, AMPA receptors are found to be disrupted in multiple neurological diseases such as Alzheimer’s disease. In this manuscript, we describe a high-content AMPA receptor trafficking protocol that takes advantage of monoclonal antibodies targeting AMPA receptor subunits and uses primary hippocampal neurons cultured in 96-well microplates. Using this method, we show altered AMPA receptor trafficking in primary neurons isolated from a transgenic knock-in mouse (ArcKR), which we previously published in the journal ***Neuron*** (Wall\_et\_al\_2018).

The protocol described here has its advantages, as it consumes much less time and materials than alternative methods and can be modified using 384-well microplates. Moreover, our assay can be expanded to measure the trafficking of other receptor types in a high-content manner. Given these advantages, our assay would be of high interest and benefit to researchers in academia and industry, thus broadening JoVE’s potential readership.

reviewers felt that our method was suitable for publication in JoVE. We are grateful for the positive comments and enthusiasm of the reviewers and editor. Reviewers requested clarification in the methods section and modification to an incorrect statement in the Introduction. We have addressed all of their points in our revised version, which significantly improved the quality of this publication.

Sincerely,



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