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vGlutI-pH imaging experiment analysis

In 1 collection

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We use this protocol and it's working

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Abstract

This protocol describes how to analyze imaging experiments using vGlutI-pH from primary neurons.

Materials

Software

Microsoft Excel (https://www.microsoft.com/en-us/microsoft-365/excel, version 2308, RRID:SCR_016137)
GraphPad Prism (http://www.graphpad.com, version 10.0.2, RRID:SCR_002798)
ImageJ (NIH, US, https://imagej.net, version 1.52p, RRID:SCR_003070)
Time Series Analyzer plug-in (https://imagej.net/ij/plugins/time-series.html, version 3.0)

vGlutI-pH image analysis

1 Open the image stacks to by analyzed with ImageJ by drag and drop.

2 Identify nerve terminals that responded to electrical stimulation, by observing their fluorescence increasing in sync with the timing of the action potential train.

3 Place ROIs on the responding terminals using the Time Series Analyzer plug-in.
We usually use ROIs of width and height 6 pixels each, with the pixel size being 400 nm in our images.

4 Get the average pixel intensity of each ROI and each image using the "Get Average" function.
Save the produced csv results as well as the ROIs selection.

5 Similarly place background ROIs surrounding the nerve terminals identified. Save the pixel intensity values and background ROIs selection.

6 Analyze the NH_4Cl images in the exact same way, using the previously saved ROIs.
In case the nerve terminals have slightly moved, the ROIs can be readjusted using the "Recenter" function.

vGlutI-pH data analysis

7 Open the produced csv files in Microsoft Excel.

8 For each time point, subtract the average background fluorescence values from the average nerve terminal vGlutI-pHl fluorescence values.

9 Calculate the ΔF values, by subtracting the initial fluorescence values before the action potential train from all values, so that the first produced values will now be around 0.

10 The data can now be further normalized. To normalize against the total sensor fluorescence, revealed by the 50 mM NH_4Cl Tyrodes solution, analyze the NH_4Cl image stack as described above and calculate the maximal fluorescence change. Dividing all fluorescence values with the calculated $\Delta F_{NH4,}$ will yield the $\Delta F_{APs}/\Delta F_{NH4}$ normalization of the data-set.
Note: It is advised to use the initial F_0 when calculating the ΔF_{NH4}, since manipulations that slow or block synaptic vesicle recycling will also increase the fluorescence of the sensor at the end of the experiment.

11 The data-set can also be normalized internally to the fluorescence peak during the action potential train. This allows to specifically look at synaptic vesicle endodytic kinetics and is unaffected by changes in exocytosis. First, calculate the ΔF_{MAX} by identifying the maximal fluorescence values around the end of the action potential train. For 10 Hz trains, we locate the F_{MAX} at a 2 second window around the end of the action potential train. Dividing all fluorescence values with the calculated $\Delta F_{MAX,}$ will yield the $\Delta F_{APs}/\Delta F_{MAX}$ normalization of the data-set.

12 To calculate the endocytic efficiency, the leftover sensor fluorescence can be calculated for all single cell data, at a specified time-point. To minimize variability due to noise, we usually use a small time window around the chosen time point of approximately 2 seconds.

13 All produced time trace data and subsequent quantifications can be plotted and presented in Graphpad Prism.