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Title: Circuit analysis using optical stimulation in ChR2 transgenic mice
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Channelrhodopsin-2 (ChR2) is a cation channel that is rapidly activated by light and has been used to photostimulate cultured neurons (Nature Neurosci. 8: 1263). We now generated ChR2 transgenic mice to allow photostimulation of neurons in more intact conditions. To test the efficacy of ChR2, *in vitro* whole-cell patch-clamp recording was used to measure responses elicited by illumination (465-495 nm) in cortical slices. In voltage-clamped neurons, illumination of ChR2-positive neurons produced inward photocurrents at holding potentials of -70 mV. No currents were generated in response to room light or to illumination with wavelengths outside the action spectrum of ChR2. In controls, no currents were generated by illumination of slices from wild-type mice that did not express ChR2. The amplitude of ChR2-dependent photocurrents increased with increases in light intensity or duration. With prolonged illumination, the photocurrent gradually inactivated with a time constant of 14.1 ± 0.1 ms. In current-clamp recordings, illumination generated action potentials. The time from the light onset to the peak of the first spike was 6.1 ± 0.3 ms, which was limited, in part, by the rise time of the light pulse. The number of action potentials increased with larger or longer light pulses. During trains of brief light pulses, action potentials could follow photostimuli up to 30 Hz. To determine whether this photostimulation method can be used to analyze circuitry, we used brief light flashes centered on the intermediate layer to activate neurons in the superior colliculus. Outward currents were completely blocked after the addition of GABAzine, indicating they were GABA_A receptor mediated IPSCs. In current clamp, the depolarization induced by blocking the IPSCs generated action potentials. We conclude that ChR2 offers good spatial and temporal resolution and is an effective means of using genetic targeting to analyze neural circuits.

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