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Supporting Information

Monitoring Fluorescent Calcium Signals in Neural Cells with Organic Photodetectors

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Materials and Methods

Experimental Section

Tissue Slice Preparation. Wild type and transgenic mice anaesthetized with isoflurane were decapitated; the brain was removed from the skull rapidly and placed in the ice-cold artificial cerebrospinal fluid (ACSF). The ACSF solution composed of (in mmol/L): NaCl 126, KCl 3.50, NaH_2PO_4 1.25, $NaHCO_3$ 25, $CaCl_2$ 2.00, $MgCl_2$ 1.30, and dextrose 5, pH 7.4. ACSF was aerated with 95% O2/5% CO2 gas mixture. Saggital slices (350 µm) were cut using a tissue slicer (Leica VT 1200s, Leica Microsystem, Germany). During slicing, brain slices were submerged in an ice-cold (< 6°C) solution consisting of (in mmol/L): K-gluconate 140, HEPES 10, Na-gluconate 15, EGTA 0.2, NaCl 4, pH adjusted to 7.2 with KOH. Slices were immediately transferred to a multi-section, dual-side perfusion holding chamber with constantly circulating ACSF and allowed to recover for 2h at room temperature (22°C – 24°C).

Synaptic Stimulation and Field Potential Recording. Slices were transferred to a recording chamber continuously superfused (5ml/min) with ACSF (30°C) with access to both slice sides. Schaffer collateral/commissures were stimulated using the DS2A isolated stimulator (Digitimer Ltd, UK) with a bipolar metal electrode. Stimulus current was adjusted using single pulses (40-170 µA, 200µs, 0.15 Hz) to induce LFP of about 50% maximal amplitude. LFPs were

recorded using glass microelectrodes filled with ASCF, placed in stratum pyramidale of CA1 area and connected to an EXT-02F amplifier (npi electronic GMbh, Germany). Synaptic stimulation consisting of a stimulus train (200μs pulses) at 10 Hz and 100 Hz, lasting 20 s and 1 s, were used to induce changes in the intracellular Ca^{2+}. To induce epileptiform activity a Kv1 channel blocker 4-aminopyridine (50 μM) was added to ACSF.

Signal-to-noise ratio. Signal's power spectral density (PSD) was estimated using Fourier analysis. For the PSD calculation, a Kaiser window with $\beta = 38$ was used and due to having a real-value signal, the PSD was one-sided. The SNR was calculated as the ratio of the magnitude of summed square to noise.

Data analysis. Data analysis was performed using Matlab 2017a.

Frequency response. The dynamic response of the photodiodes was measured in an electrically and optically shielded box with a transimpedance amplifier (FEMTO DHPCA-100) connected to a digital oscilloscope (Tektronix MDO3034). A green pulsed light was applied by a high-power LED (OSRAM Oslon1) controlled by a signal waveform generator (TGA 1242) to create a sinusoidal wave with frequencies ranging from 1 Hz to 1 MHz. The amplitude of the photocurrent was recorded over the excitation frequency.

OPD Characterization

1. Noise current spectral density characterization

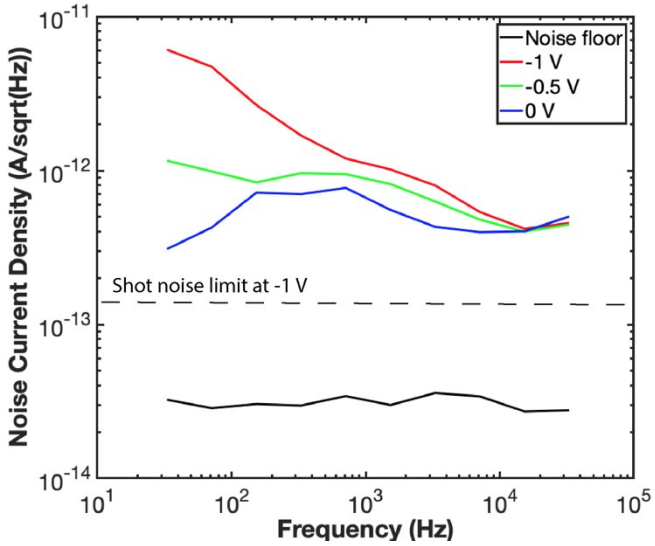

Figure SI.1. Noise current spectral density characterization of the OPD at 0, 0.5 and -1 V and dark noise characterization. The device presented an ultra-low spectral dark noise.

2. OPD Detectivity

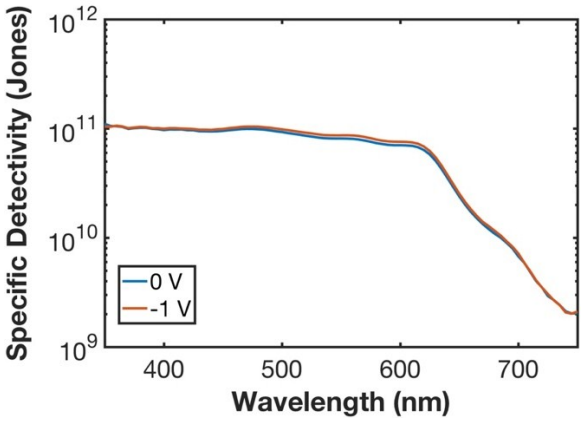

Figure SI.2. OPD detectivity curve. For detectivity measurements the noise current density was measured with a transimpedance amplifier (FEMTO PHPCA-100) and a lock-in amplifier from 30 Hz to 30 kHz in an optically and electrically shielded box. The specific detectivity was then calculated according to:

$$D^* = \frac{\sqrt{A_D}}{NEP} = \frac{R_\lambda \cdot \sqrt{A_D}}{I_n} \quad (2)$$

Here A_D is the active area of the photodiode, *NEP* the Noise equivalent power, R_λ the spectral response and I_n the noise current density.

3. OPD Baseline during recording:

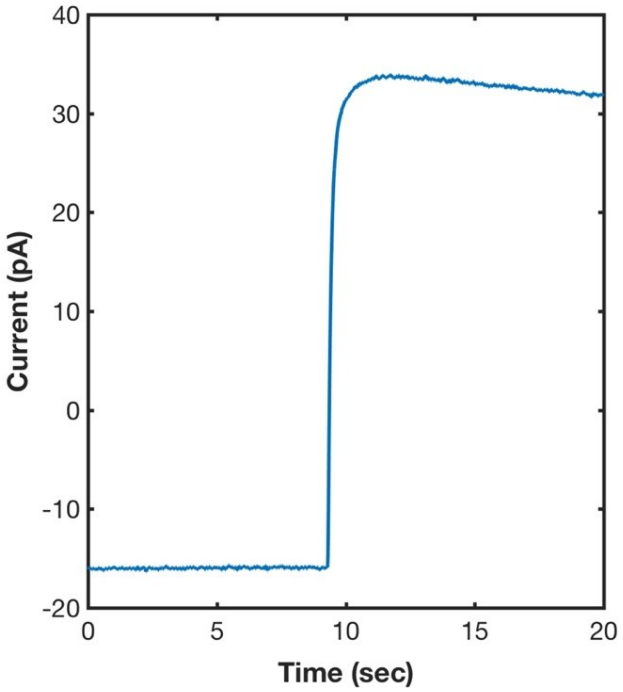

Figure SI.3. Baseline during electrophysiological recording. The PA2000 pico-ampere limit in quantifying current is 1 pA. The baseline value from 0 to ~10 s is the device's dark-current, which is showed as negative value. The negative value means that it is sub-pA, approximately between 900 to 1000 fA. The vertical part of the signal shows the switch-on of the excitation light. The last part of the signal from ~10 s to 20 s shows the baseline during illumination. This figure shows the device's stability during recording.

4. Signal-to-noise ratio

Figure SI.4 to Figure SI.7 show the corresponding power spectrum density of the recorded calcium signals in hippocampal slices. These figures illustrate the frequency components of the signals.

In general, when the amplitude of a signal increases, assuming the same noise level, the SNR increases as well. The comparison of the OPD's calcium signals in Figure 3f and Figure 4b shows that although the amplitude increases, the SNR does not change drastically (~3 dB) (Figure SI.3, Figure SI.4). This is because, during the 10 Hz electrical stimulation, there are some fluctuations in the calcium signal of Figure 4b. Furthermore, the comparison of the trend of the other calcium signals (Figure SI.3 to Figure SI.6) illustrates that by the increase (Figure SI.4 to Figure SI.5) and decrease (Figure SI.5 to Figure SI.6) of the signal's amplitude, the SNR increases and decreases accordingly.

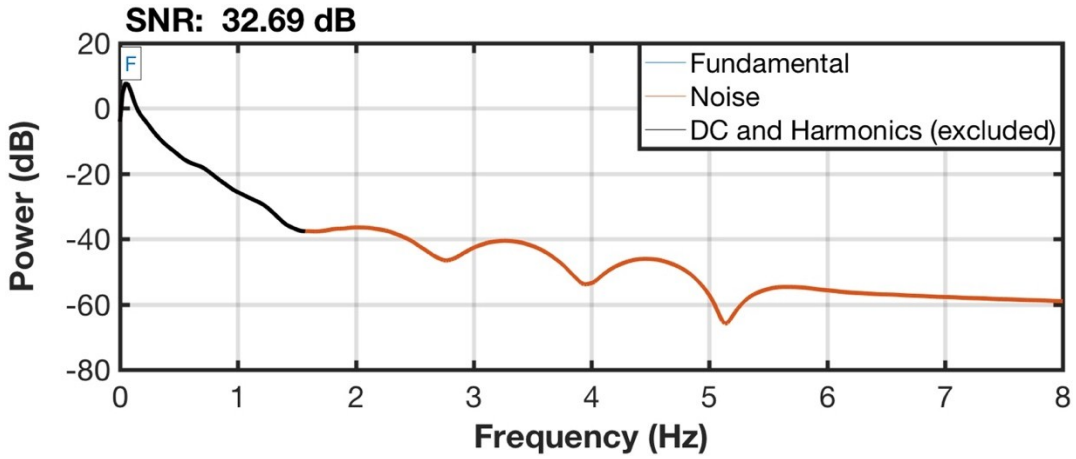

Figure SI.4. Frequency spectrum of calcium signal, reported by Fluo-4, induced by 10 Hz stimulation, with maximum fluorescent change of 8 % (Figure 3.f).

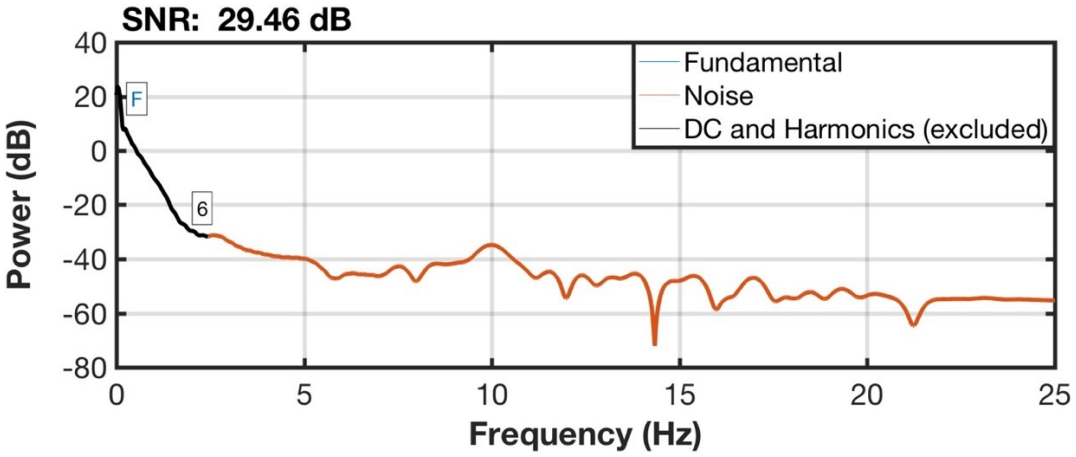

Figure SI.5. Frequency spectrum of calcium signal reported by GCaMP6f, induced by 10 Hz stimulation, with maximum fluorescent change of 23 % (Figure 4.b).

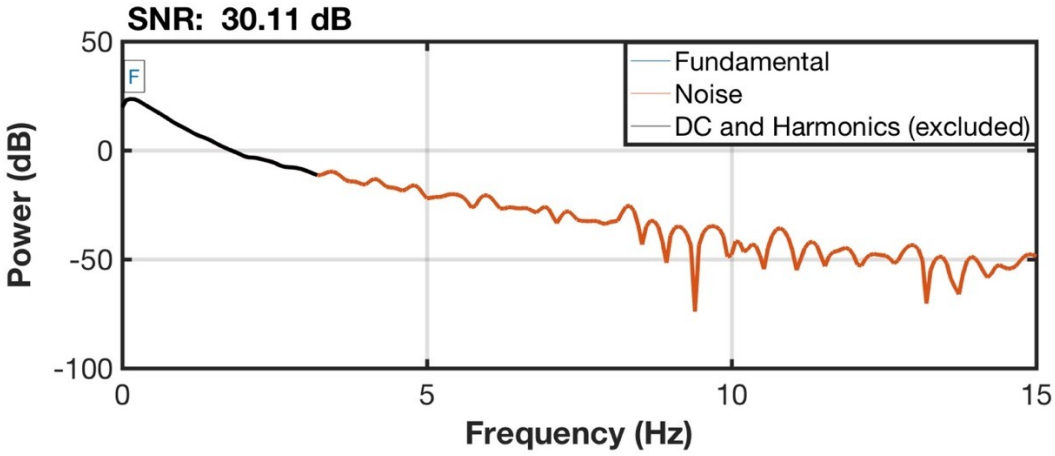

Figure SI.6. Frequency spectrum of calcium signal reported by GCaMP6f, induced by 100 Hz stimulation, with maximum fluorescent change of 80 % (Figure 4.c).

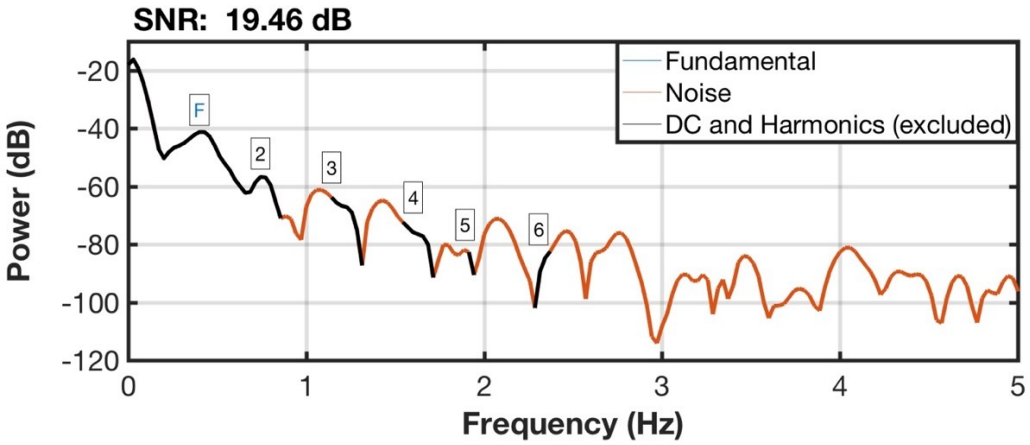

Figure SI.7. Frequency spectrum of spontaneously induced calcium signal reported by GCaMP6f, with maximum fluorescent change of 6 % (Figure 4.d).