Biophysical Journal, Volume 96

Supporting Material

Characterization of Engineered Channelrhodopsin Variants with Improved Properties and Kinetics

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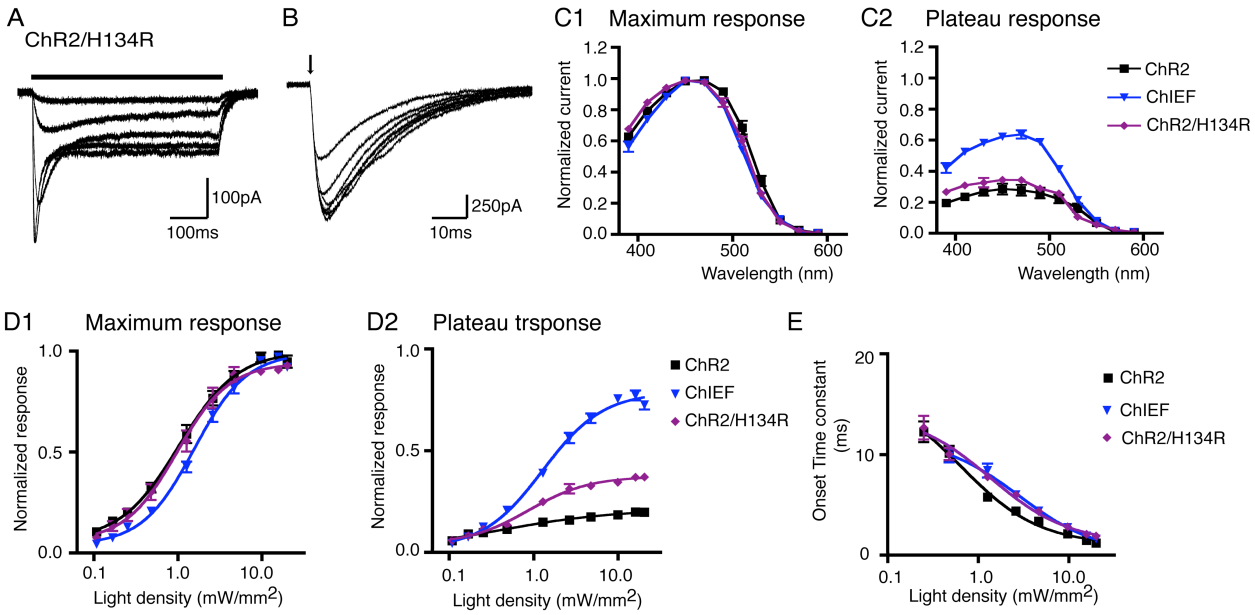
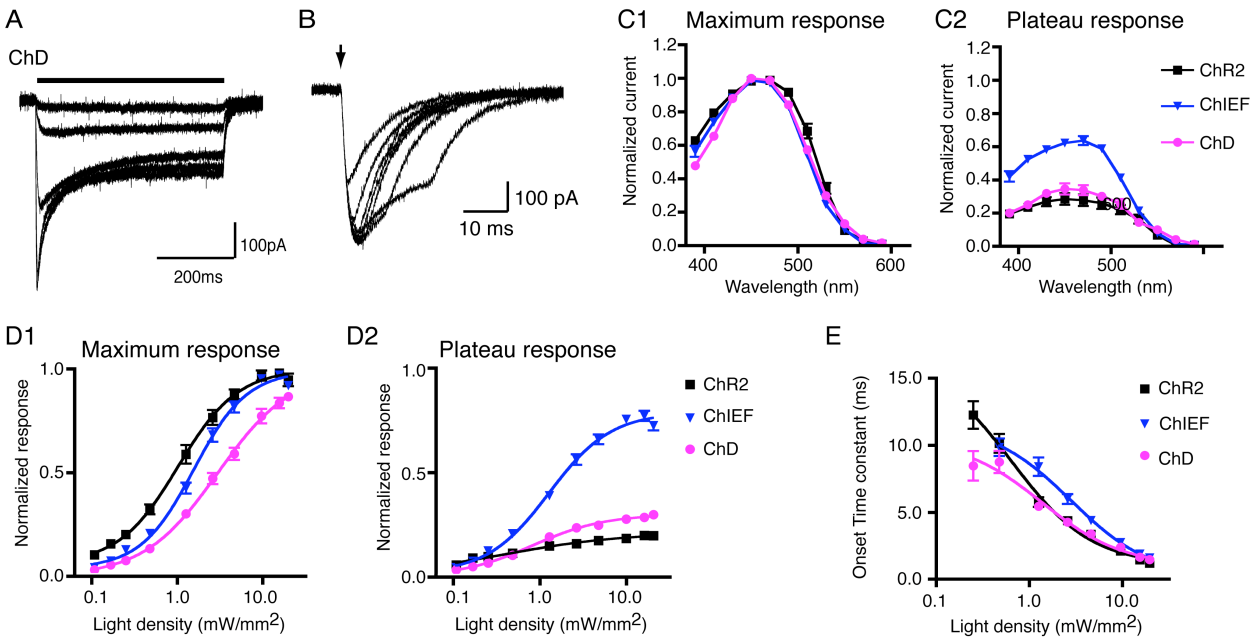
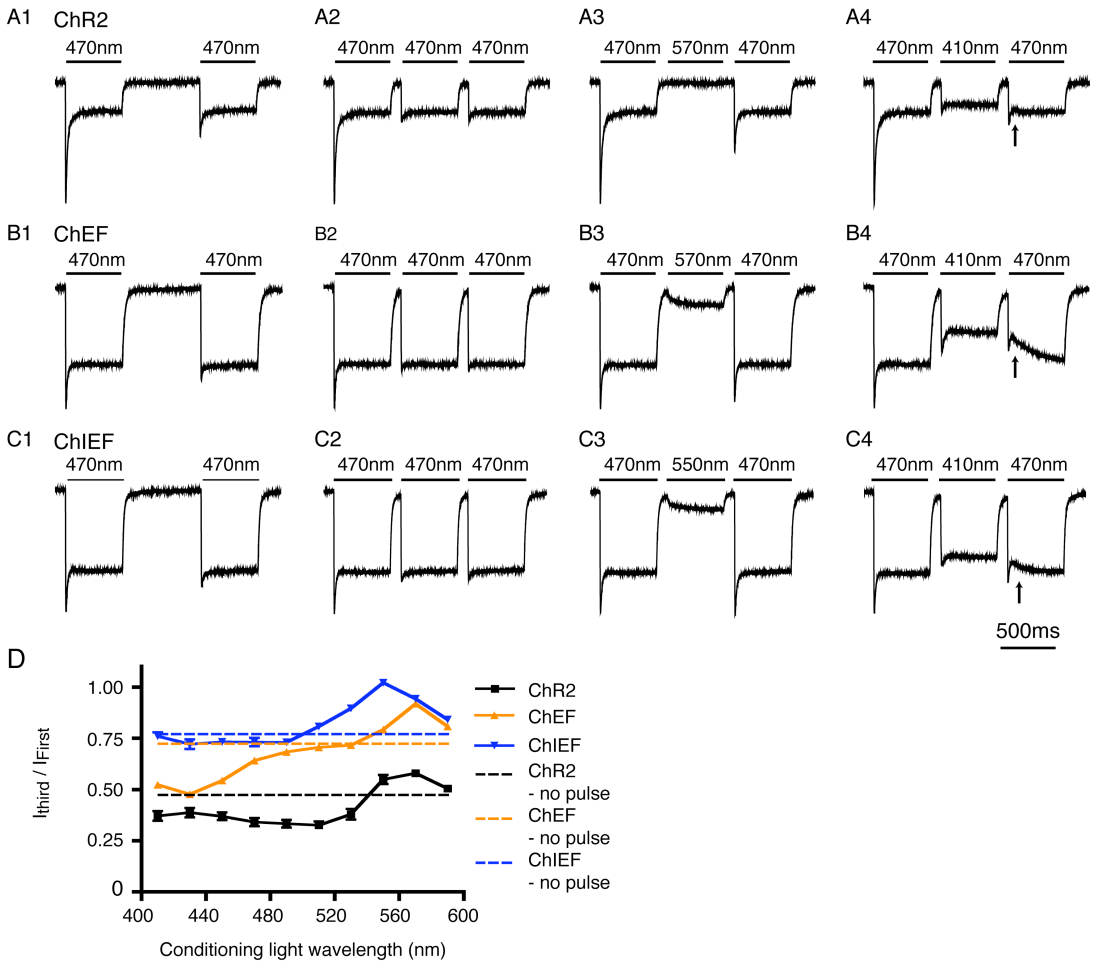


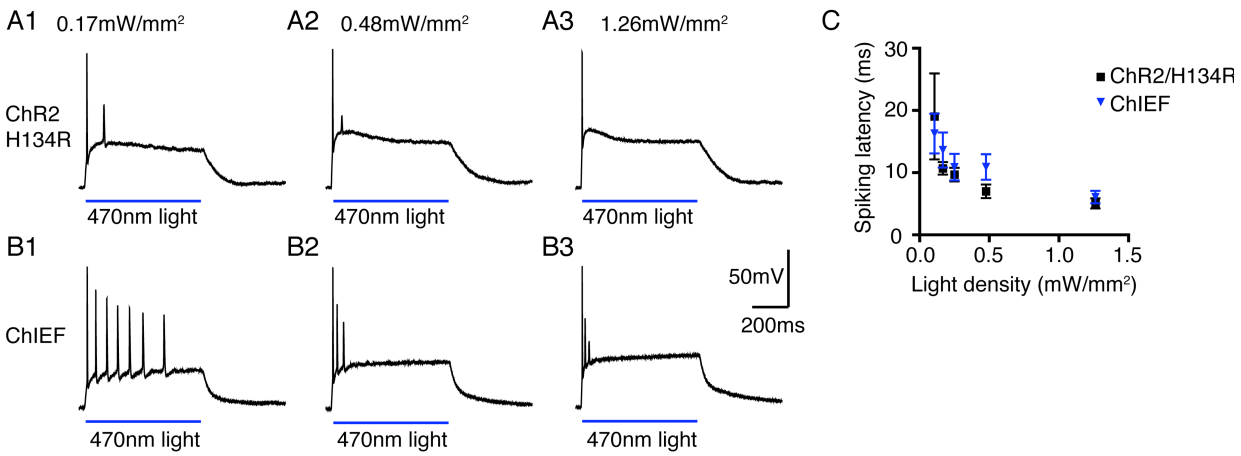
Supplementary Figure 1. Characterization of ChR2 with H134R mutation (ChR2/H134R) and the comparison to ChR2 and ChIEF. (*A*) Example of ChR2 with H134R mutation to 0.11, 0.48, 2.59, 9.64 and 19.81 mW/mm^2 of 470nm of light. (*B*) The spectral response of ChR2/H134R (n=5), ChR2 and ChIEF channelrhodopsins. (*C*) The intensity-amplitude relationship of H134R mutant (n=6) compared to ChR2 (*black*) and ChIEF (*blue*). (*D*) The intensity-onset time constant relationship of ChR2 with H134R (n=6).



Supplementary Figure 2. Characterization of ChD mutant and the comparison to ChR2 and ChIEF. (*A*) Example of ChD mutation to 0.11, 0.48, 2.59, 9.64 and 19.81 mW/mm^2 of 470nm of light. (*B*) The spectral response of ChR2, ChIEF and ChD (n=3) channelrhodopsin variants, all response normalized to the maximum response obtained from the cell tested at the various wavelengths. (*C*) The intensity-amplitude relationship of ChD mutant (n=5) compared to ChR2 (*black*) and ChIEF (*blue*). (*D*) The intensity-onset time constant relationship of ChR2 with ChD (n=5).



Supplementary Figure 3. Effects of 'conditioning' light on the subsequent responses of ChR2, ChEF and ChIEF. (*A*) The effects of conditioning light on the response of ChR2, with enhanced response by 570nm conditioning light *(A3*) and appearance of a small slow component with 410nm conditioning light (*arrowed*; *A4*). (*B*) The effects of conditioning light on the response of ChEF, with enhanced response by 570nm light (*B3*) and appearance of an obvious slow component with 410nm light (*arrow*; *B4*). (*C*) The effects of conditioning light on the response of ChIEF, with enhanced response by 550nm light (*C3*) and appearance of a slow component with 410nm light (arrow; *C4*). (*D*) Summary of ChR2, ChEF and ChIEF responses to different 'conditioning' wavelength before the third light stimulation at 470nm. 570nm of light enhanced the recovery of ChEF and ChR2, whereas 550nm light enhanced the recovery of ChIEF. Dotted lines indicate the mean value when no light stimulation was present for the same time course (ChR2, n=9; ChEF, n=7; ChIEF, n=9).



Supplementary Figure 4. The responses of ChR2/H134R (*A*) and ChIEF (*B*)-transfected cultured hippocampal neurons to continuous light pulse at different light intensity under current-clamp recording. The ChR2/H134R-transfected neurons showed initial strong depolarization and subsequent smaller depolarization due to the channel inactivation (*A1*, *A2*, *A3*) whereas ChIEF-transfected neurons showed an exponential-shaped charging of the membrane as expected from a more rectangular channelrhodopsin response (*B1*, *B2*, *B3*). (*C*) The latency of first successful action potential triggered at different light intensity of ChR2/H134R and ChIEF-transfected neurons were not significantly different at the light intensities tested.