Supplemental File: Calcium activation of cortical neurons by continuous electrical stimulation: Frequency-dependence, temporal fidelity and activation density

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**Abbreviated title:** Spatiotemporal activation by microstimulation

Key words: microstimulation, bioelectric medicine, brain-computer interface, deep brain stimulation, neuroprosthetics

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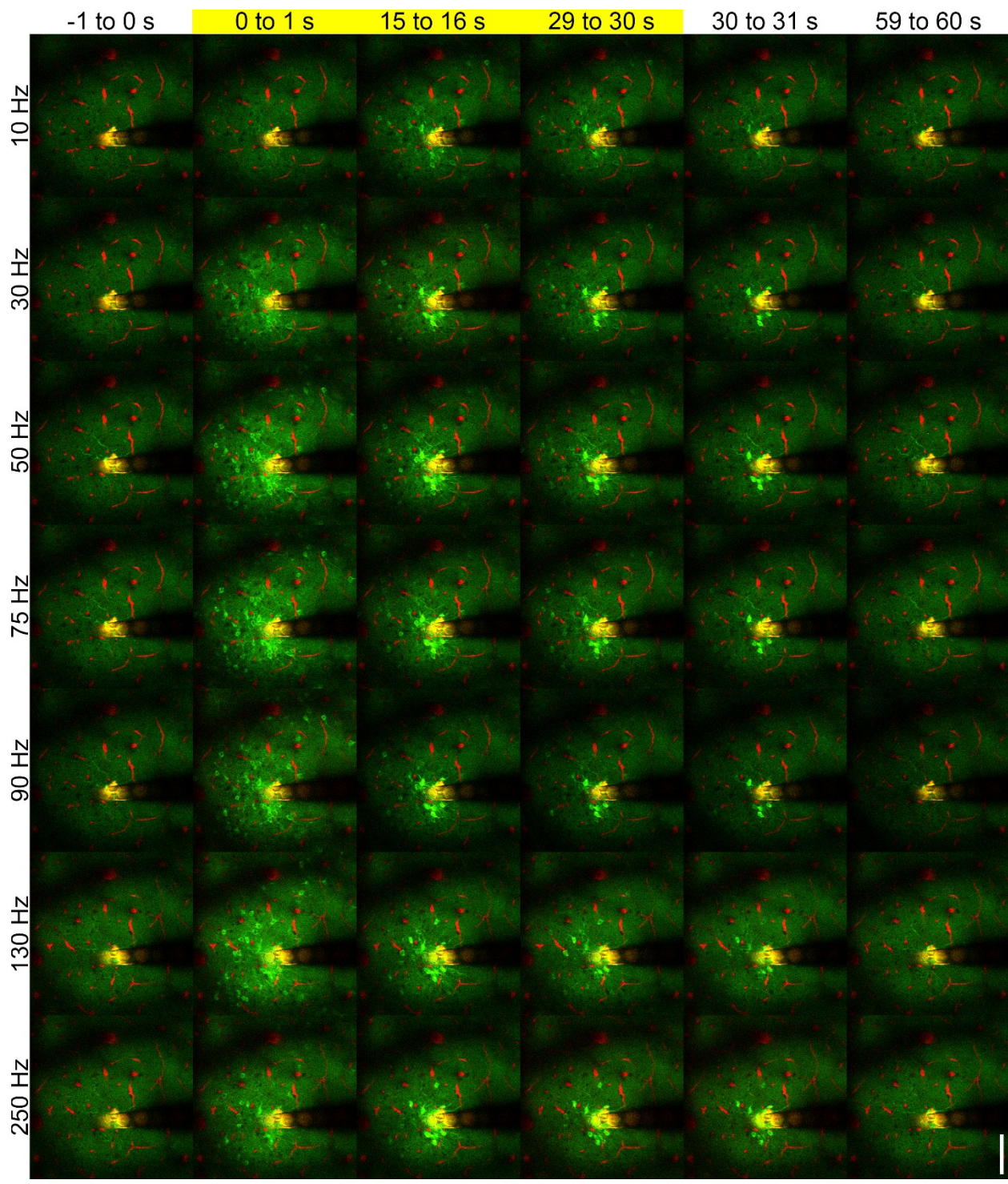
Supplemental Figure 1. Same as figure 3 for all frequencies. Scalebar = 100 μm
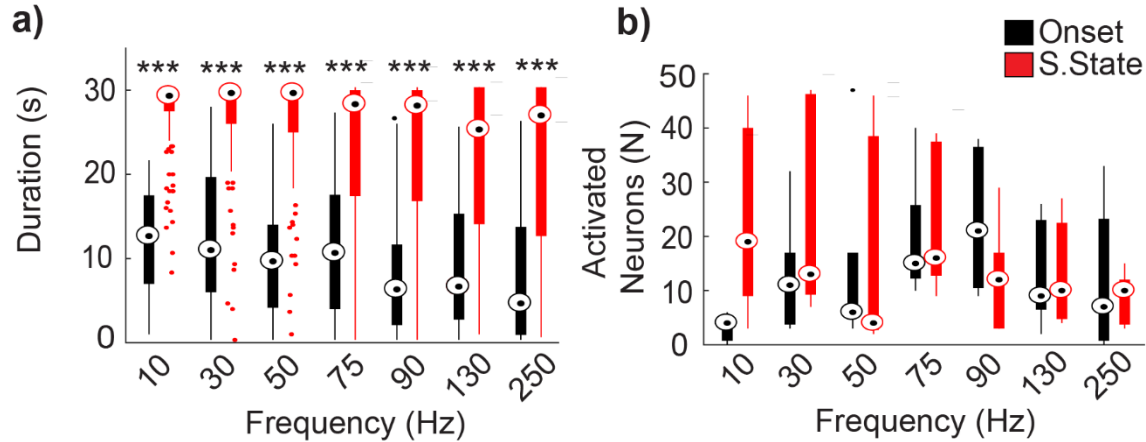
Supplemental Figure 2. (a) Average duration of onset and steady-state phase. Duration of activation phases were calculated by tracking onset-responsive (neurons that are active 0-2s into stimulation) and steady-state-responsive neurons (neurons active 28-30s into stimulation). (b) Average number of neurons activated during onset and steady-state phase for 10Hz, 30Hz, 50Hz, 75Hz, 90Hz, 130Hz and 250 Hz stimulation. For (a): repeated measures two-way (within frequencies, F=374.831, p<0.001) with post-hoc t-tests with Bonferroni correction ***p < 0.001.
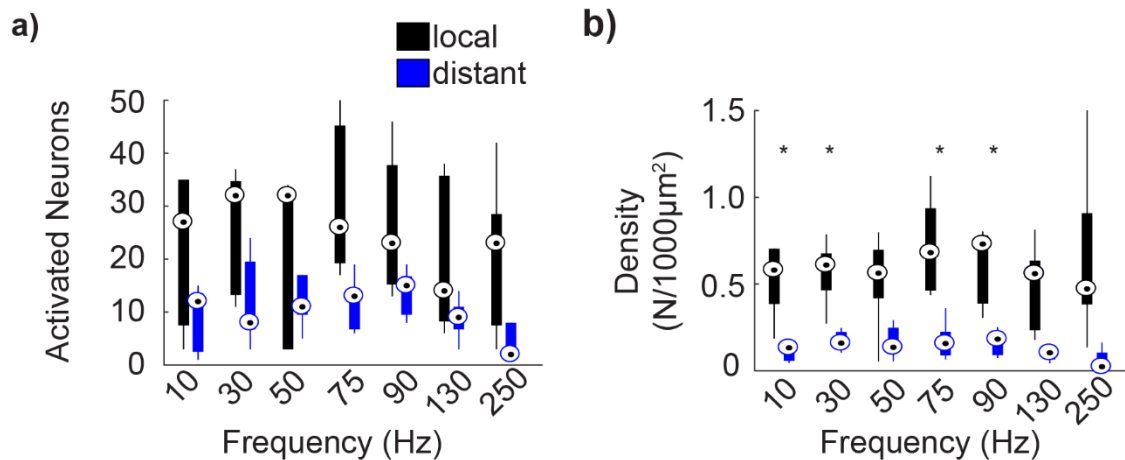
Supplemental Figure 3. (a) Average number of neurons activated that are local and distal from the electrode. (b) Average activation density of local and distal neuron population. Activation density for locally activated neurons was significantly higher for all stimulation frequencies than distally activated neurons. For (b): repeated measures two-way ANOVA (within frequencies, F=523.846, p<0.001) and post-hoc t-tests with Bonferroni correction *p < 0.05
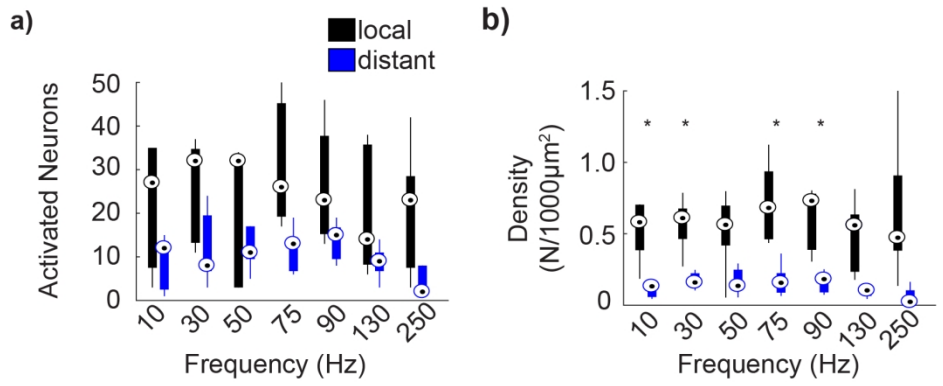
Supplemental Movies. 15 seconds of pre-stimulus period followed by 30 seconds of continuous stimulation (10-130Hz), followed by an additional post-stimulus period.

a) b)
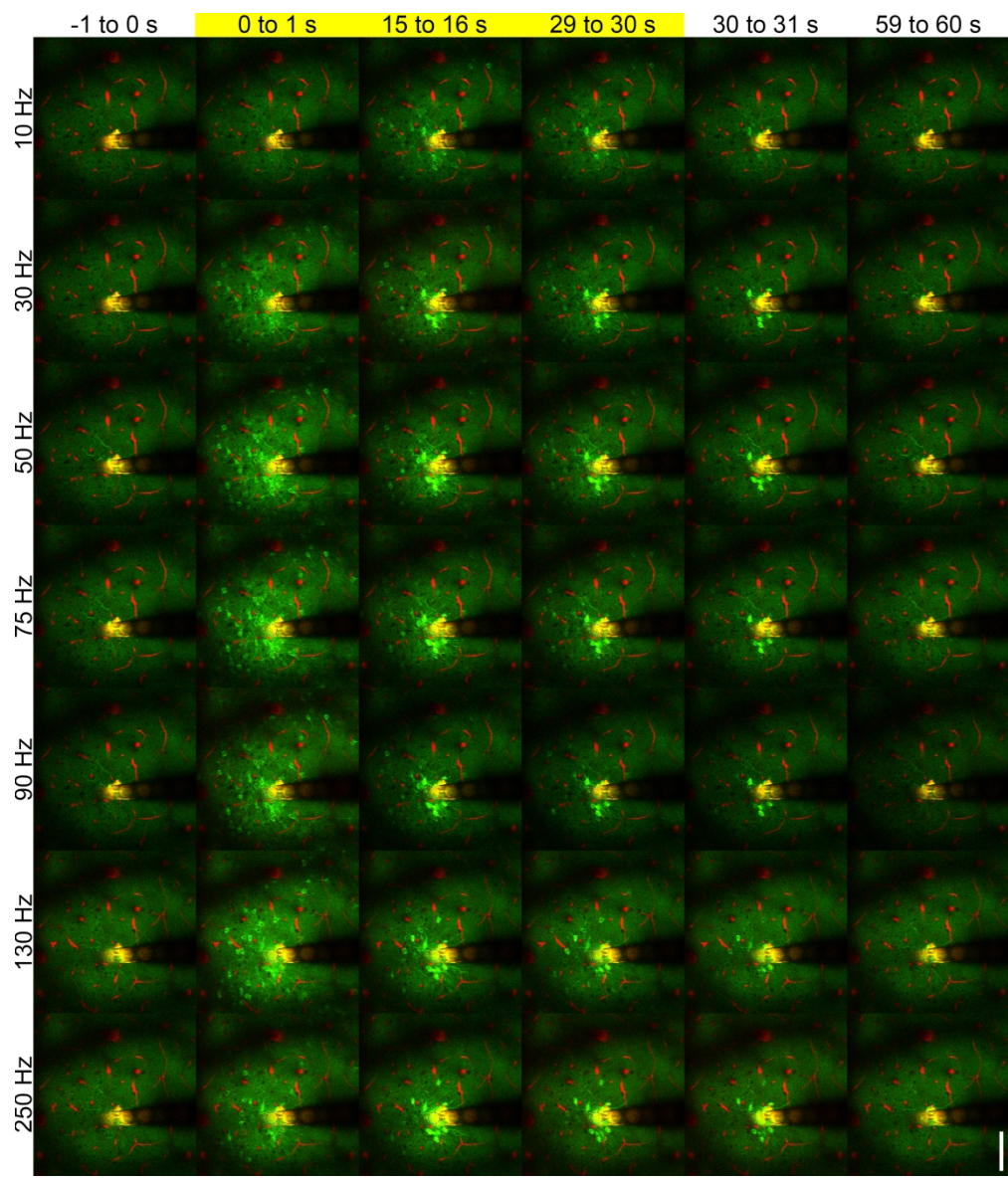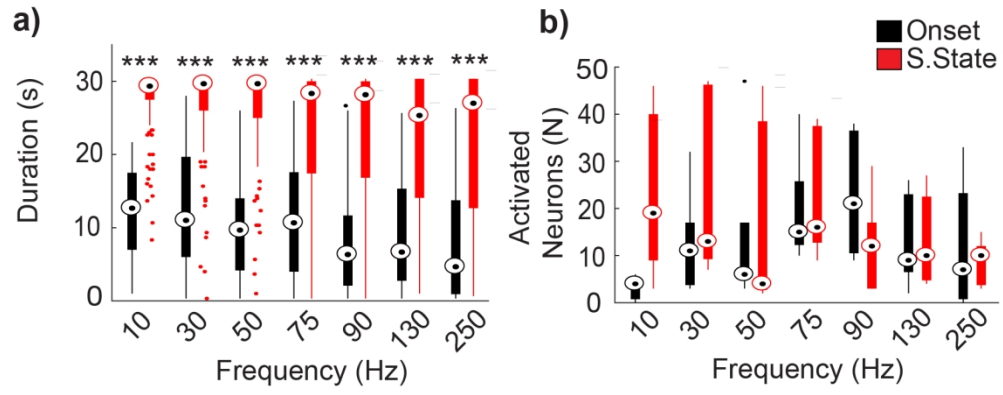
Rigorous study design and transparent reporting of results are the cornerstones of science. By maximizing the information provided in a manuscript, factors that may contribute to irreproducibility will be mitigated. The *Journal of Neuroscience Research* promotes transparency in research by strongly encouraging authors to include all relevant information about their studies (see our preprint for details). To expedite reviewer monitoring of these factors, authors submitting original research articles must complete this questionnaire.

If the manuscript is accepted and all items within the checklist are present, we will include a declaration of transparency at the end of the manuscript. This declaration reads as follows:

> *The authors, reviewers and editors affirm that in accordance to the policies set by the Journal of Neuroscience Research, this manuscript presents an accurate and transparent account of the study being reported and that all critical details describing the methods and results are present.*

To complete the checklist, fill in the right-hand column with the page and paragraph number (e.g., 'Page 3, Paragraph 2') corresponding to the checklist item. If a checklist item is not applicable to the study being reported or the authors are unable to provide that item, a reason must be supplied. Additional comments can be added at the end of the document. Upload the completed document as supplementary information for review.

Experimental and Study Design

1. Clearly state the primary and any secondary objective or hypothesis of the study	Page 3, Paragraph 2
2. For each experiment, the study design must include:	
a. Number of experimental and control groups	Page 4, Paragraph 3 [1]
b. Randomization and blinding procedures and/or steps to minimize subjective bias when allocating subjects to experimental groups	Page 18, Paragraph 4 [2]
c. Precise details of all procedures, including housing and husbandry are carried out in the experiment	Page 3, Paragraph 3
d. Is sex considered as a biological variable? See Editorial for details about proper reporting	Page 3, Paragraph 3

Experimental Subjects

3. Specify the total number of subjects in each experiment, including the number of animals, sex and age in each group	Page 3, Paragraph 3
a. Explain how the number of animals were arrived at and provide details of any sample size calculation, including power analysis	Not applicable [3]
b. Indicate the number of independent replications of each experiment, when applicable	Page 19, Paragraph 1

Data Handling

4. Indicate data collection start and stop rules:	Page 4, Paragraph 2
a. Define the criteria for data/subject inclusion and exclusion. If any outcome or condition measure used was not reported in the results section, authors must address this omission	Not applicable [4]
b. Specify reasons for any discrepancy between the number of animals at the beginning and end of the study	Not applicable [5]
c. Define and explain how outliers are handled and report if data are removed prior to analysis	Not applicable [6]

Statistical Analysis and Depiction of Continuous Data

5. Provide details of the statistical methods used for each analysis	Page 5, Paragraph 4
a. State, define and justify the statistical analysis used and specify the unit of analysis for each dataset	Page 5, Paragraph 4
b. Describe and report methods used to assess whether data met the assumptions of the statistical approach and any adjustments for multiple comparisons	Page 5, Paragraph 4
c. Fully report statistics (including exact value of N, degrees of freedom, test value and exact P-value when >0.001) and we encourage the use of effect sizes and confidence intervals	Figures 6-8 captions
d. Disaggregated data are presented for males and females	Not applicable [7]
e. Data distribution is depicted with univariate scatterplots boxplots, violin plots, or kernel density plots when presenting **continuous data** (see Editorial Publishing Transparent and Rigorous Scientific Research)	Page 5, Paragraph 4

Discussion

6. Comment on study limitations including any potential source of bias, limitations to the animal model, imprecisions associated with the results, and the inability for any reason to study possible sex influences where they may exist.	Page 18, Paragraph 4
7. Comment on possible translational implications and future research directions	Page 18, Paragraph 2

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[1] Each animal acts as its own control, as activity during stimulation is compared to activity before stimulation.

[2] Randomization and blinding procedures were not taken. This is discussed as a limitation.

[3] Formal sample size calculations or power analyses were not performed. However, this sample size was estimated to be adequate based on previous studies (Murphy, Chan, Kim, & Vazquez, 2018).

[4] All subjects with successful surgical procedures were included.

[5] No discrepancies between number of animals.

[6] Outliers were not removed prior to analysis.

[7] Only males were studied.

Murphy, M. C., Chan, K. C., Kim, S. G., & Vazquez, A. L. (2018). Macroscale variation in resting-state neuronal activity and connectivity assessed by simultaneous calcium imaging, hemodynamic imaging and electrophysiology. *Neuroimage, 169*, 352-362. doi:10.1016/j.neuroimage.2017.12.070