Coherent Control of an Opsin in Living Brain Tissue

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**Supplementary Figure 1 | Schematic of the custom-designed femtosecond laser setup used for light-evoked current measurement.**

**a.** Schematic for femtosecond pulse shaping setup. Inside the pulse shaper unit, the pulse is separated spectrally and spatially with a diffraction grating (DG) and the individual spectral components are controlled independently using a polynomial function. **b.** Spectrum of the Ytterbium laser output at 600 mW power, λ<sub>central</sub> = 1040 nm. **c.** Fiber supercontinuum output provides 380 mW power at 600 mW laser pumping power. Note that the supercontinuum was spectrally filtered by the pulse shaper. Shaded region (960-1060 nm) is the input spectrum to the SLM in the pulse shaper.
Supplementary Figure 2 | Responses from a ChR2-expressing pyramidal neuron with one-photon excitation. a. Spike response of a pyramidal neuron to step current pulses of -100, +40 and +160 pA. b. A similar spike response is observed in response to single-photon light stimulation from a 470 nm LED source with optical power densities of 0.6 mW/mm². c. Current clamp response of the neuron in the presence of TTX (0.5 μM). d. Voltage clamp response to 1200 ms single-photon light excitation at different intensities (0.0, 1.0 and 5.2 mW/mm²) show ChR2 mediated currents in the presence of TTX (0.5 μM).
Supplementary Figure 3 | Population data of neurons show no apparent thermal damage with 1200 ms laser exposure. The peak current significantly increased after phase compensation ($a_2 = 0$ fs$^2$) ($n = 6$), compared to when pulses with uncompensated phase (Uncomp) were used. For current measurements with phase compensated pulse, $a_2$ was varied in the following order: $a_2$ (in fs$^2$) = 0, -4000 or +4000, 0 (recovery). For all measurements the laser was ON for 1200 ms. The recovery was close to 100%, meaning that we obtained very similar values for both $a_2 = 0$ fs$^2$ values. This would not have been possible if the cell experienced any damage due to excessive heating due to long laser exposure. Data is represented as mean ± s.e.m.
**Supplementary Figure 4 | Phase compensation altered steady state current as well as activation rate.** a. At same average laser power, phase compensation significantly increased the steady state current evoked from pyramidal neurons; Uncompensated: 15±3 pA, Compensated: 21±3 pA, n = 6, p<0.005. All currents are negative inward currents. b. Phase compensation increased the initial activation slope of the evoked current response; Uncompensated: 3.3±0.5 pA/ms, Compensated: 4.8±0.6 pA/ms, n = 6, p<0.05. Data is represented as mean ± s.e.m. This is indicative of more efficient (faster) activation with compensated pulses.
Supplementary Figure 5 | Steady state current as well as activation rates are modulated differently by PCH pulse and NCH pulse. a. Steady state current amplitudes for compensated and PCH and NCH pulses at different a2 values. Phase modulation significantly altered steady state evoked current for a2 = 4000: a2 = +4000 fs²: -15±2 pA, a2 = -4000 fs²: -10±1 pA (n = 6, p<0.05). Moreover, steady state currents evoked by both PCH and NCH pulses are significantly reduced compared to the compensated TL pulse. b. The initial activation slope of the evoked current response with PCH is significantly different from that with NCH at a2 = 4000; a2 = +4000 fs²: 3.2±0.4 pA/ms, a2 = -4000 fs²: 1.9±0.3 pA/ms (n = 6, p<0.05). This is indicative of more efficient (faster) activation with PCH. Note that the ratio of steady state current to peak current remained unaffected through phase compensation and chirp modulation (both PCH and NCH) of the light pulse. The population average for this ratio is 0.48±0.01 (n = 54). Data is represented as mean ± s.e.m.
Supplementary Figure 6 | Ultrashort NIR light pulse evoked peak current measured at two different average laser powers. Light evoked peak current was measured from three different pyramidal neurons, each with two average powers and at a2 = 0, +4000 and -4000 fs². All three cells showed an increase in response at higher power. Percent increase from 75% power to 100% power at different a2 values were as follows: a2 = 0 fs²: 37±3%, a2 = +4000 fs²: 37±10%, a2 = -4000 fs²: 35±9% (n = 3). Data is represented as mean ± s.e.m. Similar percentage increase in current output at all chirp values showed that the cell was not stimulated at saturation laser power. However, there was only a 37% increase in current instead of 77% predicted using the TPA power square law, which indicated that we are not too far from saturation.
Supplementary Figure 7 | Comparison between evoked currents with PCH pulse, NCH pulse and pure TPA at an equivalent power. a. Intensity profiles for pulses at different a2 values indicate that with increasing a2 the pulse broadened considerably and the peak pulse intensity got reduced. a.inset (top). The pulse envelope with a2 = +2000 fs² and +4000 fs² are replotted to show the combed structure. a.inset (left). Normalized SHG intensity, which is proportional to the peak intensity of the pulse, and pulse width are plotted as a function of linear chip parameter, a2. b. Normalized evoked-current values for PCH (red) and NCH (blue) pulses (relative to a2 = 0 fs²) were plotted with normalized average of theoretically calculated and experimentally estimated TPA-probability (proportional to TPA-only current, green). As the pulse broadened and the peak pulse intensity reduced with increasing a2, the TPA-only current falls sharply (TPA-only current ∝ TPA ∝ Intensity_{peak}² · τ_{pulse}). It is worth noting that with linear chirp with higher values of a2, the experimentally measured evoked current did not decrease as significantly. This indicates that chirped pulses may enable more effective stimulation compared to an equivalent pulse of same pulse width. Data is represented as mean ± s.e.m.
Supplementary Discussion 1 | Pulse-width and peak current dependence of two-photon excitation

At constant pulse energy, changing pulse shaping parameters from $a_2 = 0$ to $a_2 = \pm 4000 \text{ fs}^2$ caused the pulse peak power to reduce by a factor of 10, and the pulse width to increase by a factor of 10 (Supplementary Fig. 7a). This understandably led to a variation of about a factor of 10 in the SHG signal (Eq. 5). If the evoked neuronal output is proportional to the two-photon absorption (TPA), we would expect the current to be also reduced by a factor of 10 at $a_2 = \pm 4000 \text{ fs}^2$ (Supplementary Fig. 7b). This would occur for an equivalent Gaussian pulse without chirp. However, that is not what we observed; the evoked current values were higher (Figure 2). One reason for this could be a change in the temporal pulse profile. Two-photon absorption depends on the temporal profile of the pulse, which varied greatly as linear chirp was introduced (Supplementary Fig. 7b). Another reason could be the use of laser power that is close to saturation. For different cells, we used 13-19 mW average laser power for TPA, always with a $\lambda_{\text{central}}$ of 1010 nm. We observed an increase in current output going from 75% to 100% average power (Supplementary Fig. 6), even though the percentage increase was less than what would be expected for an intensity-squared dependence. This led us to believe that either the peak current with chirped pulses behaved differently from a pure TPA process, or we are observing this because we are close to saturation for some of our measurements, although not at saturation.

We looked into existing TPA data with ChR2 for comparison with our data, but we did not find any that used chirped pulses or light at a 1010 nm central wavelength. Prakash et al 45 showed that at $\lambda_{\text{central}} = 940 \text{ nm}$, 20 mW average laser power could be close to saturation for ChR2 variants, which are different from what was cloned in the mouse line we used for our experiments. In contrast, Rickgauer and Tank 46 showed that integrated current from ChR2 expressed in HEK293 cells increased as expected when average power for TPA was raised from 20 mW to 40 mW at $\lambda_{\text{central}} = 920 \text{ nm}$. This indicated that 20 mW average power did not cause saturation of two-photon excitation in their experiment. It is noteworthy that due to the quadratic power dependence of two-photon excitation, average power at which the saturation is attained for a specific opsin (or any other molecule) depends on several experimental parameters. This list includes: the spectrum of the laser used for the experiment, laser alignment efficiency, laser pulse width and repetition rate, microscope objective used for the experiment, sample positioning with respect to the laser focal plane, sample thickness, and the cell/tissue microenvironment that could cause defocusing due to scattering of light. Therefore, 20 mW average laser power will not always put ChR2 at saturation. Additionally, coherent control of opsins at different field strengths2-4,34 was studied using single-photon excitation. The fluence values reported in these articles are comparable to what we used. Given that for the ChR2 system, the ratio of $\sigma_1(470\text{nm})/\sigma_2(920\text{nm})$ is $\sim 10^{31} 10,46$, we concluded that the fluence we used for two-photon excitation did not put this experiment at saturation.
Supplementary Discussion 2: How a change in linear chirp may tune spiking of FS neurons

We observed that changing linear chirp tuned spiking patterns evoked from FS neurons (Figure 3). These experiments were performed under current clamp conditions, which is more physiological compared to voltage clamp conditions. The cell under observation received a step current input (130 pA, Figure 3) and a 1200 ms long exposure of the two-photon pulsed light stimulation with different values of linear chirp (a2 = 0, -500, -4000 fs²). Since the voltage was not clamped, various known subthreshold ionic conductances (gA, gT, gM, gH etc.) were free to traverse their activation/inactivation ranges, contributing to the subthreshold dynamics that lead to spike activation. Some of these subthreshold conductances may have slower kinetics of activation that build up over time (e.g., gKCa: calcium dependent potassium conductance) and can affect the subthreshold dynamics in a delayed manner and over a longer period of time. Similarly, the occurrence of spikes give rise to different kinetics for gAHP: after-hyperpolarization conductance.

The spike firing pattern over a period of time depends on the subtle interplay of various subthreshold ionic conductances with different slow and fast kinetics. In addition, the initial activation of one or more of these conductances is affected by the linear chirp (from Ohm’s law, in the current clamp condition, different chirp value will affect the membrane voltage). Therefore, we expected that the spike firing pattern would be altered in a fairly repeatable manner with changing linear chirp, and this is what we have observed (Figure 3).

Additionally, from a nonlinear dynamical systems perspective, varying the linear chirp value (a2 = 0, -4000, -500 fs²) constitutes a slightly different initial condition that will affect the activation and interplay of inherent subthreshold ionic conductances, which in turn determines the spike firing pattern over a period of time.

References
