Calcium imaging in freely-moving mice during electrical stimulation of deep brain structures

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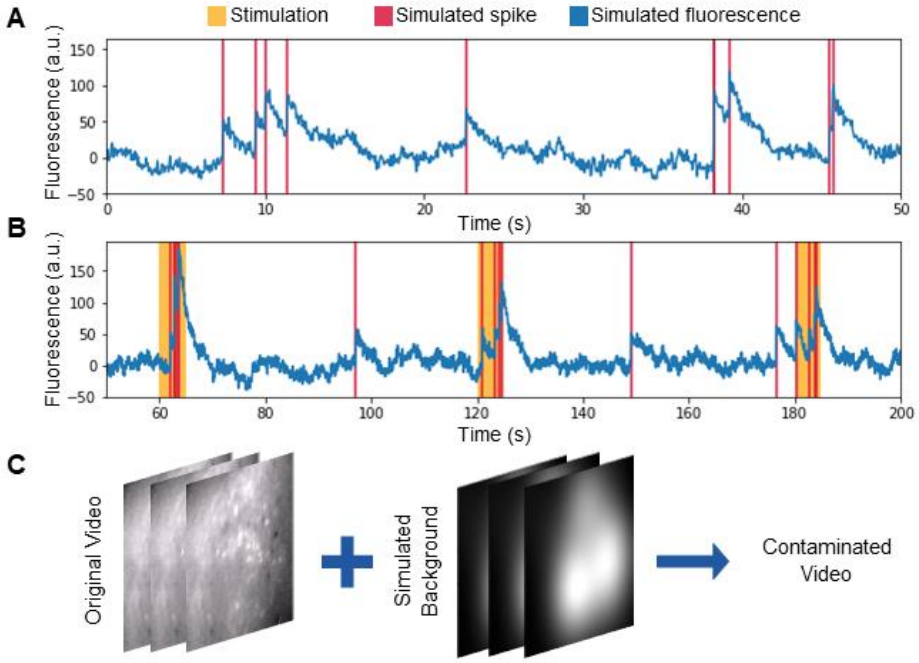

Supplementary Figures

Figure S1. Method for simulating background florescence contaminated calcium imaging data. A) Simulation of florescence changes occurring neural activity was performed according to Vogelstein et al. (2009)[68]. **B)** Simulation were performed such that the activity rate of the simulated traces increased during periods of stimulation. **C)** Simulated background fluorescence due to out-of-focus neurons was added to real calcium imaging data obtained under anesthesia to produce a contaminated video that could be analyzed via standard techniques. These data were used to determine the effectiveness of different analysis techniques for identifying cells in the presence of changes in out-of-focus fluorescence occurring during stimulation.

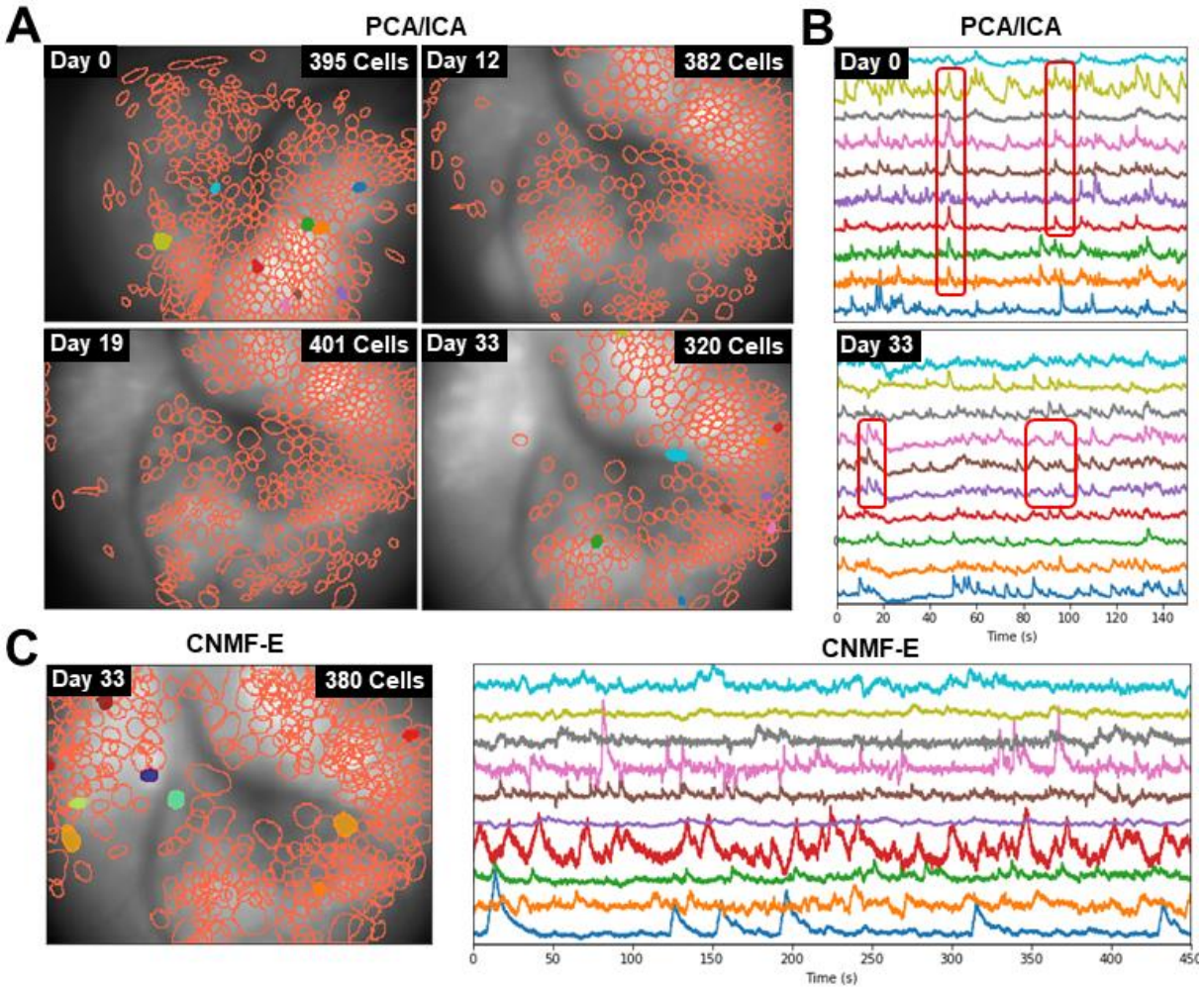

Figure S2. Difference in extracted cell activity between PCA/ICA and CNMF-E performed on calcium imaging data obtained in an open-field arena. A) Representative mouse showing that the number of striatal neurons identified by PCA/ICA was consistent over the course of the study as well as the failure of PCA/ICA to separate overlapping cell bodies. **B)** Neural signals extracted from 10 cells showing highly correlated activity (outlined in red) between nearby cells. **C)** CNMF-E was able to separate overlapping cells and did not exhibit highly correlated activity between cells.

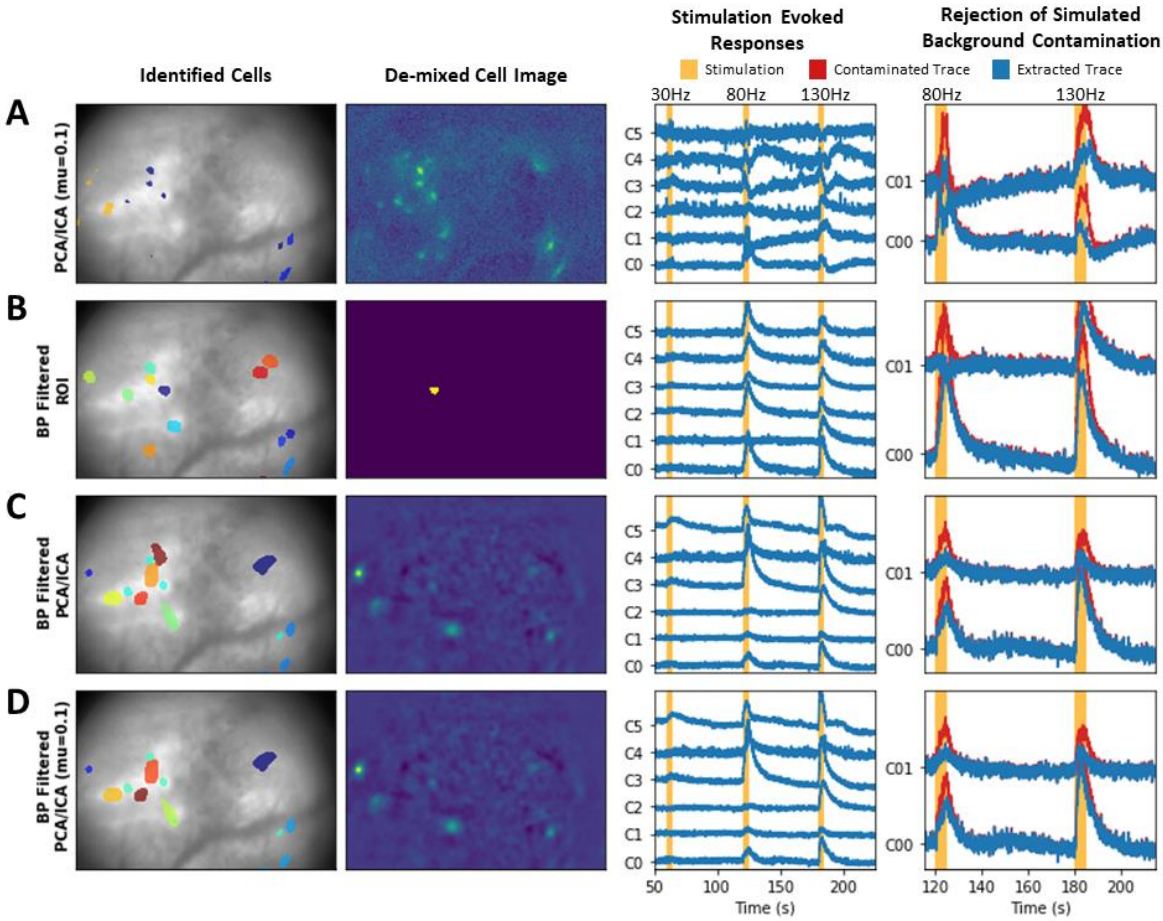

Figure S3. Additional data extraction techniques. In addition to the techniques presented in Figure 5, the capabilities of other data analysis techniques to remove simulated background fluorescence was assessed. For each technique, individual cells that were identific are shown in the first column. The second column shows, a represetnative cell image (de-mixed cell image) and the third column shows calcium traces during 30, 80, and 130 Hz stimulation trains. Lastly, the fourth column shows calcium traces identified by each analsysis technique, plotted on top of extracted traces from the same dataset but contaminated with simulated background flourescence data. **A)** PCA/ICA with spatiotemporal de-mixing value (mu) of 0.1, **B)** ROI analyis performed following band-pass filtering of the data, **C**) PCA/ICA following band-pass filtering, and **D)** PCA/ICA (mu=0.1) following band-pass filtering were assessed the capability of each analysis methods and to identify spatially compact neurons, using the identified cell-bodies and de-mixed cell images, and reject simulated background contamination.