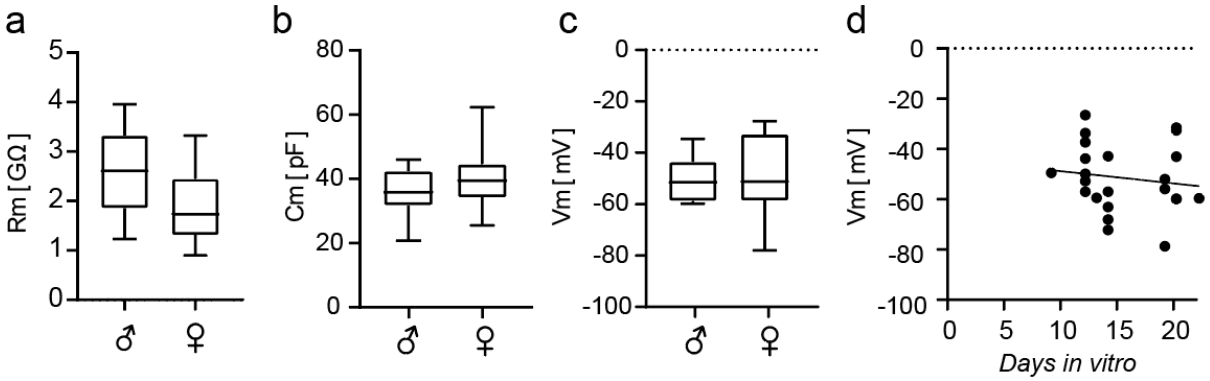
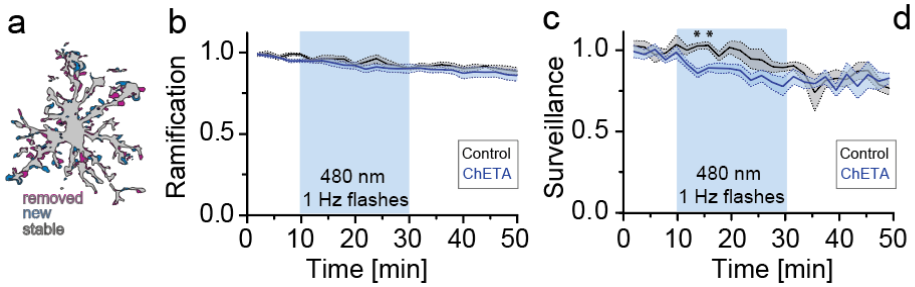
Supplementary Information



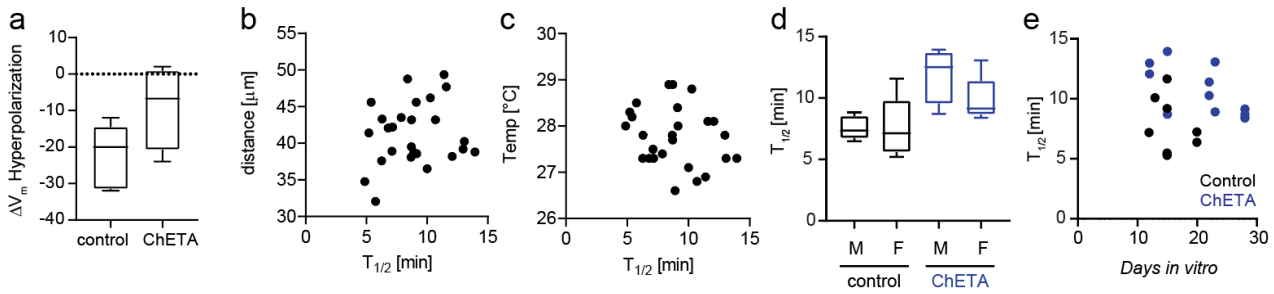
Additional file 1. Cell parameters in microglia of male and female mice show no differences in slice cultures. Duration of slice culture in vitro has no significant effect on microglia membrane potential. (a) Membrane resistance (n=14/9, male/female), (b) Membrane capacitance (n=9/12, male/female), (c) Membrane resting potential (n=12/8, male/female), (d) Membrane resting potential vs. days in vitro (n=23). Spearman's ρ = -0.24, P = 0.27, n = 23 experiments.



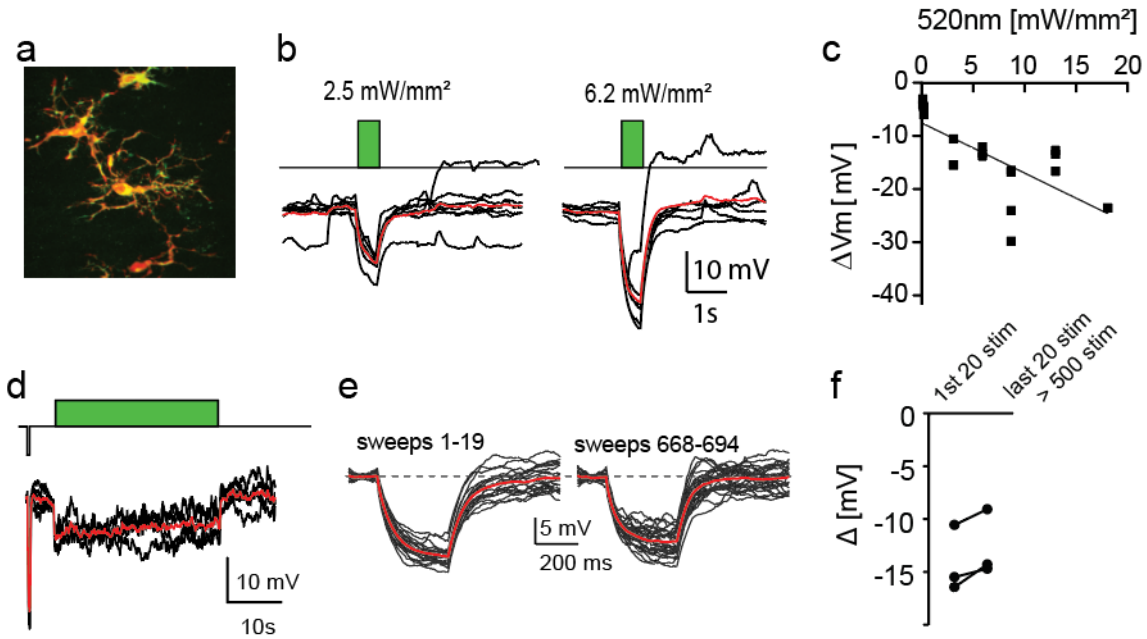
d

Ramification	control	ChETA
0-10 min	0.99±0.00	0.97±0.01
10-20 min	0.95±0.01	0.94±0.01
20-30 min	0.92±0.01	0.89±0.02
Surveillance		
0-10 min	1.00±0.01	0.97±0.01
10-20 min	1.01±0.01	0.89±0.01
20-30 min	0.84±0.04	0.81±0.03

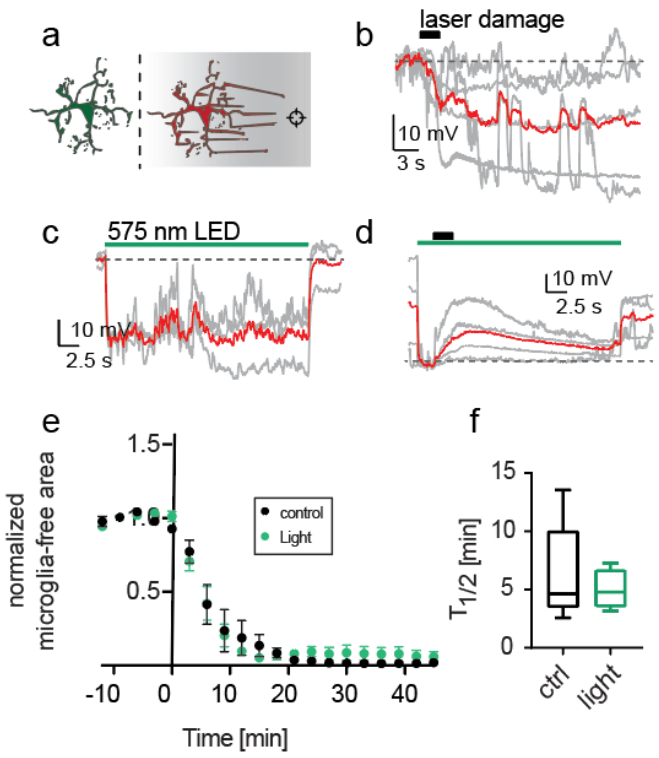
Additional file 2 – Optogenetic depolarization mildly affects microglia baseline properties. (A) Superimposed images of the same microglia at two timepoints one minute apart showing process movement during surveillance (purple, retracted; blue, extended and gray stable processes). (B-C) Relative microglia ramification (B) and surveillance index (C) over the time course of 50 min (ChETA_480, n = 4 microglia in 3 slices, control_480, n = 8 microglia in 5 slices). Light application (20 min with 480 nm) was started after 10 min of baseline recording. Statistical analysis: Two-way ANOVA with Bonferroni post-hoc comparison (*p < 0.05) (D) Overview table of relative ramification and surveillance indices in the 10 min before light application and the first 10 minutes of light application (Average ± SEM).



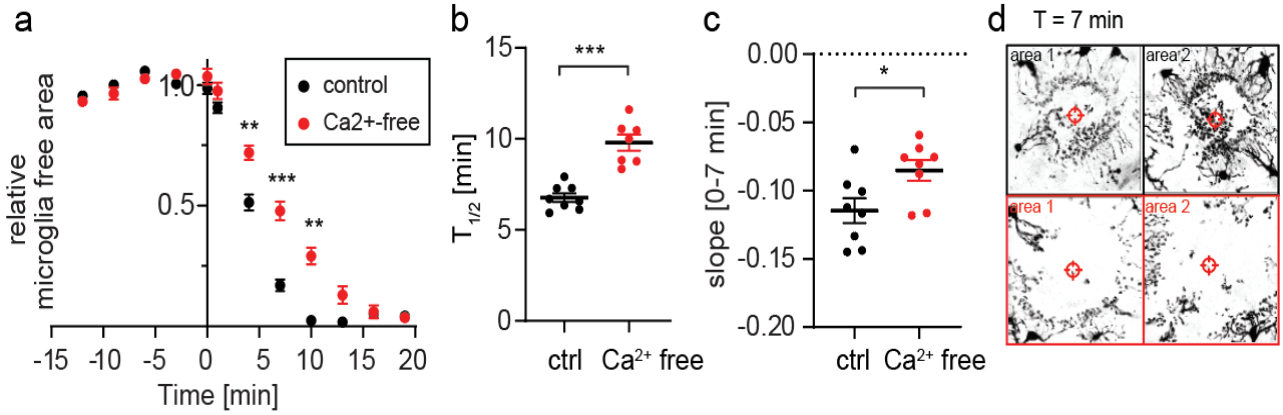
Additional file 3 - Microglia response time is independent from distance of ablation, temperature, sex, or days *in vitro*. (a) Amount of hyperpolarization of microglia membrane potential induced by laser-damage with and without activation of ChETA (n=5 controls, n=4 ChETA, DIV 20-22) **(**b) $T_{1/2}$ plotted against the distance of microglia processes from the laser damage (Spearman's ρ = 0.15, P = 0.47, n = 26 experiments). (c) $T_{1/2}$ plotted against the temperature of the extracellular solution (Spearman's ρ = −0.24, P = 0.25, n = 26 experiments). (d) $T_{1/2}$ plotted for microglia in slice cultures from male (n=5/4 control/light) and female (n=8/7 control/light) animals. (e) $T_{1/2}$ plotted against DIV for control slices (black) and ChETA slices (blue). Control: Spearman's ρ = -0.2, P = 0.66, n = 8 experiments, ChETA: Spearman's ρ = -0.6, P = 0.06, n = 11 experiments.



Additional file 4 - ArchT effectively hyperpolarizes microglia under resting conditions. (a) Immunohistochemical staining of ArchT (green) in microglia (red). (b) Light -induced currents in microglia expressing ArchT. Red lines: Average time course. (c) Light-dose dependence of currents induced in microglia. (d) Prolonged light stimulation induces robust, lasting currents in microglia. (e-f) Repetitive light stimulation induces stable light-induced currents in microglia.



Additional file 5 - ArchT activation during laser damage does not further increase hyperpolarization and has no effect on laser-damage induced chemotactic responses. (a) Schematic drawing of a chemotactic microglia response. (b) ATP-mediated hyperpolarization in microglia. Red line: Average time course. (c) ArchT light- induced hyperpolarization. (d) Combination of ArchT induced hyperpolarization with laser-damage response. (e) Chemotactic response over time while constantly applying light during acquisition of stacks. (n = 9 (5 female, 4 male)) (f) T1/2 of microglia responses in control and ArchT expressing cultures.



Additional file 6 - Microglia response kinetics towards tissue damage are slowed down in nominally Ca^{2+} free extracellular solution. (a) Relative laser damage response measured as microglia-free area in HEPES solution with 2 mM Ca^{2+} (n = 8, 3 slices, female, DIV 15-21) (black) and in nominally Ca^{2+}-free HEPES solution (n = 8, 3 slices, female, DIV 15-16) (red). 2-way ANOVA (** $p < 0.01$, *** $p < 0.001$). (b-c) Summary of $T_{1/2}$ and microglia-free area slope (0-7 min). One-way ANOVA with Tukey's post-hoc comparison (* $p < 0.05$, *** $p < 0.001$). (d) Z-projection of individual areas 7 min after laser damage. Black: microglia processes extension in control conditions with 2 mM Ca^{2+}. Red: microglia process extension under nominally Ca^{2+}-free conditions.