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Supplementary information

Fast and sensitive GCaMP calcium indicators for imaging neural populations

In the format provided by the authors and unedited

Supp. Table 3. Adult *Drosophila* L2 assay protocol

Cycle	Frequency	Off	On	Trials	Volt (Lights Off)	Volt (Lights On)	Time (s)	Description
1	0.5			10	0	5	20	Frequency
2		25	500	25	0	5	14	Dark Flash
3		8	500	25	0	5	12	Dark Flash
4		4	500	25	0	5	12	Dark Flash
5	0.5			10	0	5	20	Frequency
6	30			600	0	5	20	Frequency
7	28			560	0	5	20	Frequency
8	26			520	0	5	20	Frequency
9	24			480	0	5	20	Frequency
10	22			440	0	5	20	Frequency
11	20			400	0	5	20	Frequency
12	18			360	0	5	20	Frequency
13	16			320	0	5	20	Frequency
14	14			280	0	5	20	Frequency
15	12			240	0	5	20	Frequency
16	10			200	0	5	20	Frequency
17	8			160	0	5	20	Frequency
18	6			120	0	5	20	Frequency
19	4			80	0	5	20	Frequency
20	2			40	0	5	20	Frequency
21	1			20	0	5	20	Frequency
22	0.5			10	0	5	20	Frequency
23		2000	500	15	Ramp	5	45	Ramp
24		1000	500	15	Ramp	5	23	Ramp
25		3000	500	15	Ramp	5	67.5	Ramp
26		25	500	25	2.5	5	12	Dark Flash
27		25	500	25	0	2.5	12	Dark Flash
28		8	500	25	2.5	5	14	Dark Flash
29		8	500	25	0	2.5	14	Dark Flash
30		500	25	25	0	5	14	Light Flash
31		500	8	25	0	5	12	Light Flash
32		500	4	25	0	5	12	Light Flash
33	0.5			10	0	5	20	Frequency

Supp. Table 4. **Summary of changes to the field stimulation pipeline between jGCaMP7 publication[1] and current manuscript:**

	jGCaMP7 publication[1]	Current publication	Reason for change
Cell segmentation algorithm	Script with manually set thresholds	Cell segmentation with Ilastik[2], a machine learning-based algorithm	Better separation of somata from neuropil
Source of neurons	Neonatal rat hippocampus	Neonatal rat cortex	More neurons can be harvested from the cortex, enabling higher throughput
Imaging speed	35 Hz	200 Hz	Focus on kinetics in current publication
Quality control	Wells with low expression manually removed from analysis	Wells with low expression manually removed from analysis; dim outlier cells automatically excluded	Dim cells (F_0 near background) produce artificially high $\Delta F/F_0$ signal

1. Dana, H. *et al.* High-performance calcium sensors for imaging activity in neuronal populations and microcompartments. *Nat Methods* **16**, 649–657 (2019).
2. Berg, S. *et al.* ilastik: interactive machine learning for (bio)image analysis. *Nat Methods* **16**, 1226–1232 (2019).