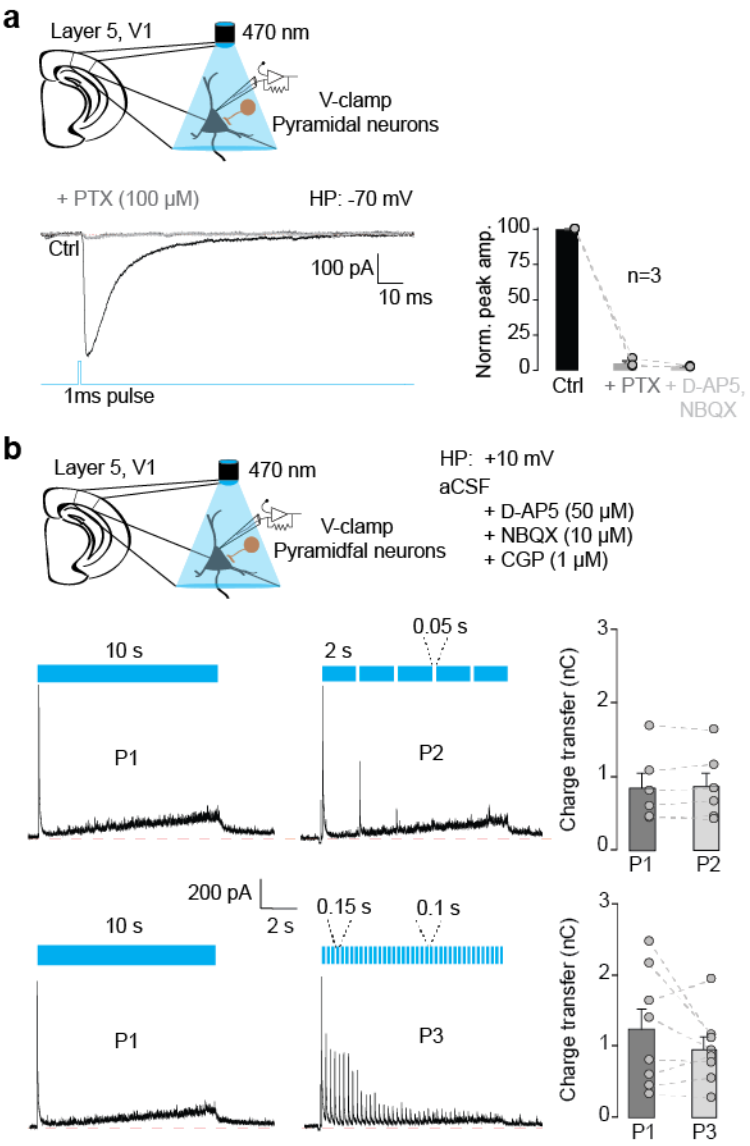
KCC2 overexpression prevents the paradoxical seizure-promoting action of somatic inhibition

SUPPLEMENTARY FIGURES / MOVIE

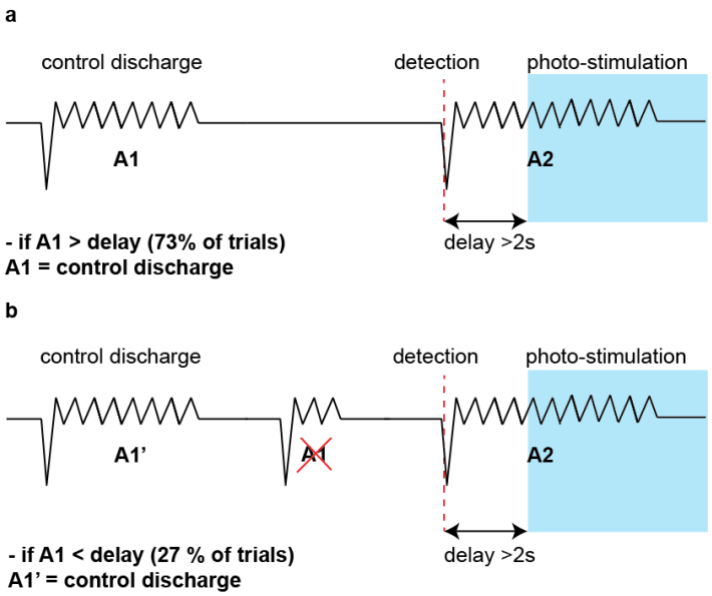


Supplementary Figure 1. Optogenetic activation of ChR2-expressing PV+ interneurons in the visual cortex V1 area.

(a) Blue light pulse-induced current responses in pyramidal layer 5 V1 neurons are abolished by the $GABA_A$ receptor antagonist picrotoxin (PTX; n = 3 cells).

(b) $GABA_A$ receptor-mediated currents in pyramidal neurons induced by 3 different optogenetic stimulation protocols (P1: continuous 10 s stimulation; P2: five 2-s-long pulses delivered with 50 ms intervals; P3: 10 s stimulation with 150 ms-long pulses separated by 100 ms intervals). Continuous photostimulation produces $GABA_A$ receptor-mediated charge transfer similar to that elicited by intermittent light pulses (P1 vs. P2: n = 5 cells; P1 vs P3: 8 cells; $p > 0.05$, paired t-test).

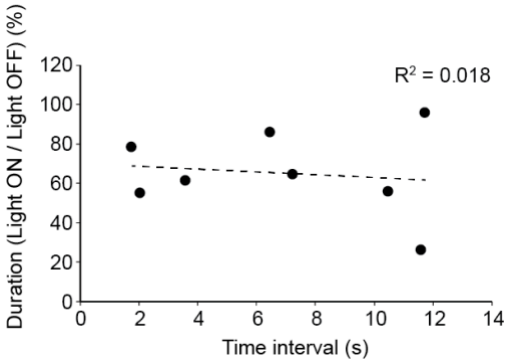
HP: holding potential.



Supplementary Figure 2. Analysis of delayed photostimulation.

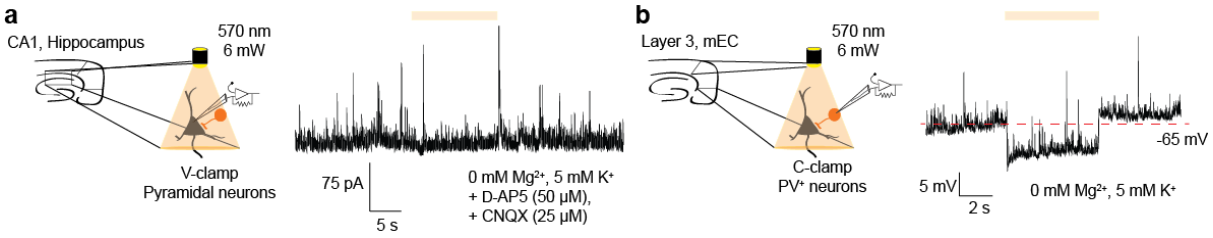
(a) In the majority of trials (73%), the duration of the photostimulated seizure was compared to that of the immediately preceding non-stimulated (control) discharge, if it was longer than the photostimulation delay.

(b) In 27 % of the trials, the control discharge was shorter than the photostimulation delay. In these cases, the nearest (within 30 s) preceding seizure of sufficient length was used for comparison.



Supplementary Figure 3. Seizure suppression by photo-depolarization of ChR2-expressing PV+ interneurons is independent of the time interval between seizures.

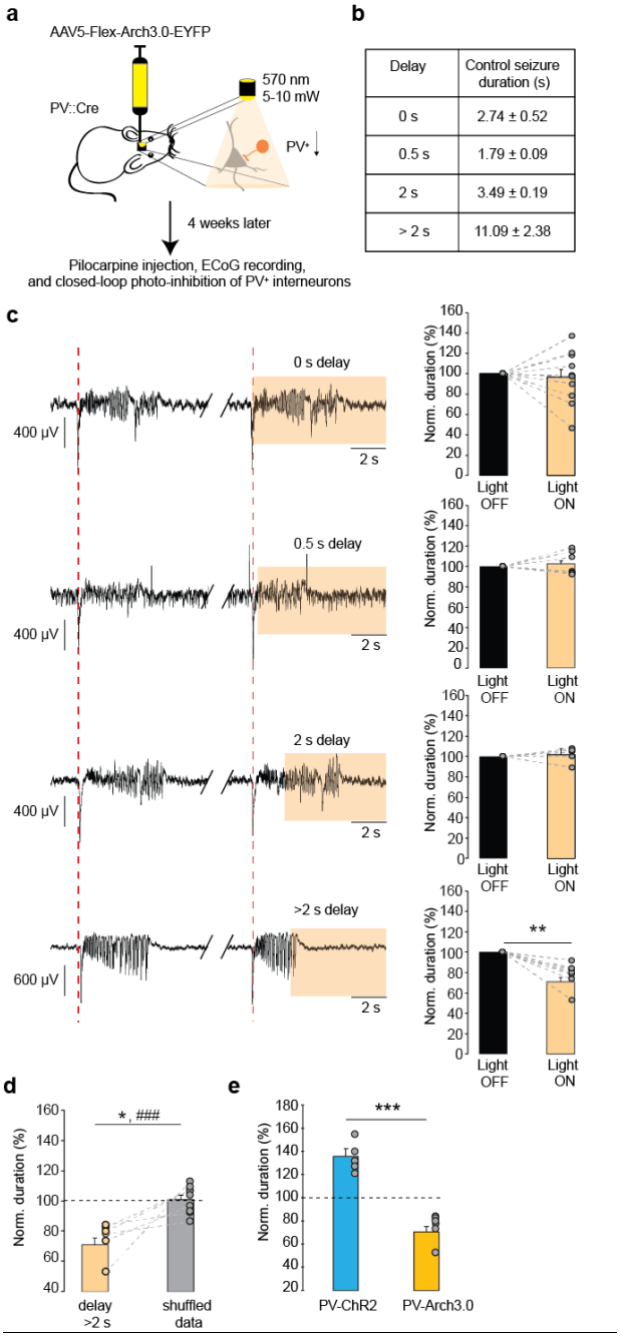
Data points represent individual animals.



Supplementary Figure 4. Optogenetic hyperpolarization of PV+ interneurons expressing Arch3.0.

(a) Continuous yellow illumination of a slice with Arch3.0-expressing PV+ cells reduces the frequency of spontaneous $GABA_A$ receptor-mediated postsynaptic currents recorded in a pyramidal neuron.

(b) Whole-cell current-clamp recording demonstrating photostimulation-induced hyperpolarization of a PV+ interneuron expressing Arch3.0.



Supplementary Figure 5. Photo-hyperpolarization of Arch3.0-expressing PV+ interneurons reduces the duration of electrographic seizures

(a) *In vivo* experimental schematic.

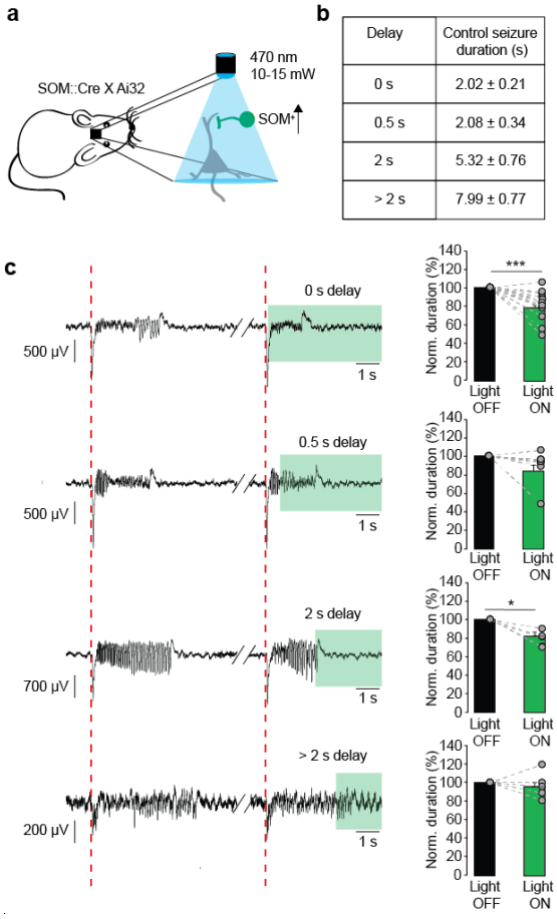
(b) Mean durations of control ictal discharges in experiments with different delays.

(c) Closed-loop photo-hyperpolarization of PV+ neurons reduces the duration of ictal discharges when delayed by more than 2 s (n = 11, 7, 5 and 6 mice for 0, 0.5, 2 and >2 s delays respectively). Sample traces illustrate pairs of consecutive seizures without and with photostimulation (intervening periods between seizures are omitted; yellow rectangles indicate photostimulation).

(d) The effect of delayed photoinhibition of PV+ interneurons compared to randomized reshuffled data analysis (* $p < 0.05$ paired t-test, n = 6; ### $p < 0.001$, unpaired t-test, n = 9, includes data from additional mice injected with the Arch3.0 construct).

(e) Summary graph comparing the effects of delayed photo-depolarization (PV-ChR2, *blue*, n = 5) and photo-hyperpolarization (PV-Arch3.0, *yellow*, n = 7) of PV+ interneurons, unpaired t-test.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; error bars represent s.e.m.



Supplementary Figure 6. Closed-loop photostimulation of ChR2-expressing SOM+ interneurons has no seizure-promoting action.

(a) *In vivo* experimental schematic.

(b) Mean durations of control ictal discharges in experiments with different delays.

(c) Closed-loop photostimulation of SOM+ interneurons with 0 s (n = 13), 0.5 s (n = 8), 2 s (n = 5), >2 s (n = 6) delays. Sample traces illustrate pairs of consecutive seizures without and with photostimulation (intervening periods between seizures are omitted; green rectangles indicate photostimulation).

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; error bars represent s.e.m.

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Supplementary Movie 1. Simultaneous ECoG and video during local pilocarpine-induced seizure.