Persistent Light-Induced Reduction of Neuronal Excitability: Implications for Non-Optogenetic control of Brain Actvity

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Abstract

The primary objective of this study was to determine whether light stimulation can induce a persistent reduction in neuronal activity within the central nervous system. Using repeated pulses of blue light (5 seconds, λ = 430 - 495nm at 19 mW, ~2.4 mW/mm²) on cortical slices, we observed a robust and sustained decrease in evoked firing activity—approximately 60% relative to baseline—in cortical neurons from both male and female mice. This inhibitory effect persisted for more than twenty minutes following stimulation. In human cortical slices, the effect was more variable. While some neurons showed reduced activity, others—particularly those from female subjects—exhibited increased firing in response to light. The long-lasting modulation of neuronal excitability appears to result from changes in both the passive (membrane resistance and resting potential) and active (voltage-dependent Na^+ and K^+ channels) properties of neurons. These findings suggest that visual light stimulation may serve as a tool to treat brain pathologies produced by neuronal hyperexcitability. We propose a roadmap for further research, including in vivo validation, exploration of stimulation parameters, and evaluation in pathological models. Importantly, the infrastructure and protocols developed for optogenetic studies provide immediate opportunities to test the therapeutic potential of light alone, without requiring exogenous opsin expression.

Introduction

Although pharmacological treatment remains the primary therapeutic approach for managing brain pathologies arising from neuronal hyperexcitability, this strategy has several limitations, including: **a)** low specificity in targeting specific brain regions, **b)** challenges in crossing the blood-brain barrier, **c)** peripheral side effects resulting from systemic administration, and **d)** the development of tolerance and dependency with prolonged use (Rezaei *et al.*, 2023; Kendre *et al.*, 2024). To address these limitations, non-pharmacological therapeutic tools have been developed. These approaches primarily focus on targeted interventions aimed at locally modifying brain activity. Examples include Deep Brain Stimulation (DBS) (Lozano *et al.*, 2019), Transcranial Magnetic Stimulation (Cantone, 2024), and Ultrasound Neuromodulation (Zhang *et al.*, 2021). These tools are now widely used in both preclinical and clinical research, and in specific cases—such as DBS for Parkinson's disease—they have become cornerstone therapies for managing advanced stages of the disease in carefully selected patients (Malvea *et al.*, 2022). A lesser-known approach to locally modifying brain function is the use of near-infrared (NIR) light stimulation. This technique, termed brain photobiomodulation, appears to rely on the ability of NIR light to influence neuronal metabolism by reducing oxidative stress, modulating inflammatory responses, and promoting neurogenesis (Pierroz & Folcher, 2018; Ramezani *et al.*, 2022) . Recent studies have also demonstrated that the mammalian brain is sensitive to light stimulation within the visible spectrum. Specifically, a decrease in neuronal firing activity has been observed in various central nervous system (CNS) neuron types when stimulated in the yellow-blue light range (Ait Ouares *et al.*, 2019; Owen *et al.*, 2019; Mazo *et al.*, 2022; Lightning *et al.*, 2023). This effect is typically transient, with neuronal activity recovering shortly after the light is switched off. Futher, the inhibition appears to coincide with a light-induced hyperpolarizing membrane current and modifications in action potential (AP) amplitude and latency (Ait Ouares *et al.*, 2019; Lightning *et al.*, 2023). The underlying mechanisms responsible for these light-induced effects on neuronal activity remain under investigation but experimental evidence suggests that they result from tissue heating produced by the light, which alters the gating properties and/or permeability of cellular membrane channels, thereby affecting neuronal electrical activity (Stujenske *et al.*, 2015; Senova *et al.*, 2017; Ait Ouares *et al.*, 2019; Owen *et al.*, 2019; Lightning *et al.*, 2023). Additionally, there may be a GABAergic component involved (Leszkiewicz & Aizenman, 2003; Sun *et al.*, 2016).

The magnitude of the visible light-induced neuronal inhibition effect increases with both light power and stimulation duration (Stujenske *et al.*, 2015; Senova *et al.*, 2017; Ait Ouares *et al.*, 2019). Furthermore, continuous LED stimulation has been shown to produce a stronger effect than pulsed LED stimulation (Lightning *et al.*, 2023). Interestingly, preliminary results suggest that repeated continuous blue light pulses of relatively long duration (5–10 s) can induce a sustained inhibition of neuronal firing in mitral cells of the olfactory bulb, persisting for several minutes beyond the stimulation period (see supplementary figure in Ait-Ouares et al., 2019). If confirmed and extended to other neuronal types, the long-lasting inhibition of neuronal activity produced by visible light could open new therapeutic possibilities for treating the neuronal hyperexcitability associated with several neurological disorders in humans. In the present report we investigate this possibility by looking at the effect produced by 5s blue light (19 mW power) stimulation on the electrophysiological activity of both mouse and human cortical neurons.

Material and methods

Animals.

Male and female C57Bl6/J mice (Janvier Laboratories, France) aged between 60 and 90 days were used. All procedures were in accordance with European Union recommendations for animal experimentation (2010/63/UE). Mice were housed in groups of up to six in standard laboratory cages and were kept on a 12-hour light/dark cycle (at a constant temperature of 22°C) with food and water *ad libitum*.

Human Tissue.

For the investigation of human cortical neurons, we obtained living cortical tissue in collaboration with surgeons from two hospitals in Bron, Auvergne-Rhône-Alpes, France. Tissue samples were collected from patients (aged 3–54 years) undergoing surgical treatment for drug-resistant epilepsy or tumor removal surgery. All patients, or their legal guardians when applicable, provided informed consent for the use of their excised cortical tissue in research. The tissue was sourced exclusively from cortical areas that were already being removed as part of the standard surgical procedure.

Optical stimulation.

The light stimulation applied in this study was single-photon (1P) visible blue light stimulation, peaked at 470 nm (emission spectrum: 430–495 nm). Stimulation was delivered using a Dual Port OptoLED system (CAIRN, UK) with a 495 nm dichroic mirror, with a power output of 19 mW measured at the x40 objective. Due to the presence of artificial cerebrospinal fluid (ACSF), power loss at the working distance (2.5 mm from the objective) was empirically estimated at 13%. The average power density in the tissue was estimated by dividing the power at the working distance by the empirically measured illumination area (~7 mm^2). After accounting for the 13% power loss, the final estimated average power density in the tissue was ~2.4 mW/mm^2 for the 19 mW blue LED.

Slices preparation.

Slice preparation of mouse cortical neurons.

Mice were anaesthetized with an intra-peritoneal injection of ketamine (50 mg/ml) and decapitated. The head was quickly immersed in ice-cold (2-4°C) artificial cerebrospinal fluid (CutACSF) with the following composition: 125 mM NaCl, 4 mM KCl, 25 mM NaHCO3, 0.5 mM $CaCl_2$, 1.25 mM NaH_2PO_4, 7 mM $MgCl_2$ and 5.5 mM (1g/L) glucose (pH = 7.4, oxygenated with 95 % O_2/5 % CO_2). The osmolarity of the solution was adjusted to between 300 and 320 mOsm with sucrose. The brain was removed from the dissected animal and the cerebellum, midbrain, olfactory bulb, and approximately 1 – 2 mm of the anterior cerebral cortex were removed using an Unger 4 cm razor blade in order to create a flat plane for adherence to the vibratome chamber used in the preparation of the slices. Cortical coronal slices (400µm thick) were prepared with the vibratome (Leica) and cut in half to divide the hemispheres approximately along the corpus collosum. Slices were then incubated in a recovery chamber at 30 ± 1°C using an ACSF solution with a composition similar to the Cut ACSF, except for changes to $CaCl_2$ and $MgCl_2$ concentrations (1.2 mM and 0.7 mM, respectively).

Slice preparation of human neurons.

Approximately 0.5-2 cm^3 of cortical tissue, containing both gray and white matter, was removed by the neurosurgeon and transferred to an oxygenated transport container filled with ice-cold (2–4°C) CutACSF solution with the same chemical composition used for mouse tissue. During transportation from the hospital to the research laboratory, the solution was continuously oxygenated (95% O_2, 5% CO_2) using a portable oxygen tank. Upon arrival at the laboratory, the tissue and Cut ACSF were transferred to a glass Petri dish and cut as needed to

fit the vibratome chamber. In most cases, though not always possible, the tissue was sectioned to retain both gray and white matter, aiding in the eventual orientation of the tissue beneath the microscope. Following this, 400-µm-thick slices were prepared using the Leica vibratome and transferred to a recovery chamber at 30 ± 1°C, containing an ACSF solution with a composition similar to CutACSF, except for adjusted $CaCl_2$ (1.2 mM) and $MgCl_2$ (0.7 mM) concentrations.

Determination of light-induced tissue temperature change

Light induced temperature modification produced by LED stimulation was measured by placing the temperature probe connected to a ThermoClamp-1 device (Atomate Scientific) on cortical tissue beneath the microscope objective, which was held at the exact same distance as present in the electrophysiological experiments. The slices were stimulated with 19mW continuous blue light for 5s and the evolution of tissue temperature was recorded throughout. Temperature modification was calculated as the difference between the average temperature of the slice during LED stimulation and the average temperature in the one second period preceding the light stimulation.

Electrophysiological recordings.

Slices were transferred to a recording chamber mounted on an upright microscope and continuously perfused with oxygenated ACSF (2-4 ml/min) at 36 ± 1°C. Neurons were visualized using a microscope (Zeiss axioscope) with a x40 objective (Zeiss Plan-APOCHROMAT). Data were acquired with the amplifier RK 400 BioLogic at full sampling frequency of 25 kHz using a 12-bit A/D-D/A converter (Digidata 1440A, Axon Instruments) and PClamp software (PClamp10, Axon Instruments). Patch-clamp whole-cell recordings were achieved with borosilicate pipettes having a resistance 5-9 MΩ and filled with: 126 mM K-gluconate, 5 mM KCl, 10 mM HEPES, 1 mM EGTA, 1 mM MgCl2, 2 mM ATP-Na2, 0.3 mM GTP-Na3, and 10 mM phosphocreatine (pH = 7.3, 290 mOsm).

Procedure 1: Determination of the light effect on the evoked firing rate of cortical neurons.

This procedure was used for both mouse and human neurons. Cells were recorded in current-clamp mode at their resting membrane potential. If spontaneous firing was present beforehand, a hyperpolarizing current was injected to suppress spontaneous activity. To program the protocol and ensure consistent stimulation timing, the procedure was divided into predefined sweeps (Supplementary Figure 1). Each electrophysiological sweep lasted 38 seconds. During each sweep, two consecutive 5-second trains (separated by a 15 second interval) of action potentials were evoked using 5-second depolarizing current steps. At the

beginning of each sweep, a hyperpolarizing current step of -0.1 nA (1-second duration) was applied to assess membrane resistance (Rm). In the pre-light period, nine sweeps were recorded per neuron as controls. This was followed by a light period consisting of either ten or six sweeps, during which a 5-second continuous blue light stimulation (19 mW) was applied concurrently with the first depolarizing step (referred to as StimLED), but not during the second depolarizing step (referred to as StimCtr). Additionally, four seconds after the end of the second depolarizing step, neurons received an extra 1-second pulse of continuous light to assess the effect of illumination on the resting membrane potential. Finally, a post-light period with a variable number of sweeps was recorded to evaluate the long-lasting effects of light. A custom Python script (/https://osf.io/unj9k/) was used to calculate the temporal evolution of firing by normalizing the firing rate induced by the depolarizing steps to the median firing rate of the control period. The effect of light stimulation on membrane potential was analyzed by subtracting the average membrane potential (Vm) during the 1-second light stimulation at the end of the sweep from the average Vm in the 1 second preceding the light stimulation. Membrane resistance (Rm) was calculated in each sweep using Ohm's law: $R=\Delta V/\Delta I$ where ΔV was the difference between the membrane potential measured in the last 100 ms of the hyperpolarization and the membrane potential in the 200 ms preceding the hyperpolarizing step.

Procedure 2: Determination of light effect on voltage dependent currents participating in the action potential generation

This procedure was applied only to mouse cortical neurons in voltage-clamp mode (see Supplementary Figure 2). Neurons were held at -70 mV. A voltage command was used to generate a fake action potential (AP) that mimicked the shape of real APs recorded in current-clamp configuration. This fake AP was preceded by 10 repetitions of itself at a -0.1 scale, producing inverted fake APs of small amplitude (ranging from -8.5 mV to +0.4 mV) to ensure that they elicited only passive currents. The total passive current generated during the fake AP was calculated as the sum of the passive currents from the 10 reduced fake APs. This passive current was then subtracted from the total recorded current during AP generation, allowing for the isolation of the active current involved in AP generation. This approach revealed an inward, putative Na^+ and Ca^{2+} current associated with AP depolarization and an outward, putative K^+ current associated with AP repolarization. The control was established over 13 sweeps, followed by 6 sweeps with LED stimulation and a 25-minute post-led recovery period. The eventual modification of the recorded currents produced by the modification of the access resistance (R_A) during the recording was corrected by multiplying the current trace of each

sweep by the AR ratio (R_A first sweep/ R_A recorded sweep). The fake action potential (AP) was preceded by a 1-second hyperpolarizing step of -5 mV, used to monitor membrane resistance. This resistance was calculated in each sweep using Ohm's law: $R=\Delta V/\Delta I$ where ΔV=-5mV and ΔI was the difference between the median current in the last 100 ms of the hyperpolarization and the median current in the 200 ms preceding the step. Access resistance A_R was calculated as $A_R=\Delta V/\Delta I_A$,where ΔI_A is the difference between the peak of the transient current and the median current in the 200 ms preceding the step. The analysis was performed using custom scripts written in Python (https://osf.io/unj9k/).

Statistics:

The different biophysical parameters were compared across pre-light, light, and post-light conditions using repeated measures ANOVA, followed by Holm post hoc comparisons. Correlations were assessed using Pearson's test. A 2×2 repeated measures ANOVA was employed to assess the light effect as a function of sex. A χ^2 test was used to compare proportions. For frequentist statistics, the α risk was set at 5%. The absence of an effect was assessed using the Bayes factor (BF_{01}), representing the likelihood of the null hypothesis over the alternative hypothesis (Keysers *et al.*, 2020). Statistical analysis was performed with JASP software (JASP Team, 2022) and is available at : https://osf.io/rx5vs, https://osf.io/6jqye/ ,and https://osf.io/nf6g4.

Exclusion criteria.

We excluded from analysis the following electrophysiological profiles:

For the action potential firing analysis, cells were excluded when an instability with respect to AP frequency along the different sweeps occurs during the pre-light period., individual sweeps from a cell's dataset were excluded if spontaneous firing activity occurring between current steps obscured the activity induced by the depolarizing steps. For the effect of light on Vm individual sweeps within a cell's data where excluded when spontaneous action potential firing occurred during the 1s LED stimulation. For currents analysis cells, were excluded when an instability with respect to current amplitude was present in the pre-light period.

Results

Blue light stimulation produces a long-lasting reduction of the firing activity of male mouse cortical neurons

To explore the impact of repetitive 5s blue LED stimulation at 19 mW on adult cortical neurons, we conducted current clamp recordings following the protocol outlined in Figure 1A. A negative current step was employed to monitor membrane resistance (Rm), followed by two depolarizing steps inducing firing activity. The first depolarizing step was correlated with LED stimulation during the light period. Additionally, a test LED pulse was utilized to assess the acute modification of membrane potential by light (refer to Supplementary Figure 1 for additional details). As depicted in Figure 1B, the LED caused a slight and temporary increase in tissue temperature of 1.8° ± 0.5 C (tested on separate slices). Ten repetitions of LED stimulation (with 32 s intervals between stimuli) resulted in a long-lasting decrease in firing activity (Fig 1Ci, N=17). The reduction in firing varied somewhat among different neurons, and in a few cases, the activity reverted back to or even exceeded the pre-LED condition several minutes after cessation of LED stimulation. In one neuron, the firing activity increased upon LED stimulation. This neuron does not exhibit distinguishable biophysical characteristics compared to neurons showing decreased firing in response to light (see https://osf.io/8g3rt). Similar effects were observed when six repetitions of LED stimulation were applied (Fig 1Cii, N=6). In this condition, firing continued to decrease in the few minutes following the last stimulus. Extended recording time in these experiments highlighted the persistence of the firing decrease. An overall reduction to 38% ± 23% of the pre-LED activity was observed between 10 and 16 minutes after the initiation of the first LED stimulation ($p < 0.001$; N = 23, see Fig. 1Ciii).

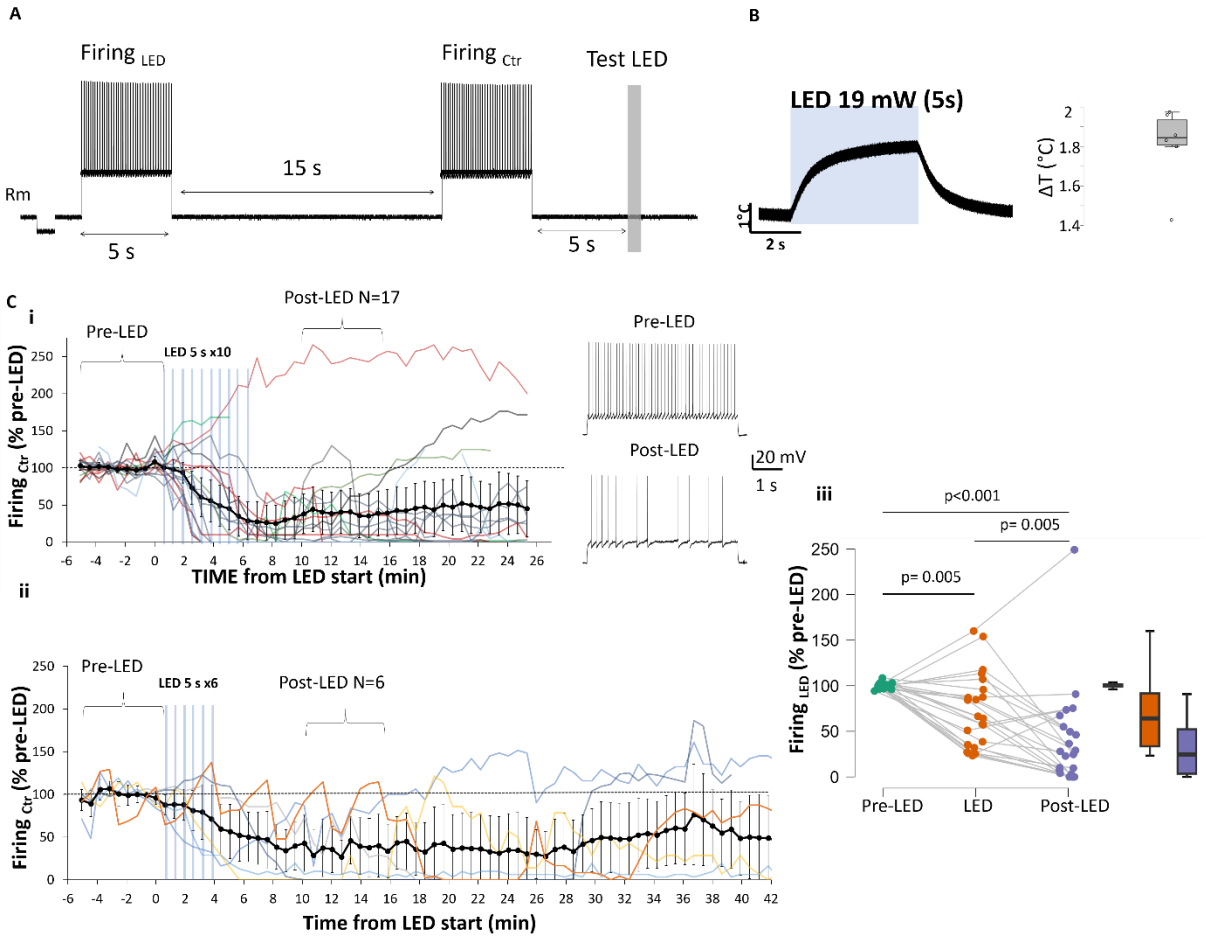

Figure 1. *Light pulses induce a prolonged reduction in firing activity in mouse cortical neurons.* ***A)*** *The current-clamp protocol used to study the effect of light on firing activity. This protocol included a negative current step to monitor membrane resistance, followed by two depolarization steps to elicit firing activity. The first depolarization (LED firing) occurred simultaneously with LED stimulation during the light period. In addition, a 1-second test LED pulse was used to evaluate the acute change in membrane potential caused by light.* ***B)*** *Transient increased tissue temperature following 5 seconds of LED stimulation.* ***C)*** *Repeated LED pulses resulted in a long-lasting decrease in firing activity.* ***Ci****. Time course of the modification of firing activity induced by 10 LED pulses. Representative traces of the light effect are shown on the right.* ***Cii****. Time plot illustrating the modification of firing activity induced by 6 LED pulses.* ***Ciii****. Statistical quantification of the light effect on firing activity in the three time periods. (Post LED = 10-16 minutes); (ANOVA test followed by Holm post hoc comparisons). Black dots and bars represent the mean and 95% confidence interval, respectively. Colored lines represent single cell responses. Raw data and analysis are available at: https://osf.io/uh73f/*

Previous reports have shown that 1-second light pulses at intensities ranging from 13 to 15 mW lead to a temporary decrease in firing activity, lasting only for the duration of the light

stimulation (Ait Ouares *et al.*, 2019; Owen *et al.*, 2019). However, in the present investigation, the firing activity during LED stimulation (Firing $_{LED}$) never dropped below the firing rate observed during the subsequent depolarization (Firing $_{Ctr}$) (see Supplementary Figure 3). This indicates that the long-lasting reduction in firing activity begins to manifest within the 15-second interval separating the two depolarizing stimuli. The light stimulation results in a significant and enduring average reduction of neuronal Rm (-17 ± 15 MΩ, p=0.001, N=22). However, this effect varied among different neurons (see Fig 2Ai and Aii). Interestingly, the modification of firing produced by LED stimulation correlates with the Rm modification (r=0.52, p=0.013, N=22, see Fig 2Aiii), suggesting that the LED effect on Rm accounts for approximately 27% of the variability in the LED effect on firing. On the other hand, the average resting membrane potential (Vrest) was not consistent affected by the light (Fig 2Bi and Bii) and the modification of this parameter only slightly correlate with the modification of firing (r=0.36, p=0.097, N=22, see Fig 2Biii). As previously reported (Ait Ouares *et al.*, 2019; Owen *et al.*, 2019; Lightning *et al.*, 2023), during test LED we observed a transient hyperpolarization of the membrane potential (Vm) (-0.31 ± 0.09 mV, p<0.001, N=38, see Fig. 2Ci), which reverts at -100 mV ± 8 mV (N=14, data not shown). Interestingly, this acute effect of light on Vm did not correlate with the long-term effect on firing (see Figure 2Ciii), suggesting that the two phenomena rely on different cellular mechanisms. Moreover, this lack of covariation also suggests that the observed variability in the light effect on neuronal firing is not due to methodological artifacts (e.g., the health of the recorded neuron or its depth in the slices).

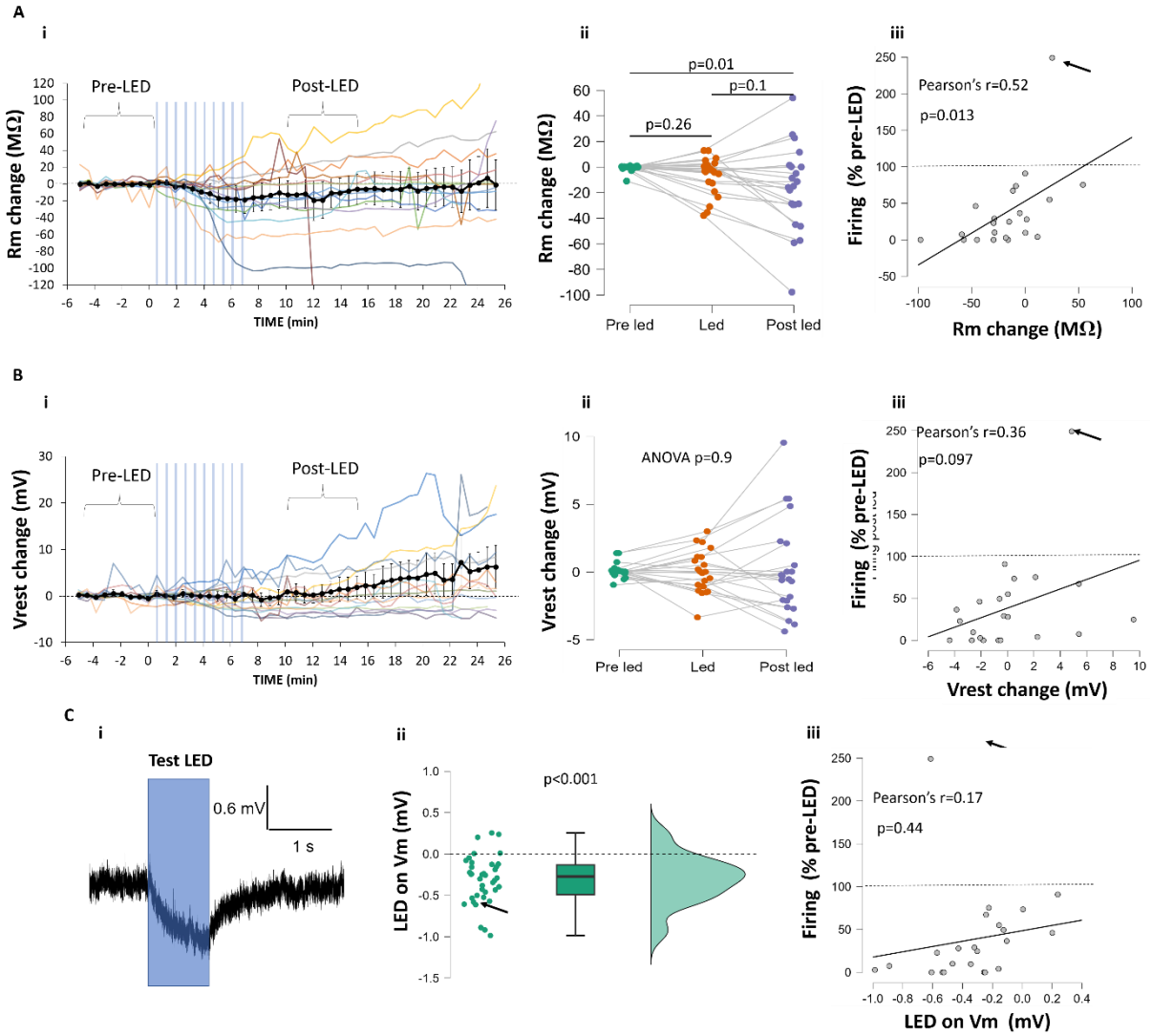

Figure 2. *Changes in Rm explain part of the light effect on neuronal firing. A) Repeated LED pulses resulted in a decrease in Rm.* ***Ai.*** *Time course of Rm modification induced by 10 LED pulses.* ***Aii.*** *Statistical quantification of the light effect on Rm in the three time periods. Aiii. LED-induced change in firing activity correlated with LED-induced change in Rm.* ***B)*** *Repeated LED pulses on Vrest.* ***Bi.*** *Time course of Vrest modification.* ***Bii.*** *Statistical quantification of the light effect on Vrest in the three time periods.* ***Biii.*** *LED-induced modification of firing activity does not correlate with LED-induced modification of Vrest.* ***C)*** *Light induces transient membrane hyperpolarization.* ***Ci.*** *Example of transient modification of Vm during light stimulation* ***Cii.*** *Quantification of the transient Vm change.* ***Ciii.*** *LED-induced modification of firing activity does not correlate with LED-induced transient modification of Vm. B and C: (post LED = 10-16 min) ANOVA test followed by Holm post hoc comparisons. Black dots and bars represent mean and 95% confidence interval, respectively. Colored lines represent single cell responses. Arrows depict the values for the neuron for wich light produced long lasting increase of firing. Raw data and analysis are available at :* https://osf.io/uh73f/

Blue light stimulation induces a long-lasting modification of voltage dependent inward and outward currents participating in the AP in male mouse neurons

Both voltage-gated sodium and potassium channels contribute to action potential (AP) generation. Therefore, the effect of light on firing activity could be mediated by changes in the function of these channels. To investigate this, voltage-clamp recordings were performed to isolate voltage-gated (VG) outward (putative K^+) and inward (putative Na^+) currents. These currents were elicited by experimentally modifying the membrane potential to mimic an AP waveform representative of cortical pyramidal neuron activity. In this protocol, two consecutive APs were generated for each electrophysiological recording and only the first, the AP_{LED}, received light illumination during the LED period (Figure 3A, see Supplementary Figure 2 for more details). As shown in Figure 3B, LED stimulation produced a long-lasting reduction in VG-inward current amplitude (66% ± 28% of the pre-LED period, N=11). This effect developed slowly, was heterogeneous between different neurons (Fig. 3Bii, Biii) and was associated with a slowing of VG-inward current kinetics (rise time from 4.4 ± 1.4 nA/ms to 3.1 ± 1.2 nA/ms; decay time from 3.4 ± 1.4 nA/ms to 2.2 ± 1.2 nA/ms, N=11, Fig. 3Biv, 3Bv). On the other hand, LED stimulation did not alter the threshold of VG-inward current, i.e. the membrane potential at which the VG-inward current starts to develop (Figure 3Biv). To isolate possible transient LED effects from the long-lasting LED effects, the VG-inward currents produced by the AP_{LED} were compared with those produced by the AP_{Ctr}. As shown in Figure 3C (i-iii), the kinetics of the VG-inward current were slightly faster during LED stimulation than in the control condition (LED rise time = 4.5 ± 1.4 nA/ms, Ctr rise time = 4.2 ± 1.2 nA/ms; LED decay time = 3. 4 ± 1.4 nA/ms, Ctr decay time= 3.1 ± 1.4 nA/ms, N=11), while the VG-inward current amplitude and threshold were not altered (Fig. 3Civ, 3Cv).

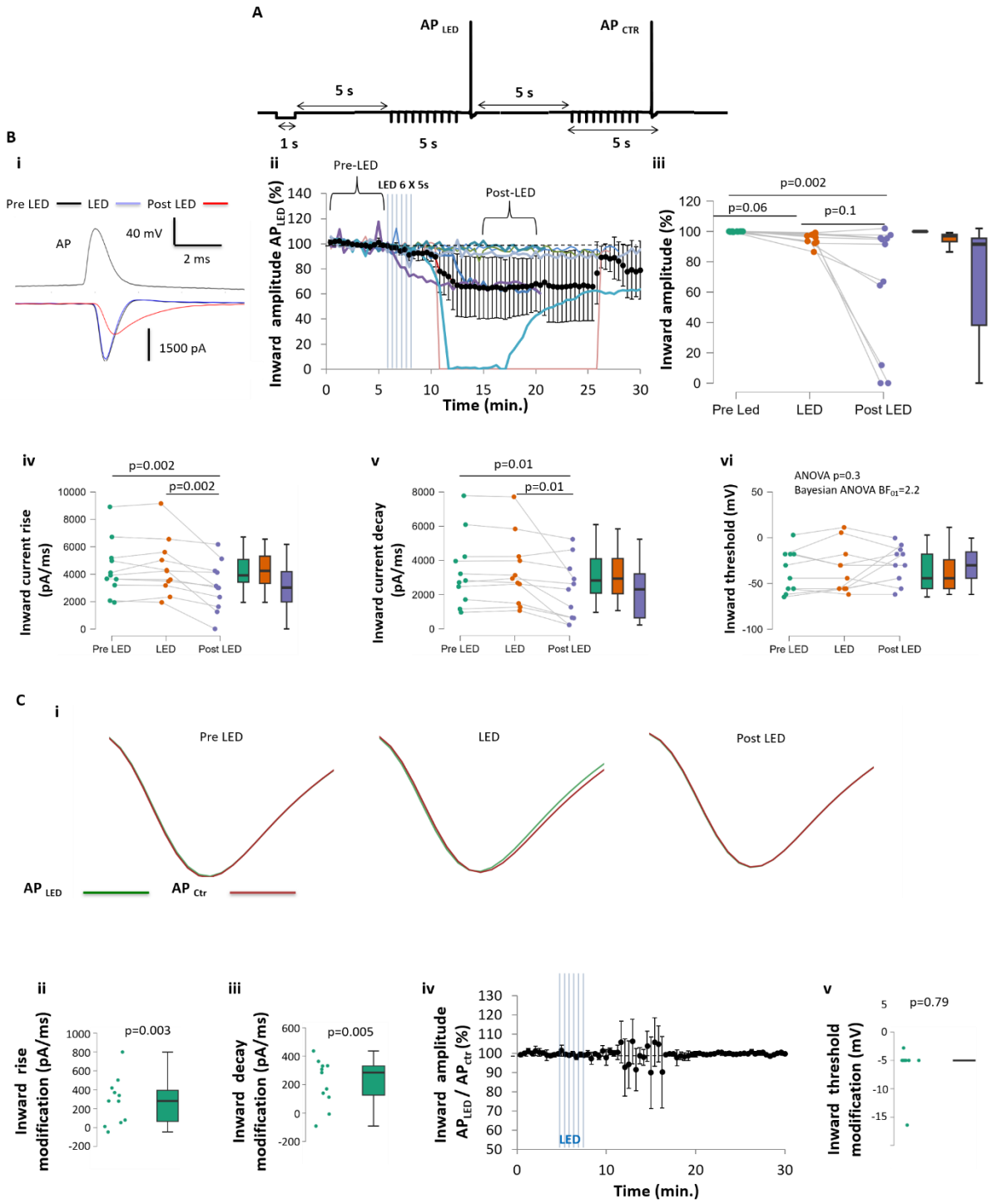

Figure 3. *Light stimulation alters the voltage-gated inward current involved in AP generation.* ***A)*** *Voltage clamp protocol used to isolate VG currents. Two consecutive AP potentials were generated by voltage command.* AP_{LED} *was generated simultaneously with light stimulation during the LED period. VG-currents were isolated from resistive and capacitive currents using the method shown in Supplementary Figure 2.* ***B)*** *LED stimulation produces a sustained reduction in VG inward current amplitude and kinetics.* ***Bi.*** *Example of VG-inward current modification by LED stimulation.* ***Bii****. Temporal evolution of VG inward current amplitude. Black dots and bars represent mean and 95% CI, respectively. Coloured lines represent single-cell evolution.* ***Biii****. Statistical*

quantification of the average VG inward current amplitude in the three periods. ***Biv****. Statistical quantification of the average VG inward current rise time in the three periods.* ***Bv****. Statistical quantification of the average membrane potential threshold for VG inward current generation in the three periods.* ***Bvi****. Statistical quantification of the average VG inward current rise time in the three periods. (Post LED = 15-20 minutes); (Friedman test followed by Conover post hoc comparisons).* ***C)*** *LED stimulation produces a short-term increase in VG-inward current kinetics.* ***Ci****. Example of overlap of VG-inward currents produced by* AP_{LED} *and APCtr in the three periods..* ***Cii.*** *Statistical quantification of the change in VG-inward current rise time during LED stimulation.* ***Ciii.*** *Statistical quantification of the change in VG-inward current decay time during LED stimulation.* ***Civ.*** *Temporal evolution of the ratio between* AP_{LED} *and APCtr VG-inward current amplitudes* ***Cv****. Statistical quantification of the LED-induced modification of the membrane potential threshold for VG-inward current generation. (N=11). Raw data and analysis are available at https://osf.io/pt4br/*

The LED effect on VG-outward currents was more heterogeneous. As shown in Figure 4, during LED stimulation the amplitude of VG-outward current increased in six of the recorded neurons and decreased in two, but the inverse effect was observed thereafter. Because of this heterogeneity, the long-lasting population effect did not reach significance (average decrease of 45 ± 39 %, p=0.3 after Holm correction, N=8) (Figure 4Aii, Aiii). The comparisons of AP_{LED} and AP_{Ctr} currents during LED period show a clear transient increase in the amplitude VG-outward current during the LED stimulus (131 ±7 % during LED, Fig 4B) showing a short-lasting effect of light on this parameter.

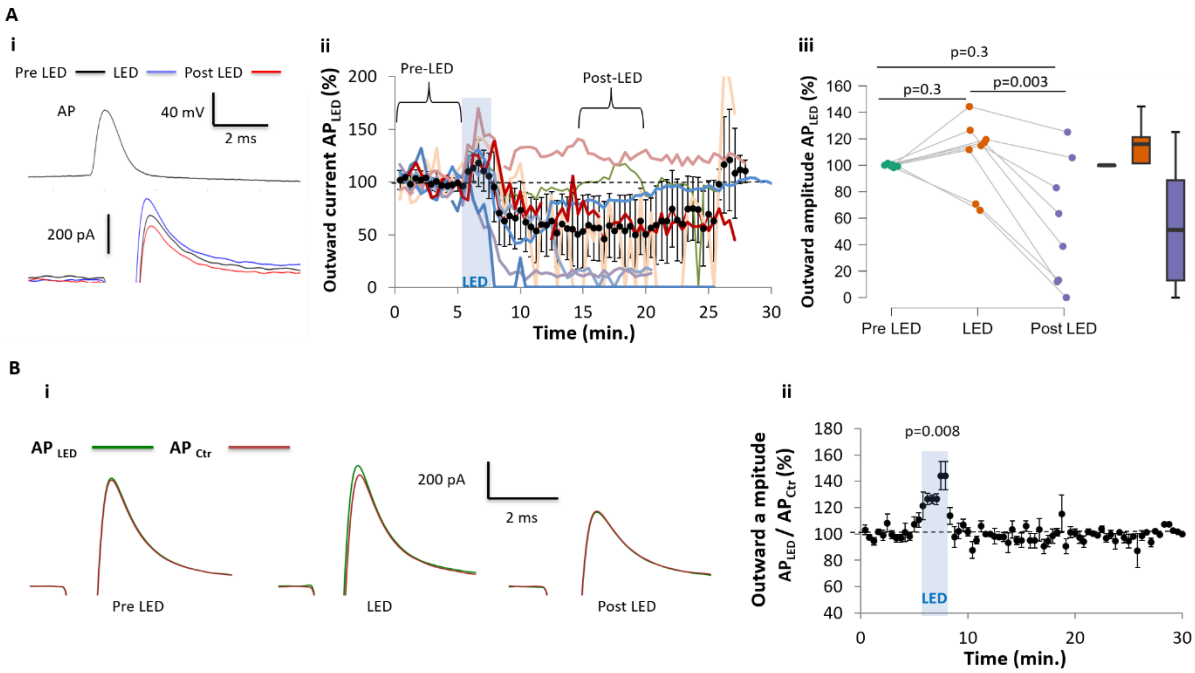

Figure 4. *Light stimulation modifies the voltage-gated outward current involved in AP generation.* ***A)*** *LED stimulation produces a long-lasting change in the amplitude of the voltage-gated outward current. Ai. Example of outward current modification by LED stimulation. Aii. Temporal evolution of VG outward current amplitude. Black dots and bars represent mean and 95% CI, respectively. Coloured lines represent single-cell evolution. Aiii. Statistical quantification of the average outward current amplitude in the three periods (Friedman test followed by Conover post hoc comparisons).* ***B)*** *LED stimulation produces a transient increase in VG outward current amplitude.* ***Bi.*** *Example of overlap of VG outward currents produced by* AP_{LED} *and APCtr in the three periods.* ***Bii.*** *Temporal evolution of the ratio between* AP_{LED} *and APCtr VG outward current amplitudes. Raw data and analysis are available at* https://osf.io/pt4br/

In agreement with the current clamp experiment, the LED stimulation produced a long-lasting decrease in Rm, as monitored during voltage clamp recording (-60 ± 45 MΩ at 15'-20', N=10, Fig. 5).

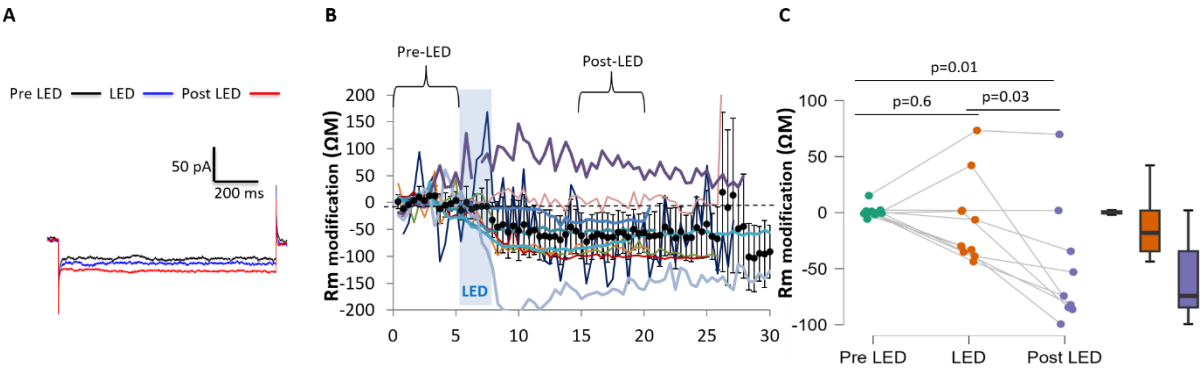

Figure 5. *Light stimulation produces a long-lasting decrease of membrane resistance.* ***A)*** *Example of passive membrane currents induced by 5 mV membrane hyperpolarisation used to calculate Rm.* ***B)*** *Temporal evolution of the Rm change. Black dots and bars represent mean and 95% CI, respectively. Coloured lines represent single cell evolution.* ***C)*** *Statistical quantification of the average Rm changes in the three periods (Friedman test followed by Conover post hoc comparisons). Raw data and analysis are available at https://osf.io/pt4br/p*

Taken together, these results suggest that light causes both transient and long-lasting changes in the biophysical properties of voltage-gated channels involved in the AP, as well as in channels involved in resting membrane permeability. Transient effects are likely to be produced by the light-induced temperature rise, leading to a faster activation and inactivation rate of the VG-inward current, an increase in the amplitude of the VG-outward currents and a heterogeneous modification of Rm. Long-term changes include a decrease in the amplitude and kinetics of VG-inward currents, a heterogeneous effect on VG-outward current amplitude dominated by a decrease of the latter, and a predominant decrease in Rm. The theoretically expected effects of changing these three parameters on the direction of firing change are illustrated in Figure 6A. In order to evaluate their reciprocal contribution to the light-induced long-lasting decrease of firing, we plotted the modification of evoked firing activity, that was monitored before and at the end of voltage clamp protocol, as a function of the modifications of the VG-currents and the Rm (Fig 6B). This analysis suggests that the light-induced decrease of firing rate is a multifactorial process with the modification of VG-inward current being the principal actor. A large decrease in VG inward currents was always associated with a large decrease in firing rate. On the other hand, a small decrease in inward currents was associated with either a strong, medium, small or no decrease in firing rate, depending on the direction and magnitude of the modification of VG-outward currents and Rm.

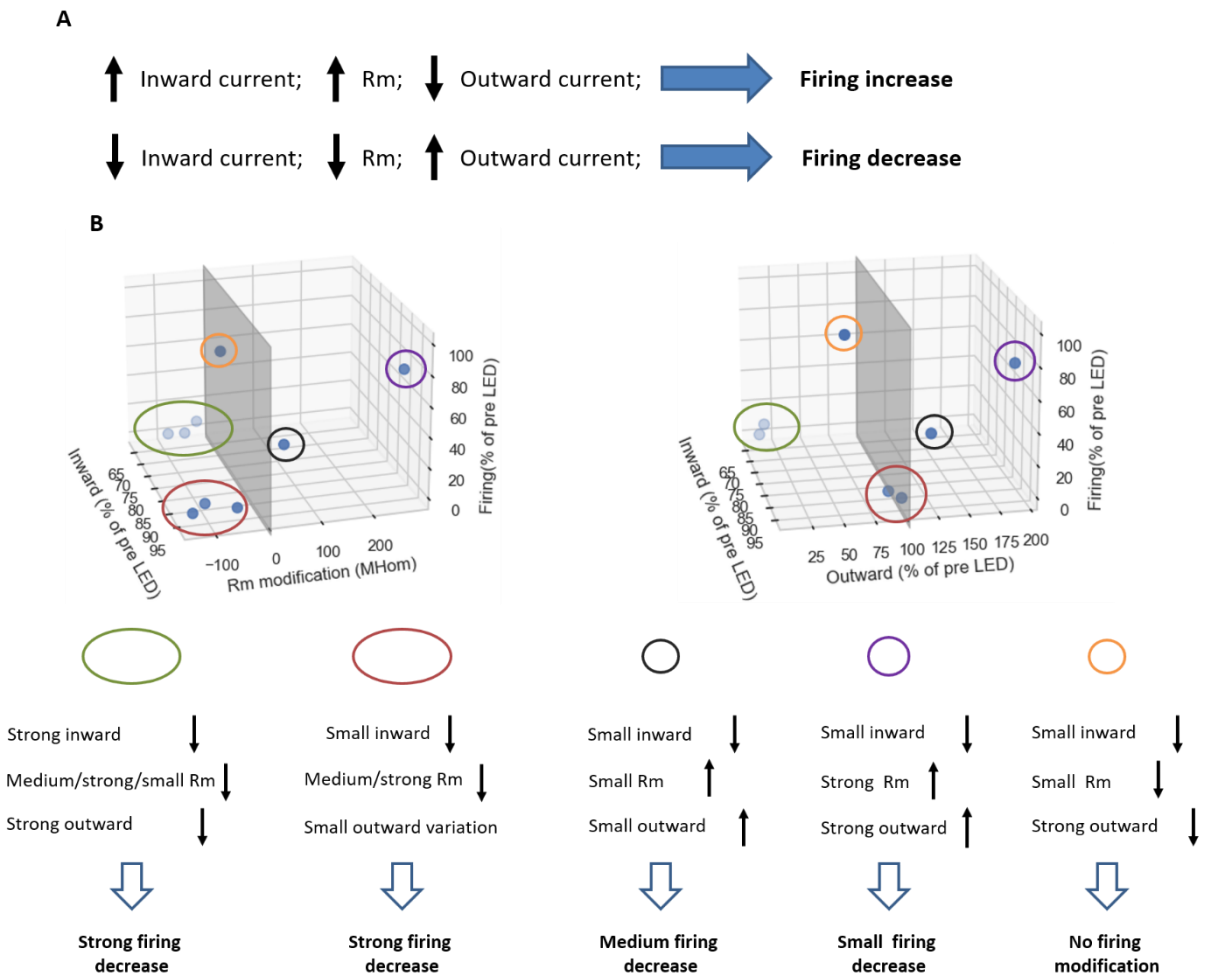

Figure 6. *Relative contribution of the light-induced changes in VG-currents and Rm to the modification of neuronal firing rate.* ***A)*** *Expected impact of VG-currents and Rm modifications on the direction of firing rate change.* ***B)*** *Top, 3D plot illustrating the light-induced modification of firing rate as a function of the light-induced modification of VG-inward, VG-outward and Rm. Bottom, Classification of the effect on firing activity associated with the different combinations of observed changes in VG currents and Rm.*

Heterogenous effect of blue light stimulation on cortical human neurons

The effect of blue light stimulation on human cortical neurons was investigated using a current clamp configuration, following the same protocol as for mouse neurons (Figure 1A). Tissue samples came from nine patients differing in age, sex, pathology, and the explanted cortical region. Ten repetitions of LED stimulation resulted in a heterogeneous modification of firing activity in the fifteen neurons recorded. Specifically, there was a significant long-lasting reduction of firing in 8 neurons (55%) and a significant long-lasting increase in firing in 4 neurons (27%) (Figure 7A). This proportion of excited and inhibited neurons was significantly different from what was observed in mice ($p<0.001$, χ^2 test). Exploratory analysis did not reveal any distinguishable biophysical properties explaining this difference (for more details, see

https://osf.io/q9bfv). However, the proportion of excited neurons was significantly higher in tissue from female patients compared to male patients (83% vs 12%, respectively, p=0.008, χ^2 test, Figure 7B, see also Supplementary figure 4 A-B). Moreover, the difference between human and mouse samples disappeared when female neurons were removed from the human sample (6% vs 12% increase in firing, respectively, Bayesian statistic, BF_{01}=2.85), suggesting that sexual dimorphism could be the cause of the heterogeneity in the effect of LED stimulation on firing in the human sample.

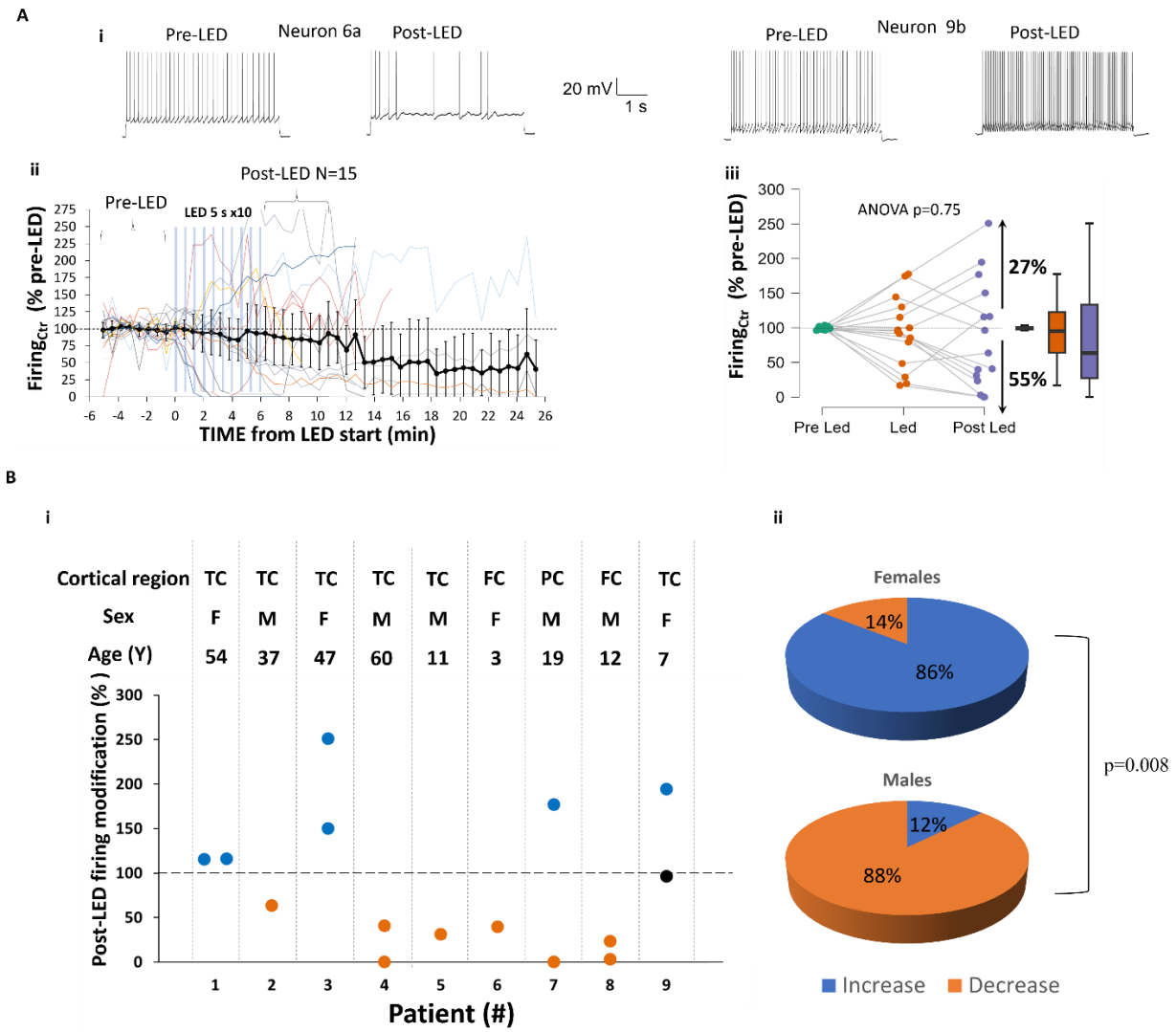

Figure 7. *Light Pulses Induce a Prolonged and Heterogeneous Modification in Firing Activity in Human Cortical Neurons.* ***A)*** *Repeated LED pulses induce a long-lasting decrease in firing activity in 55% of recorded neurons and an increase in firing in 27% of recorded neurons.* ***Ai)*** *Representative traces showing the effect of light on two different neurons.* ***Aii)*** *Time course of the firing activity modification induced by 10 LED pulses.* ***Aiii)*** *Statistical quantification of the light effect on firing activity across three time periods (Post LED = 6-11 minutes).* ***B)*** *Sexual dimorphism in the long-lasting effect of light on the firing modification of human neurons.* ***Bi)*** *Long-lasting LED-*

induced firing modification in neurons from different patients. ***Bii)*** *Long-lasting LED effect on firing activity as a function of the sex of the donor patient. Blue, orange, and black dots in B respectively represent neurons that showed a significant increase, a significant decrease, or no change in firing activity. Raw data and analysis are available at : https://osf.io/2wfy5/*

Modification of Rm upon light stimulation in human neurons was also heterogeneous but did not correlate with changes in firing activity (Supplementary Figure 5A). A slight, long-lasting membrane depolarization was produced by blue light (2 mV ± 2 mV), and the change in this parameter only slightly correlated with the modification of firing ($r = 0.35$, $p = 0.20$, $N = 15$, Supplementary Figure 5B).

To test whether the sex difference in light effects is also present in mice, ten additional cortical neurons were recorded from female mice. As shown in Supplementary Figure 4 C-D, light stimulation produces a similar long-lasting inhibition of firing activity in mouse neurons from both sexes (sex effect $p = 0.87$; $BF_{01} = 3.5$).

Interestingly, human neurons also differ from mouse neurons in their acute response to light on membrane potential. In 4 out of 23 (17%) human neurons, blue light produced a depolarization of the membrane potential (Figure 8Ai, Aii), an effect that was never observed in mouse neurons in the present study (Figure 8B, $p<0.001$, χ^2 test) nor previously reported in other studies. The reversal potential of this effect was estimated at -35 mV (N=1). When these neurons were excluded from the analysis, a global membrane hyperpolarization was observed (Vm = -0.22 mV ± 0.094 mV, $p < 0.001$; Figure 8Aiii), consistent with the finding in mouse neurons (Bayesian statistic, Mice vs. Human, $BF_{01} = 1.9$). The proportion of neurons exhibiting the depolarizing effect was slightly larger in females compared to males, although this difference did not reach significance (25% in females vs. 9% in males, $p=0.31$ χ^2 test, $BF_{01}=1.76$). Similar to observations in mice, the light-induced modification of Vm did not correlate with the light-induced long-lasting modification of firing (Figure 8Aiv).

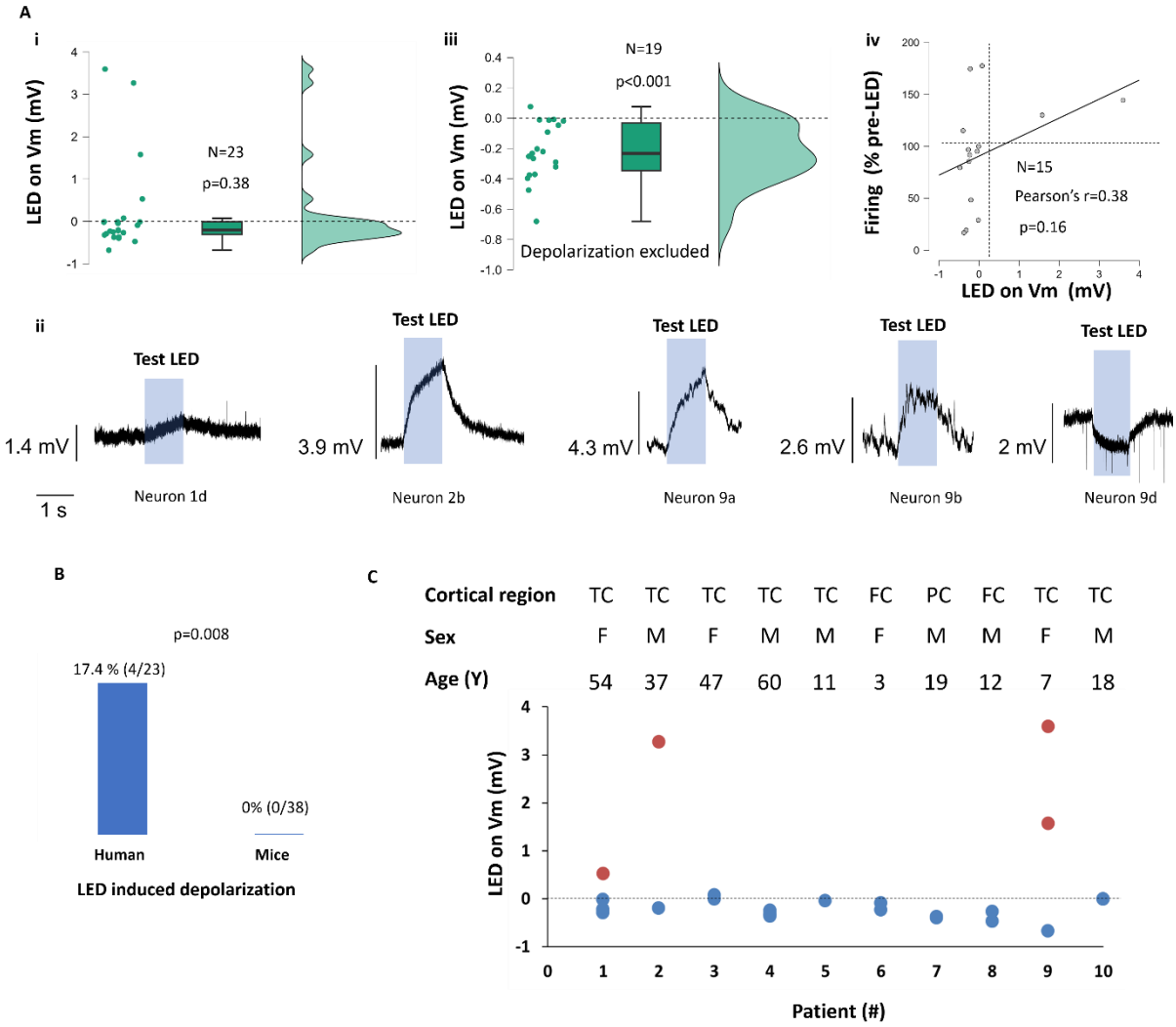

Figure 8. Heterogeneous modification of the membrane potential by LED stimulation in human heurons. ***A)*** *Light-induced membrane potential modification in human neurons.* ***Ai)*** *Raincloud plot showing the effect of light on the entire sample.* ***Aii)*** *Average traces illustrating the light-induced membrane potential (Vm) modification in four neurons showing depolarization and one neuron showing hyperpolarization (average effect produced by ten stimulations) .* ***Aiii)*** *Raincloud plot of the light effect excluding the four depolarizing neurons.* ***B)*** *The proportion of light-induced membrane depolarization was significantly higher in neurons from humans compared to those from mice.* ***C)*** *LED-induced Vm modification in neurons from different patients (in red the four neurons showing Vm depolarization).* *Raw data and analysis are available at :* https://osf.io/2wfy5/

Discussion

The primary objective of this study was to determine whether light stimulation can induce a persistent reduction in neuronal activity within the central nervous system. We show that repeated blue light stimuli, each lasting 5 seconds at an intensity of 19 mW (~2.4 mW/mm²), consistently reduce evoked firing activity in cortical neurons from both male and female mice by approximately 60% relative to baseline levels. This reduction persists for several tens of minutes after the cessation of light stimulation. The inhibitory effect of light on neuronal activity appears to be less pronounced in neurons from human cortical slices, primarily because some human neurons exhibited increased activity upon light stimulation. This excitatory effect was more prominent in neurons derived from female subjects. The long-lasting effect of light appears to be due to multiple factors, including changes in passive properties (Rm and Vrest) and active properties (voltage-dependent Na^+ and K^+ channels) of the neurons.

Key light pattern features for prolonged suppression of neuronal activity

Previous studies have demonstrated a reduction in the firing activity of several CNS neurons during continuous light stimulation within the visible spectrum (Ait Ouares *et al.*, 2019; Owen *et al.*, 2019; Mazo *et al.*, 2022; Lightning *et al.*, 2023). However, these modifications were transient, as firing activity generally reverted rapidly to pre-light levels after stimulation ceased. This rapid recovery was particularly evident in the study by Owen et al. (2019), where green light (532 nm) at 15 mW was used. Interestingly, recovery occurred regardless of whether a one-second (*in vitro*) or a five-second (*in vivo*) continuous pulse was applied. In the present study, long-lasting firing inhibition was induced by five-second continuous pulses of blue light (430–495 nm) at 19 mW. Although we cannot rule out that the difference in inhibition duration between the two studies is due to the 4 mW difference in light power, a more likely explanation is the difference in wavelength, with blue light carrying more energy than green light. In the

present study, we show that ten or six repetitions of light stimulation are equally effective in producing long-lasting firing inhibition. Notably, as shown in Figure 1Cii, firing activity continues to decrease in the minutes following the sixth pulse, suggesting that light acts as a triggering signal for an effect that requires several minutes to fully develop. On the other hand, the effect also appears rapidly after the first pulse, as a long-lasting reduction in firing activity begins to manifest within the 15-second interval separating the LED and CTR depolarizations (see Supplementary Figure 3). This suggests that a single or a few light pulses may already be sufficient to produce the maximal effect on firing reduction

Phototoxicity and its implications for long-lasting effects of light

Although the energy carried by visible light is insufficient to cause ionization and directly damage DNA or other cellular structures, it can still trigger photochemical reactions and oxidative stress, leading to cellular damage. For instance, exposure of cultured epithelial cells to continuous blue light (2.8 mW/cm²) for 6 hours resulted in approximately 10% cell mortality, potentially due to mitochondrial generation of reactive oxygen species (ROS) (Godley *et al.*, 2005). In cortical neuronal cultures, 1 or 6 hours of intermittent blue light stimulation (3.9 mW/mm²), but not red or green light, led to increased expression of several neuronal activity-regulated genes, which the authors interpreted as a cellular response to oxidative stress (Tyssowski & Gray, 2019). These findings were later replicated by Duke *et al.*, 2020 who also observed increased neuronal mortality associated with light exposure. However, these effects of light where not caused by a direct interaction with neuronal cells but rather mediated by ROS generation due to the interaction of light with the culture media (see also Stockley *et al.*, 2017). Therefore, to our knowledge, the potential cytotoxic effect of visible light on neurons, particularly in slice and in vivo conditions, remains unproven. Our results do not support this possibility. Although light stimulation affected the biophysical properties of the recorded neurons, cell viability did not appear to be compromised at least in the short term.

Neuronal death is typically associated with a rapid loss of membrane polarization; however, only a slight depolarization of the membrane potential was observed following light stimulation. Moreover, in most recorded neurons, firing activity although reduced remained detectable and, in some cases, partially or fully recovered over time.

Cellular mechanisms of light effect on firing activity

Among the various mechanisms that could contribute to the light-induced, long-lasting reduction in firing activity, we highlight the decrease in membrane resistance and the reduction of inward active currents involved in action potential depolarization. Pearson correlation suggests that the former accounts for approximately 27% of the LED effect on firing. Interestingly, the kinetics of the light effect on these two parameters appear to be slower than the kinetics of firing reduction (compare Figure 1 with Figures 2A, 3B, and 5), suggesting that other mechanisms may contribute to the initial effect of light on firing. The effects of light on membrane resistance are likely due to a modification of the permeability of leak membrane channels. This could involve an increase in the permeability of inwardly rectifying potassium channels (Kir), which have already been suggested to contribute to the transient membrane hyperpolarization induced by light stimulation (Owen *et al.*, 2019). This mechanism could also explain the slight hyperpolarization of the resting membrane potential observed in several neurons (Figure 2B). Concerning the long-lasting modification of the inward current, we observed a decrease in its amplitude, accompanied by an increase in its duration (increase of rise and decay times). On the other hand, we did not observe any evident modification in the inward current threshold. This suggests a modification in the permeability and activation-inactivation properties of the Na^+ or, potentially, Ca^{2+} channels, which are primarily involved in action potential generation in cortical neurons, without altering their voltage sensitivity (Catterall, 2023). Interesting inward currents become faster (with decreased rise and decay times) during LED stimulation. Simultaneously, an increase in the amplitude of the outward,

putative potassium current is observed. These acute effects are likely due to the transient temperature increase during light stimulation (Volgushev, Vidyasagar, Chistiakova, Yousef, *et al.*, 2000). On the other hand, the transient temperature increase during LED stimulation is unlikely to be the cause of the long-lasting effects on firing activity and membrane properties, since it has been shown that temperature-dependent modifications of neuronal activity rapidly revert to their initial values once warming ceases (Volgushev, Vidyasagar, Chistiakova, & Eysel, 2000). Moreover, it should be noted that the temperature change produced by LED stimulation (1.8°C) remained within the range of natural physiological fluctuations (2-3°C, Andersen & Moser, 1995), excluding any thermal toxicity to the neurons.

Species and sex differences in light effects

We report two differences in light response between mouse and human neurons. The first is a light-induced, long-lasting increase in firing activity observed in approximately 30% of recorded human neurons, which was almost absent in mice. This species-specific discrepancy appears to be driven by a sex effect present only in humans, where cortical neurons from female patients are more likely to exhibit increased firing in response to light compared to those from male patients. The second difference is an acute depolarizing effect of light, observed in a minority (17%) of human neurons but never in mouse neurons. The differences in the biophysical properties of human neurons compared to rodent neurons, as previously reported (Testa-Silva *et al.*, 2014; Eyal *et al.*, 2016; Gidon *et al.*, 2020; Mihaljević *et al.*, 2021) could explain the different effects of light on these cells. While it has been previously proposed that the hyperpolarizing effect of light is mainly due to an increased permeability of Kir channels (Owen *et al.*, 2019), the depolarizing effect produced by light in some human neurons is likely associated with a modification of channels permeable to Na^+ and/or Ca^{2+}, such as Na^+ leak channels or TRP channels. The sexual dimorphism observed in the long-lasting effect of light on human neurons may be attributed to functional, anatomical, biochemical, genetic, and

electrophysiological differences between male and female brains (Iezzi *et al.*, 2024; Lafta *et al.*, 2024). However, this finding requires further confirmation (see next).

Limitations and cavate

The main limitations of the present study stem from the experiments performed on human neurons, primarily due to the low availability and inherent heterogeneity of these samples. This heterogeneity includes differences in sex, age, brain pathology, pharmacological treatments, as well as variability in the cortical regions sampled and the plane of brain dissection. In many cases, this prevented clear identification of the cortical layer from which the recorded neuron originated. However, the heterogeneity of light-induced effects observed in human neurons is unlikely to be solely due to differences in neuronal localization, since mouse neurons, despite also being sampled from various cortical regions and layers, showed a more consistent response to light stimulation. The principal caveat of the study lies in the interpretation of the results regarding the dimorphic response of human neurons to light stimulation. While statistical analysis supports this interpretation, it arises from an exploratory approach in which several other parameters—such as age, pathology, and the biophysical characteristics of the recorded neurons—were investigated as potential causes of the observed variability in neuronal responses to light stimulation. This necessitates replication of this result to ensure robust scientific validation.

Perspectives and conclusions

This work was designed to explore whether visual light stimulation could serve as a tool to induce durable changes in neuronal activity, thereby providing insight into its potential for clinical application. The results observed here underscore the relevance of this approach and provide a rationale for further studies to investigate its therapeutic potential. Building on these

findings, we propose a conceptual roadmap to guide and structure future research efforts, with the ultimate goal of translating this approach into clinical practice:

- *Confirming light effects on human neurons.* As previously discussed, the results obtained on human neurons need to be replicated in a larger *in vitro* sample using a confirmatory approach. Future studies should specifically assess the sex-related differences reported in the present work and consider the potential influence of patients' pharmacological treatments as a factor that could explain the variability in light-induced effects. Human brain organoids (Chen *et al.*, 2019) could eventually be used to increase tissue availability, reduce sample variability through more standardized procedures, and investigate light-induced effects and safety over longer time scales.
- *Assessing long-lasting light effects in vivo.* Although transient reductions in neuronal firing following light stimulation have been observed in vivo (Owen *et al.*, 2019; Mazo *et al.*, 2022), it remains important to determine whether the long-lasting reduction reported in the present study can also be reproduced *in vivo*. To address this, direct *in vivo* electrophysiological recordings and/or indirect assessments through light-induced behavioral modifications should be conducted using animal models.
- *Assessing the duration of light-induced firing modulation.* From a therapeutic perspective, it is important to determine the duration of light-induced effects over extended time periods and whether the reduction in neuronal excitability can be reinstated by a subsequent stimulation after recovery. Since acute brain slices offer only a limited follow-up window, alternative models should be considered. These may include neuronal cultures, organotypic slices, brain organoids, or in vivo models equipped with chronically implanted electrodes, as well as behavioral outcomes used as indirect readouts of light effects. Furthermore, these models would provide a more comprehensive assessment of the safety profile of light

stimulation over longer time periods, encompassing both cellular toxicity and possible behavioral effects.

- *Investigating the potential of light to counteract brain pathologies.* Given its sustained inhibitory effects, visual light therapy appears particularly promising for brain disorders characterized by hyperexcitation, such as epilepsy, migraine, autism spectrum disorder, and possibly schizophrenia (Aurora 2007; Demarquay 2017; Takarae 2017; Hussein 2023; Inan 2012). Interesting, several research groups worldwide are already using optogenetic approaches in animal models of epilepsy to suppress pathological brain activity (Chiang *et al.*, 2014; Bui *et al.*, 2017; Chang *et al.*, 2017; Choy *et al.*, 2017; Streng & Krook-Magnuson, 2020; Ledri *et al.*, 2023; Lévesque *et al.*, 2023). As these studies rely on well-established protocols, infrastructure, and expertise for delivering and controlling light in the animal brain, testing whether light alone—without the need for exogenous opsin expression—has intrinsic antiepileptic effects could be pursued immediately, without requiring any additional technical development.
- *Investigating alternative patterns of light stimulation.* In the present study, only 5-second pulses of blue light at 19 mW were used. To optimize the efficacy of light-induced, long-lasting reductions in neuronal excitability, it will be important to explore alternative stimulation parameters, including variations in wavelength, power, and duration. In particular, the use of longer wavelengths could improve tissue penetration, enabling the targeting of deeper and larger brain volumes (Deng *et al.*, 2014).

Ours findings highlight the promise of visual light stimulation as a non-invasive neuromodulatory tool with potential clinical applications. Future research, guided by the proposed roadmap, will be key to validating its efficacy, optimizing protocols, and advancing toward therapeutic use in brain disorders.

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Conflict of interest disclosure

The authors declare that they have no financial or non-financial conflicts of interest in relation to the content of the article.

Data, scripts, code, and supplementary information availability

All raw electrophysiological traces, code for the analysis, analysis and statistics are accessible via the Open Science Framework website (https://osf.io/6jqye/)

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