Supplemental Information for 'Elucidating distinct ion channel populations on the surface of hippocampal neurons via single-particle tracking recurrence analysis'

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1 Supplemental methods

1.1 Cell culture

Dissections were performed after anesthesia with isoflurane followed by decapitation. Hippocampal tissue was dissected from the brains of E18 embryos and neurons were plated on glass-bottom 35-mm dishes with No. 1.5 coverslips (MatTek, Ashland, MA) that had been coated with poly-L-lysine (Sigma-Aldrich, St. Louis, MO) for 1 hr, rinsed with sterile water, then allowed to air dry for 15 min. Neurons were grown in Neurobasal Medium (Gibco/Thermo Fisher Scientific, Waltham, MA) with penicillin/streptomycin antibiotics (Cellgro/Mediatech, Manassas, VA), GlutaMAX (Gibco/Thermo Fisher Scientific), and NeuroCult SM1 Neuronal Supplement (STEMCELL Technologies, Vancouver, BC, Canada). For imaging, the media was replaced by neuronal imaging saline (NIS) consisting of 126 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 0.6 mM $MgSO_4$, 0.15 mM NaH_2PO_4, 0.1 mM ascorbic acid, 8 mM glucose, and 20 mM HEPES (pH 7.4).

1.2 TIRF microscopy

Total internal reflection fluorescence (TIRF) images were acquired using a Nikon Eclipse Ti fluorescence microscope equipped with a Perfect-Focus system, acousto-optic-tunable-filter (AOTF)-controlled 647 nm diode laser, an Andor iXon EM-CCD DU-897 camera, and a Plan Apo TIRF 100, NA 1.49 objective. Emission was collected through a filter wheel containing the appropriate bandpass filter. For excitation, an incident angle of 63° was used.

2 MATLAB codes and example data

We provide the MATLAB codes for analyzing single-particle trajectories. The raw data provided as example consists of 29 Kv1.4 trajectories, 'Kv14data.txt'. The raw data is arranged as an ASCII file with the odd columns being x coordinates and the even columns y coordinates. That is, the data is arranged as $x1, y1, x2, y2, ...$ and it is in nm. Frame time is 50 ms. The first row of the spreadsheet is a header for convenience. The file 'Kv14data.txt' can be read with the following script so that then it is directly used in the codes provided.

```
tracks_headers = 1; %Number of headers in the tracks file
file_name='Kv14data.txt';
A0 = importdata(file_name, '\t', tracks_headers);
A0 = A0.data;
```

2.1 Suggested parameters to be used with example data

classification3.m This is the main code, which classifies the trajectories according to the fraction of time; **th1**=11, **N**=2.

fractionTime2.m Fractions of time in one state.

CircleMethod3 Recurrence analysis.

regime_variance2.m Hypothesis test for single regime; **x**=**f**, **alpha**=0.05, where **f** is the vector with fractions of time.

sil2.m Silhouette analysis for optima number of classes. **stats**=**f'**, **range**=2:6, where **f** is the vector with fractions of time.

Note that the data provided in the example yield an optimal number of four classes because the number of trajectories is relatively small.