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Supporting Material

Imaging Inhibitory Synaptic Potentials Using Voltage Sensitive Dyes

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Improved sensitivity of voltage signals with 532 nm laser excitation

A critical step in the improvement in sensitivity (expressed as the signal-to-noise ratio, S/N) of the voltage imaging technique, which was mandatory for detection of membrane potential changes as small as 1 mV, was the replacement of the broad band excitation from a xenon arc-lamp with the 532 nm monochromatic excitation from a diode-pumped solid state (DPSS) laser (model MLL532, 300 mW, CNI, Changchun, China) in the epi-illumination wide-field microscopy mode. The relative fluorescence change in response to a given membrane potential change is the function of the excitation wavelength and is maximal at the wings of the absorption spectrum (1,2). Furthermore, a monochromatic illumination at a near-optimal wavelength is more efficient than any collection of wavelengths from an arc-lamp. In the case of JPW-1114, using the bandwidth of wavelengths selected from the output of 150 W xenon arc lamp by a 525±25 nm interference filter (3,4), fluorescence originates mostly from excitation at the shortest wavelengths, closer to the peak of the absorption spectrum. This part of the absorption spectrum is significantly less sensitive to membrane potential changes compared to the single wavelength excitation at 532 nm. The fractional change of fluorescence (ΔF/F) associated with a given membrane potential change for the two different excitations was measured in the experiment shown in Fig. S1A. In this experiment, one action potential of ~100 mV was evoked by current injection while measuring ΔF/F over the exact same region of a CA1 hippocampal pyramidal neuron apical dendrite either:

1. using 532 nm excitation (12.5% of the full power) or
2. using a 150 W arc lamp and a 525±25 nm interference filter (full light and average of 4 trials to obtain a comparable shot noise).

In this experiment, the peak ΔF/F signal was 6.24% at 532 nm excitation and 2.51% at 525±25 nm. This test illustrates the dramatic improvement in the sensitivity of the dye (~2.5 times) obtained with the laser excitation. The improvement in the sensitivity of the dye translates directly into the increase in the S/N without the need for potentially harmful increase in light intensity. An additional improvement in the S/N can be achieved simply by increasing the excitation, and hence the emission light intensity. The extent of this improvement is limited by the potential photodynamic damage which is a function of light intensity but also depends on preparation and the exposure duration. The exposure duration will clearly be determined by the phenomenon under study.

Evaluation of photodynamic damage with 532 nm laser excitation

Several studies using the filtered light from a lamp have reported that a large number of repetitive short exposures, with sufficient time between consecutive exposures, did not produce significant photodynamic damage (3,5,6). Because the intensity of the excitation light using a 300 mW 532 nm laser is more than 10 times higher, the number and the duration of the exposures before a photo-dynamic damage is detected are reduced. To preserve the integrity of the preparation, one can reduce the intensity of the excitation (using neutral density filters) and/or the exposure time.

To check the physiological integrity of a neuron, the monitoring of the kinetics of an action potential (if available) is ideal (5,6). In the representative example shown in Fig. S1B, an action potential signal, evoked by electrical stimulation, was measured from the apical dendrite immediately after the loading procedure and after the cell was exposed 33 times for 100 ms to 12.5% of the full light and for 4 times for 50 ms to 25% of the full light. The interval between two consecutive exposures was 30-60 s. As shown in the figure, the change in kinetics of the action potential was undetectable after these 37 recording trials. The limit to photo-toxicity may vary from experiment to experiment. We found that CA1 hippocampal pyramidal neurons are typically resistant to at least 40 exposures of 100 ms at 6% or 12.5% light, 10 exposures of 50 ms at 25% of the light, or 5 exposures of 25 ms at full light. Thus, careful use of a 300 mW, 532 nm laser allows investigating electrical signaling in neurons with negligible perturbation of physiological integrity.

References of the Supporting material

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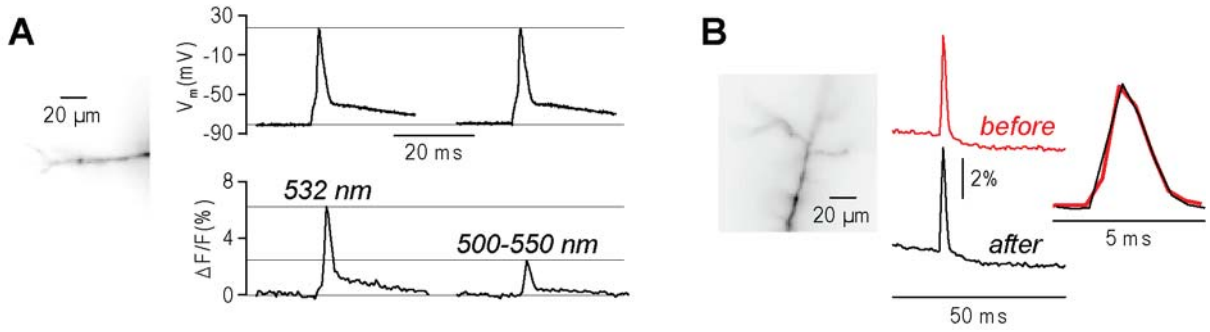

Figure S1. Sensitivity of the dye and the extent of photodynamic damage during optical recordings with a 300 mW – 532 nm laser. (**A**) Left: Dendritic region of a CA1 hippocampal pyramidal neuron in recording position (~125 µm X 125 µm, apical dendrite). Right: Action potentials evoked by current injection during a patch recording (top) and associated ΔF/F signals (bottom) using 532 nm laser excitation or 525±25 nm light from a 150 W xenon arc-lamp. (**B**) Left: Dendritic region of a CA1 hippocampal pyramidal neuron in recording position (~125 µm X 125 µm, apical dendrite). Right: Optical recordings of an action potential evoked by electrical stimulation before (red) and after (black) 33 exposures of 100 ms at 12.5% of the full light and 4 exposures of 50 ms at 25% of the full light. Left: same signals on an expanded time scale.