Persistent light-induced reduction of neuronal excitability in cortical neurons

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Abstract

Visible light is widely used in neuroscience, yet its direct effects on neuronal activity in the absence of optogenetic manipulation remain incompletely understood. Here, we investigated whether light stimulation can induce sustained changes in neuronal excitability. Using ex vivo electrophysiological recordings, we show that repeated pulses of blue light (5 s, 430–495 nm, 19 mW) produce a robust and persistent reduction in evoked firing activity in cortical neurons from both male and female mice, with an average decrease of ~60% relative to baseline. This inhibitory effect persisted for more than 20 minutes following stimulation and was associated with changes in both passive membrane properties and active ion channel conductance. In human cortical neurons, responses were more heterogeneous. While a subset of neurons exhibited similar inhibitory effects, others showed increased excitability, with this response occurring more frequently in neurons from female patients, suggesting a potential sex-dependent effect. In addition, a transient depolarizing response to light was observed in a minority of human neurons but not in mice. These findings indicate that visible light, independently of exogenous opsins, can induce long-lasting modulation of neuronal activity without evidence of acute cytotoxicity under the conditions tested. This raises the possibility that visible light may provide a previously underappreciated, opsin-independent mechanism for modulating neuronal activity, with potential relevance for disorders characterized by neuronal hyperexcitability. We outline a framework for future investigations, including validation in human systems, in vivo studies, optimization of stimulation parameters, and assessment of therapeutic potential in pathological models.

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Running title: Light inhibition of neuronal activity

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Abbreviations: ACSF= artificial cerebrospinal fluid; AP = action potential; LED=Light-Emitting Diode ; Rm= membrane resistance; VG= voltage gated; Vm= membrane potential; Vrest = average resting membrane potential. R_A =access resistance

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 [1,2]. 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)[3], Transcranial Magnetic Stimulation[4], and Ultrasound Neuromodulation [5]. 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 [6]. 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[7,8]. 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 [9–12]. 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 [9,12]. 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 [9,10,12–14]. The magnitude of the visible light-induced neuronal inhibition effect increases with both light power and stimulation duration [9,13,14]. 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[9]). 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.

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 (Cut ACSF) 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) Cut ACSF 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. The delay between resection and slicing was between ten and twenty minutes. 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. Visual inspection of the neurons did not reveal any evident degradation compared with mouse tissue.

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.

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). Recordings were performed on cortical neurons from various regions and layers, displaying the morphological and electrophysiological characteristics of excitatory glutamatergic cells: pyramidal shape, high coefficient of variation of interspike intervals (ISI > 0.3), pronounced firing frequency adaptation (median ISI of the last spike / first spike > 1.5), and long action potential duration (FWHM > 0.5 ms). 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).

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 except for one neuron where a small 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 Fig 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. At the beginning of each sweep, a hyperpolarizing current s of -0.1 nA (1-second duration) was applied to assess membrane resistance (Rm). In the pre-light period, nine sweeps (6 minutes in total) 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 (from 20 to 42 minutes in total) 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.

Determination of light effect on the currents participating in the action potential generation

This procedure was applied only to mouse cortical neurons in voltage-clamp mode (see Supplementary Fig 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 having an amplitude of 78 mV (data from Lightning *et al.*, 2023a) . This waveform was preceded by 10 repetitions of the same AP scaled by a factor of −0.1. These inverted reduced APs (ranging from −7.8 mV to + 0.4 mV) were designed to elicit only passive membrane currents. The total passive current generated during the fake AP was calculated as the sum of the passive currents from the 10 inverted-reduced 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 t ratio (R_A first sweep/ R_A recorded sweep), the efficacy of this procedure to compensate for the modification in amplitude and kinetics of the recorded current is illustrated in supplementary Fig 2D. 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 $\Delta V=-5mV$ 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. R_A was calculated as $\Delta V/\Delta I_A$, 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 [15]. 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 a consistent increase or decrease in AP frequency over time was visually detected across sweeps 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 were excluded when spontaneous action potential firing occurred during the 1s LED stimulation. For currents analysis, cells were excluded when a consistent increase or decrease of recorded current over time was visually detected across sweeps during the pre-light period

Results

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

To explore the impact of repetitive 5s blue LED stimulation at 19 mW on cortical neurons from adult male mice, we conducted current clamp recordings following the protocol outlined in Fig 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 Fig 1 for additional details). As depicted in Fig 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 resulted in a long-lasting decrease in firing activity (39% ± 27% of the pre-LED activity, $p<0.01$, N=17, Fig 1Ci). Similar effects were observed when six repetitions of LED stimulation were applied (Fig 1Cii, 37 ± 29 % of the pre-LED activity, $p=0.015$, N=6). 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). However, no significant change in firing activity was observed when only a single light pulse was delivered (Fig. 1C iv, $p = 0.52$; N = 6). Suggesting that the repetitions of LED stimulation is required to produce the long-lasting effect. To assess the potential contribution of spontaneous drift to the firing changes observed after light stimulation, we performed a series of recordings in which, after a stable baseline activity had been established, neuronal firing was monitored for an additional 10 min prior to LED stimulation. As shown in Fig. 1C (vi–vii), no average modification were observed (138 ± 63% of baseline activity, $p=0.26$, N=9). Importantly, subsequent LED stimulation still produce firing decrease (-40 ± 30% from the drift value, $p= 0.04$).

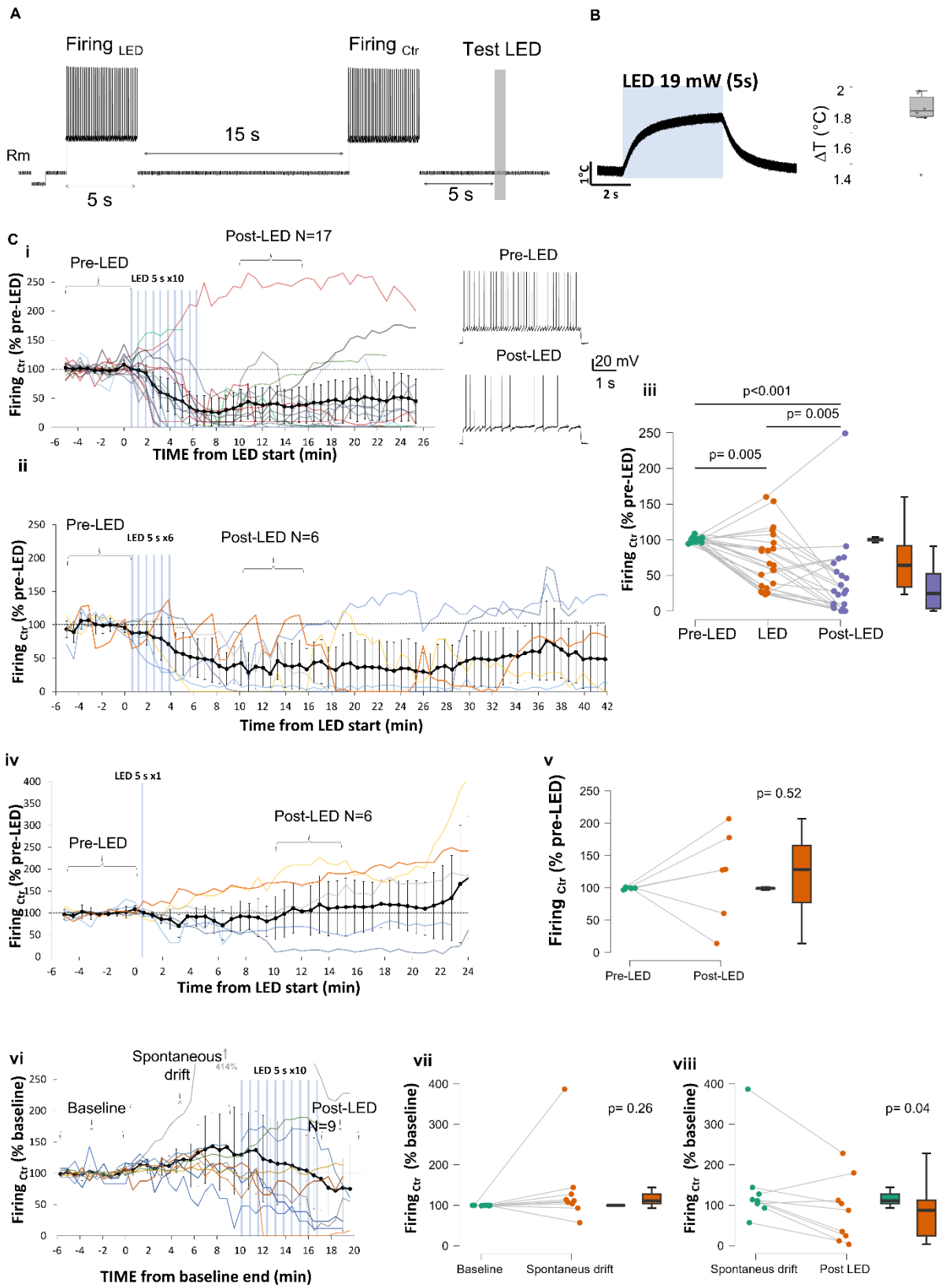

Figure 1 Light pulses induce a prolonged reduction in firing activity in mouse cortical neurons. **(A)** The current-clamp protocol used to study the effect of light on firing activity. This protocol

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. **Civ** Time plot illustrating the absence of modification of firing activity induced by 1 LED pulses. **Cv** Statistical quantification of the effect of 1 light pulse. **Cvi** Time plot illustrating the spontaneous evolution of firing activity after stable baseline period. **Cvii-Cvii** Statistical quantification of spontaneous evolution and the subsequent LED effect. (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 depict the evolution of the firing rate across trials for each recorded neuron. Raw data and analysis are available at: https://osf.io/uh73f/

The light stimulation results in a significant and enduring average reduction of neuronal Rm (-17 ± 15 MΩ, p=0.001, N=22, Fig 2A). Interestingly, the modification of firing produced by LED stimulation correlates with the Rm modification (r=0.52 [0.12;0.77], p=0.013, N=21, see Fig 2Aiii). These results suggest 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 [9,10,16], during test LED we observed a transient hyperpolarization of the membrane potential (Vm) (-0.31 ± 0.09 mV, p<0.001, N=38, 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 Fig 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).

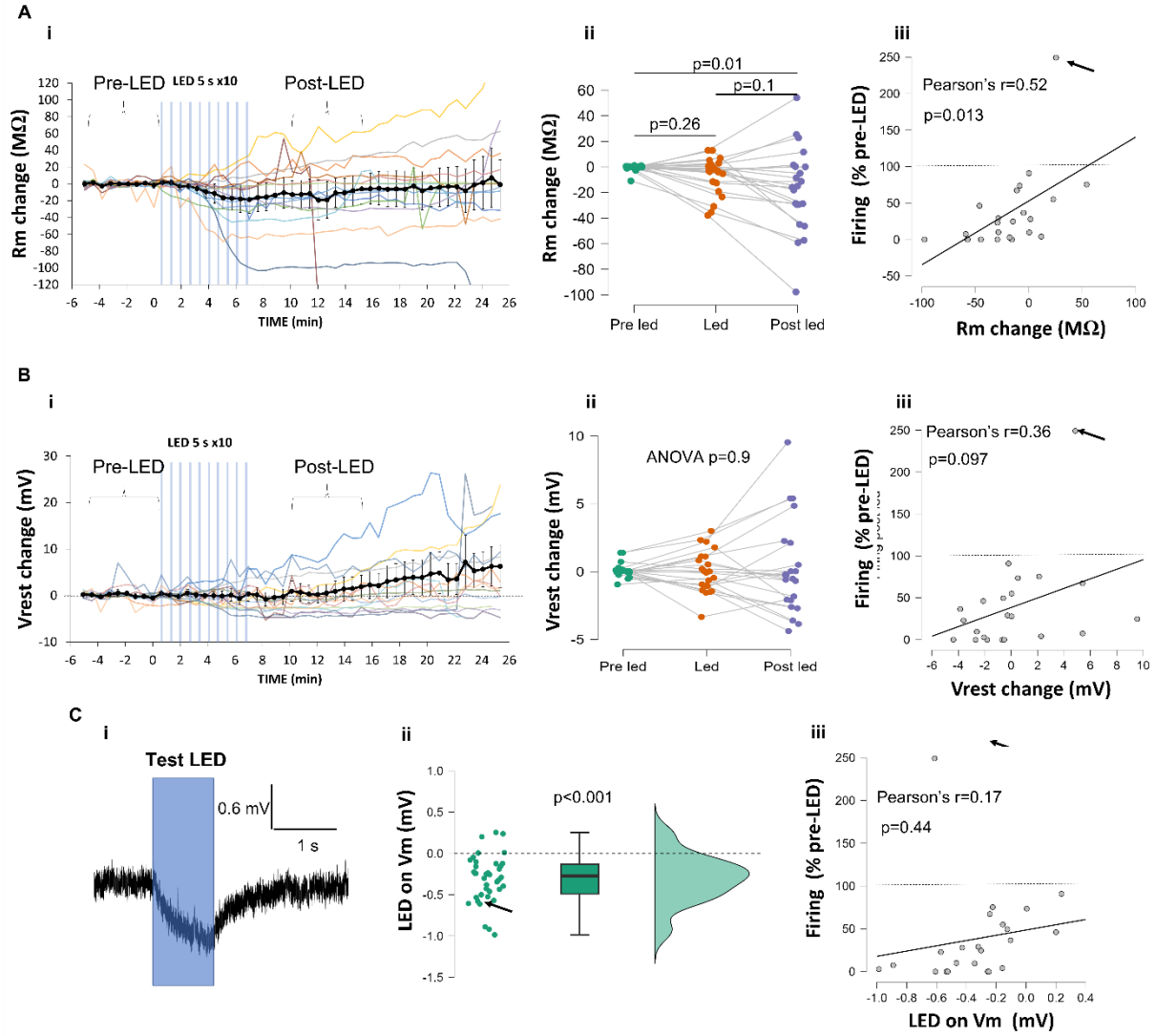

Figure 2. Changes in Rm explain part of the light effect on neuronal firing. (A) Repeated LED pulses resulted in a decrease in Rm. Ai. Time course of Rm modification induced by 10 LED pulses. Aii. Statistical quantification of the light effect on Rm in the three time periods. Aiii. LED-induced change 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 induces lasting changes in voltage-dependent currents underlying APs

To investigate the involvement of voltage-gated sodium and potassium channels on the effect of light on firing activity, voltage-clamp recordings were performed to detect 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 (Fig 3A, see Supplementary Fig 2 for more details). As shown in Fig 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, requiring around seven minutes after the first LED stimulus to reach the maximum average reduction (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). Interestingly, the inward current was completely abolished in two recorded neurons (18%), which closely matches the proportion of neurons (17%) in which firing activity fully disappeared in current-clamp experiments. It should be noted that in some neurons, the reduction of inward current following LED stimulation can revert back (see blue trace in Fig 3Bii). 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 Fig 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 was not altered (Fig. 3Civ, 3Cv).

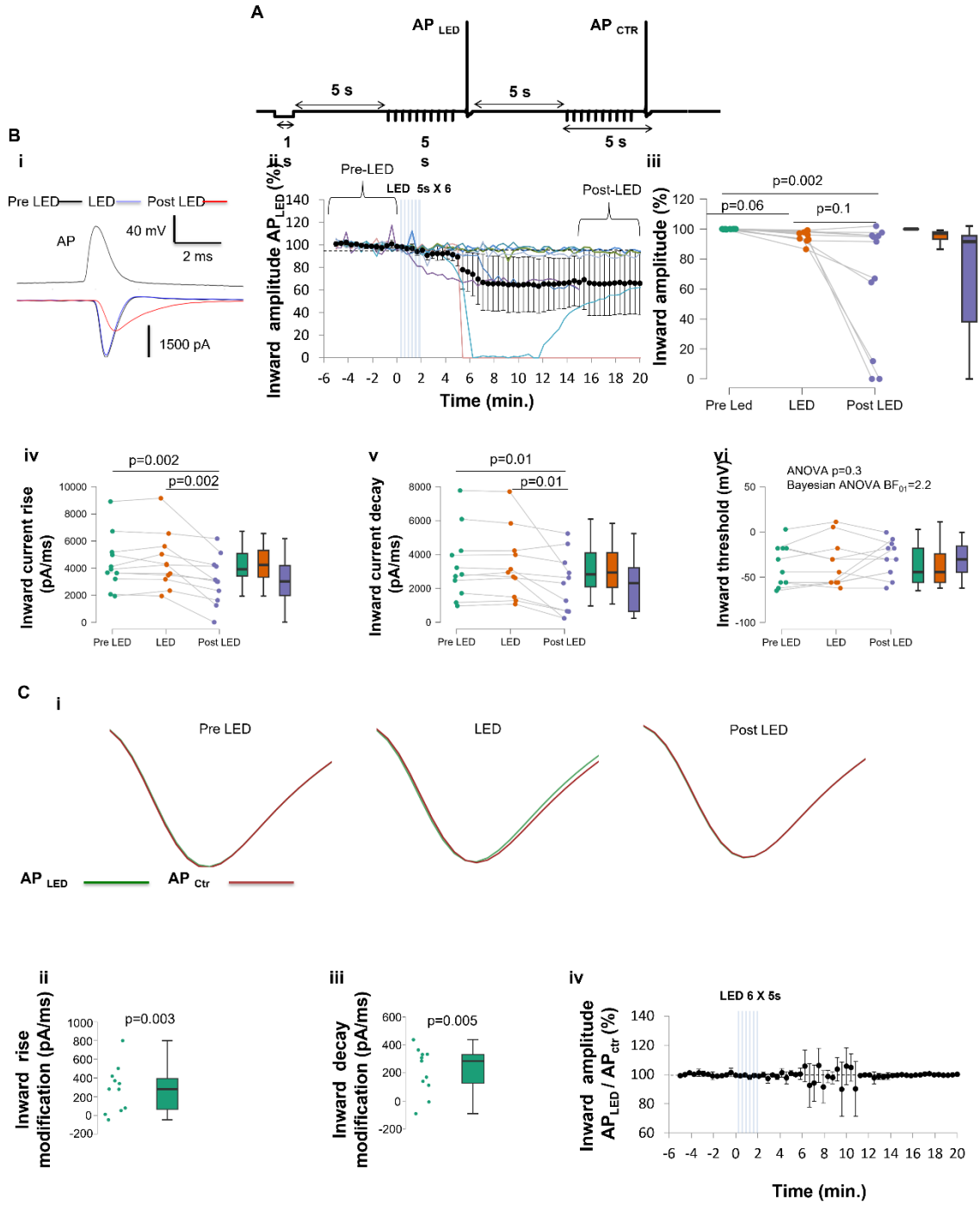

Figure 3. Light stimulation alters the voltage-gated inward current involved in AP generation. (A) Voltage clamp protocol used to isolate VG currents. Two consecutive AP potentials were generated by voltage command. AP_{LED} was generated simultaneously with light stimulation during the LED period. VG-currents 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 (n=11). Raw data and analysis are available at https://osf.io/pt4br/

As shown in Fig 4, LED stimulation induced a slight, long-lasting reduction in voltage-gated outward currents that did not reach statistical significance (average decrease of 45 ± 39 %, p=0.3 after Holm correction, N=8) (Fig 4Aii, Aiii). However 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.

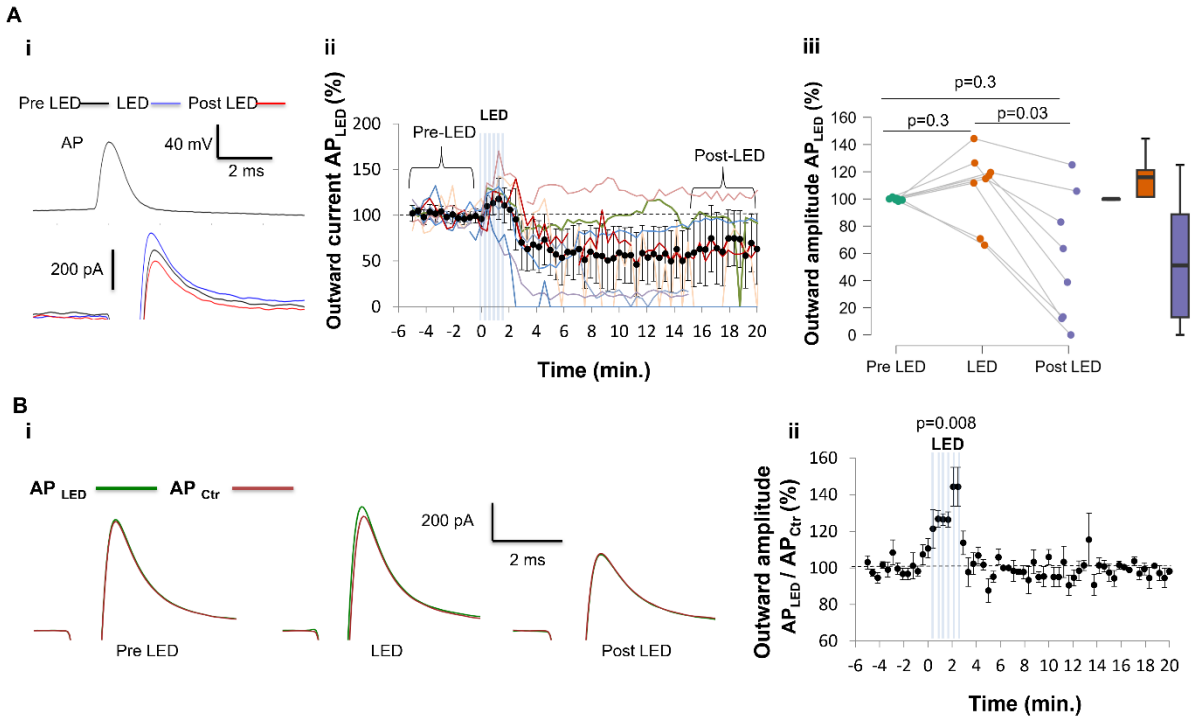

Figure 4. Light stimulation modifies the voltage-gated outward current involved in AP generation. (**A)** LED stimulation produces a long-lasting change in the amplitude of the voltage-gated outward current. Ai. Example of outward current modification by LED stimulation. Aii. Temporal evolution of VG outward current amplitude. Black dots and bars represent mean and 95% CI, respectively. Colored 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).

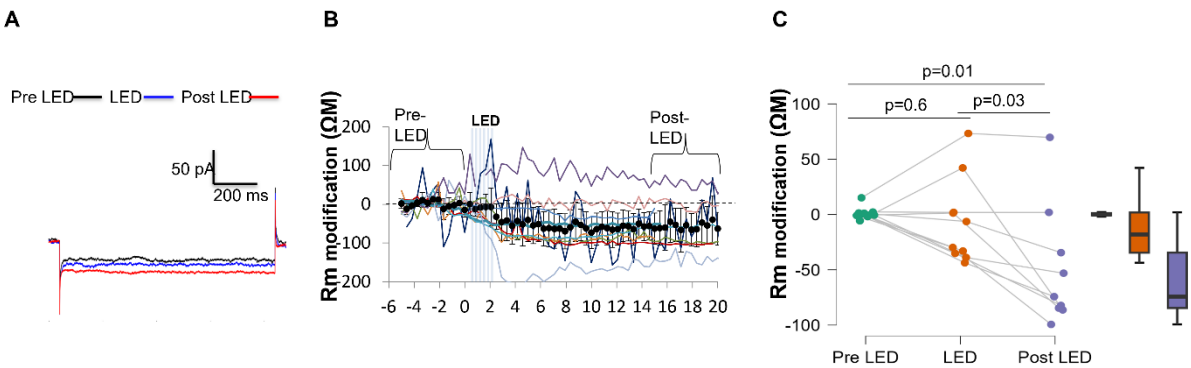

Figure 5. Light stimulation produces a long-lasting decrease of membrane resistance. (A) Example of passive membrane currents induced by 5 mV membrane hyperpolarization used to calculate Rm. (**B)** Temporal evolution of the Rm change. Black dots and bars represent mean and 95% CI, respectively. Colored 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 and an increase in the amplitude of the VG-outward currents[17]. 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 Fig 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.

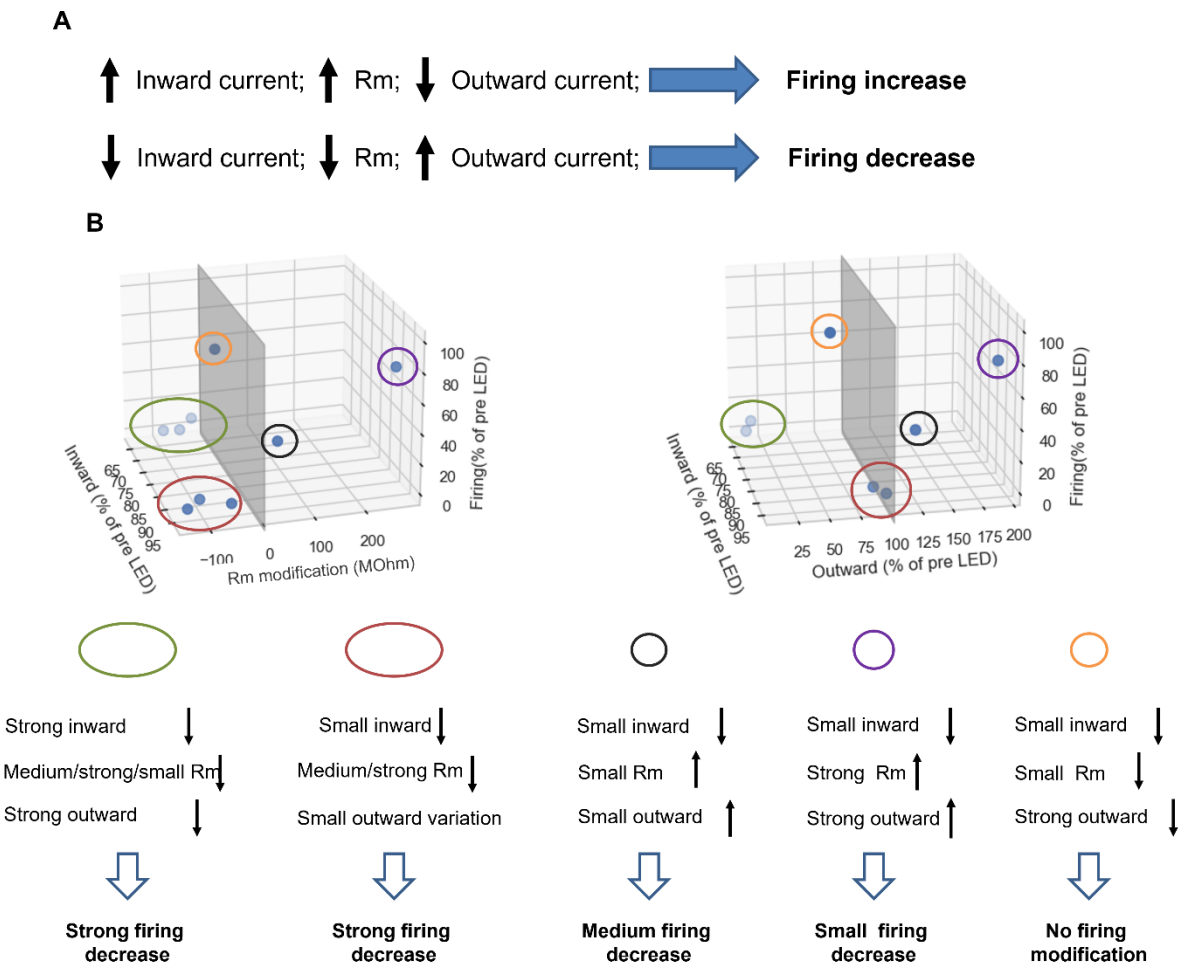

Figure 6. Relative contribution of the light-induced changes in VG-currents and Rm to the modification of neuronal firing rate. (**A**) Expected impact of VG-currents and Rm modifications on the direction of firing rate change. (**B**) Top, 3D plot illustrating the light-induced modification of firing 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 (Fig 1A). Tissue samples came from nine patients differing in age, sex, pathology, and the explanted cortical

region. 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%) (Fig 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, Fig 7B, see also Supplementary Fig 3 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, BF01=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. Other factors, including, patient age, and underlying pathology, may also act as confounding variables explaining the sex-related difference in the effect of light on neuronal firing. However, exploratory analyses show only moderate correlation between age and light effect and no effect due to the donor pathology (Fig 7 C-D). LED stimulation did not produce any modification of Rm or Vrest in human neurons (Supplementary figure 4).

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 Fig 3 C-D, light stimulation produces a similar long-lasting inhibition of firing activity in mouse neurons from both sexes (sex effect $p = 0.87$; BF01 = 3.5).

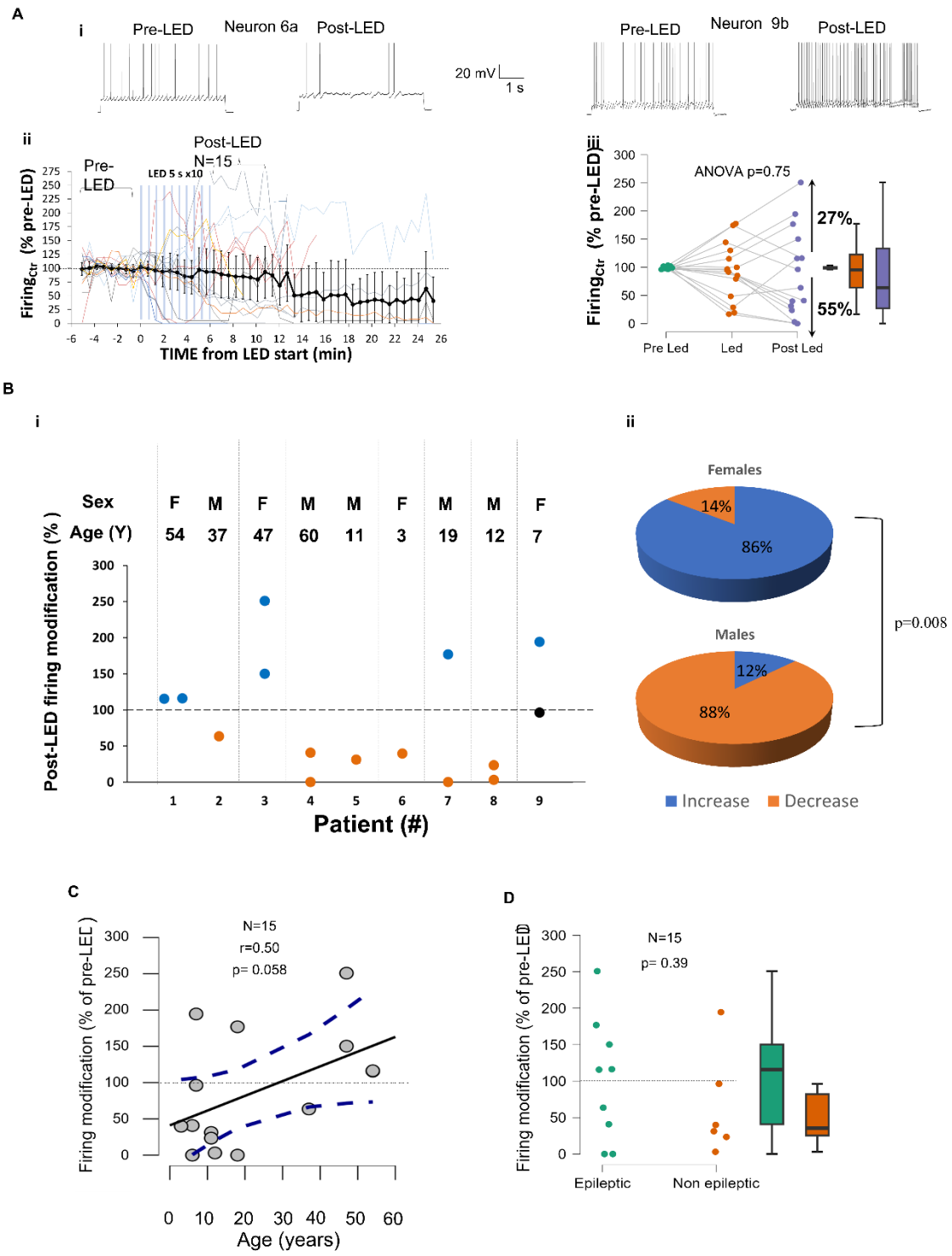

Figure 7. Light Pulses Induce a Prolonged and Heterogeneous Modification in Firing Activity in Human Cortical Neurons. (A) Repeated LED pulses induce a long-lasting decrease in firing activity in 55% of recorded neurons and an increase in firing in 27% of recorded neurons. Ai Representative traces showing 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. **(C)** Linear correlation between the effect of light and patient age **(D)** Long lasting effect of light on neuronal firing do not depend on patient pathology .Blue, orange, and black dots in B respectively represent neurons that showed a significant increase, a significant decrease, or no change in firing activity. Data are presented as mean ± 95% CI. Raw data and analysis are available at: https://osf.io/2wfy5/.

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 (Fig 8Ai, Aii), an effect that was never observed in mouse neurons in the present study (Fig 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, $p < 0.001$; Fig 8Aiii), consistent with the finding in mouse neurons (Bayesian statistic, Mice vs. Human, BF01 = 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, BF01=1.76).

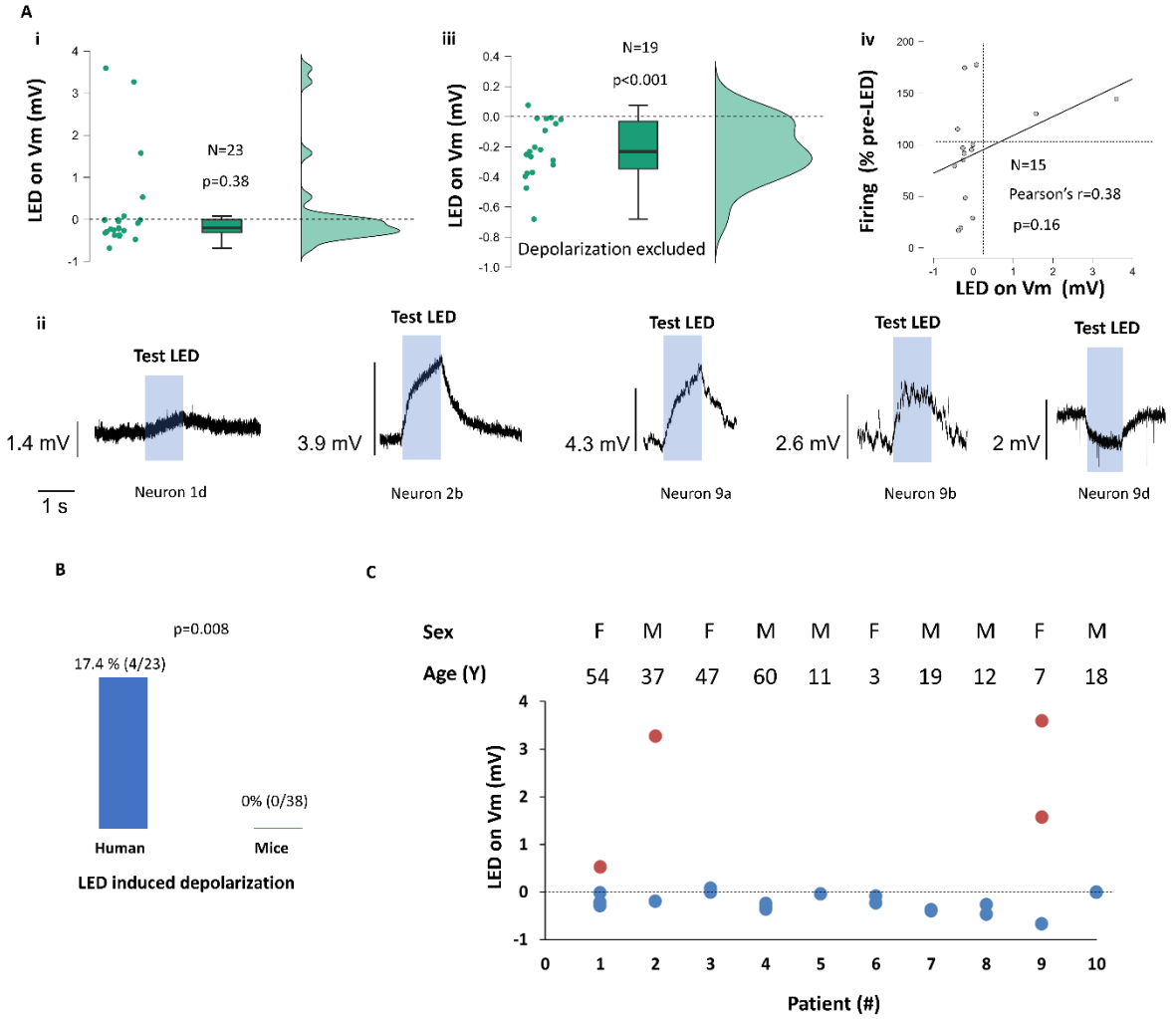

Figure 8. Heterogeneous modification of the membrane potential by LED stimulation in human neurons. **(A)** Light-induced membrane potential modification in human neurons. **Ai** Raincloud plot showing the effect of light on the entire sample. **Aii** Average traces illustrating the light-induced membrane potential 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 present study sought to determine whether light stimulation can induce a sustained reduction in neuronal excitability within the central nervous system. We show that repeated blue light exposure elicits a marked and persistent decrease in evoked firing activity in cortical neurons from both male and female mice, with a reduction of approximately 60% relative to baseline. This effect endures for tens of minutes following the cessation of stimulation. In human cortical neurons, however, the response was more variable, with a subset of neurons—particularly those derived from female subjects—exhibiting increased excitability in response to light. The prolonged modulation of neuronal activity is likely mediated by combined alterations in passive membrane properties (including membrane resistance, Rm) and active membrane conductance, notably voltage-gated Na^+ and K^+ channels.

Light Pulse Parameters Driving Sustained 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 [9–11,16]. 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. [10], where green light at 15 mW was used. In the present study, long-lasting firing inhibition was induced by 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. We show that ten or six repetitions of light stimulation are equally effective in producing long-lasting firing inhibition. Notably, as shown in Fig 1Cii, firing activity appears to continue decreaseing 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. However, a single LED stimulation was not sufficient to produce long-lasting modifications. In contrast, six repetitions of LED stimulation produced effects similar to those observed after tens of repetitions. This suggests that 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 resulted in approximately 10% cell mortality, potentially due to mitochondrial generation of reactive oxygen species (ROS) [18]. In cortical neuronal cultures, blue light stimulation 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 [19]. These findings were later replicated by [20] 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 [21]). To our knowledge, the potential cytotoxic effects of visible light on neurons, particularly under slice or in vivo conditions, remain insufficiently established. Our findings do not support a marked acute cytotoxic effect. Although light stimulation altered several biophysical properties of recorded neurons, there was no clear evidence of compromised cell viability in the short term. Neuronal death is typically associated with a rapid membrane depolarization; however, only a modest depolarization was observed following light exposure. In addition, the light-induced change in membrane resistance (−9%) was comparable in magnitude to variations reported under other experimental conditions [22,23]. Furthermore, while firing activity was reduced, it remained detectable in most neurons and, in some cases, showed partial or complete recovery over time. Nevertheless, we cannot exclude the possibility that repeated LED stimulation may affect neuronal survival over longer timescales. Addressing this question will require complementary approaches beyond in vitro electrophysiology (see Perspectives).

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. 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 [10]. 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). This suggests a modification in the permeability and activation-inactivation properties of the Na^+ which are primarily involved in action potential generation in cortical neurons [24]. 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 [17]. 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 [25]. 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[26]), excluding any thermal toxicity to the neurons. Finally, one possible mechanism leading to firing reduction could be the reported increase in GABAergic transmission in cortical neurons in response to visible light stimulation [27]. Although this effect reverted within 3–5 minutes, repetition of the stimulation and/or an increase in light power could account for the long-lasting reduction observed in the present study. Further experiments performed in the presence of GABA receptor blockers are required to address this possibility.

Species and sex differences in light effects

We identified two main differences in light responses between mouse and human cortical neurons. First, a long-lasting increase in firing activity was observed in approximately 30% of human neurons, whereas this effect was largely absent in mice. This apparent species difference may be influenced by a sex-specific effect observed only in human tissue, with neurons from female patients more frequently exhibiting increased excitability in response to light than those from male patients. Second, an acute depolarizing response to light was detected in a subset of human neurons (17%) but was not observed in mice. These differences may reflect known divergences in the biophysical properties of human versus rodent neurons[28–31]. While previous studies have suggested that light-induced hyperpolarization primarily involves increased permeability of inwardly rectifying K^+ (Kir) channels[10], the depolarizing responses observed in some human neurons are more consistent with modulation of channels permeable to Na^+ and/or

Ca^{2+}, such as Na^{+} leak channels or transient receptor potential (TRP) channels. The observed sex-related differences in the long-lasting effects of light may arise from established functional, anatomical, biochemical, genetic, and electrophysiological distinctions between male and female brains[32,33]. However, this interpretation remains tentative and requires further investigation.

Limitations and cavate

A key limitation concerns the interpretation of the apparent sex-dependent differences in human neuronal responses to light. Although supported by statistical analysis, this finding arises from an exploratory approach in which multiple factors, including age, pathology, and intrinsic electrophysiological properties, were examined as potential sources of variability. In addition, differences in age and cortical sampling between male and female subjects may act as confounding factors, thereby limiting the strength of this conclusion. Replication in larger and more controlled cohorts will therefore be required to confirm this observation. Finally, technical constraints inherent to the patch-clamp technique, including cytoplasmic dialysis and seal instability [23,34], may introduce variability in electrophysiological recordings and affect the accurate quantification of light-induced effects, particularly over prolonged durations. Alternative approaches will thus be necessary to more reliably assess the persistence of the firing changes described here (see following section).

Perspectives and conclusions

The present work was designed to assess whether visible light stimulation can induce durable changes in neuronal activity, and thereby inform its potential as a therapeutic strategy. The findings support the relevance of this approach and provide a basis for further investigation into its clinical applicability. Building on these results, we outline a conceptual framework to guide future research towards translational development:

First, the effects of light on human neurons require confirmation in larger and more standardized in vitro cohorts. In particular, the apparent sex-related differences observed here should be specifically addressed, alongside the potential influence of pharmacological treatments. The use of human brain organoids [35] may help increase tissue availability, reduce variability through standardized protocols, and enable the assessment of both efficacy and safety over extended time scales.

Second, it will be important to determine whether the long-lasting reduction in neuronal excitability observed ex vivo can be reproduced in vivo. While transient inhibitory effects of light have been reported in animal models [10,11], direct electrophysiological recordings and behavioural assessments will be necessary to evaluate the persistence and functional relevance of these effects in intact systems.

Third, the duration and reversibility of light-induced modulation of neuronal firing should be systematically characterized. From a therapeutic perspective, it is essential to establish whether these effects can be maintained over prolonged periods and re-induced following recovery. Given the limited temporal window of acute brain slices, alternative models—including neuronal cultures, organotypic slices, brain organoids, and in vivo preparations with chronic recordings—should be considered. These approaches would also allow a more comprehensive evaluation of long-term safety, encompassing both cellular integrity and possible behavioral effects.

Fourth, the sustained inhibitory effects of light suggest potential applications in neurological disorders characterized by neuronal hyperexcitability, such as epilepsy, migraine [36,37], autism spectrum disorder[38,39], and possibly schizophrenia[40] . Notably, optogenetic strategies are already widely used to suppress pathological activity in animal models of epilepsy [41–47]. In this context, it would be of particular interest to determine whether light alone, without exogenous opsin expression, can exert intrinsic antiepileptic effects using existing experimental platforms.

Finally, optimization of stimulation parameters will be required to maximize efficacy. The present study employed 5-s pulses of blue light at 19 mW; future work should explore variations in wavelength, intensity, and duration. In particular, longer wavelengths may offer improved tissue penetration, enabling modulation of deeper and more extensive brain regions [48].

Our findings support the potential of visible light stimulation as a non-invasive strategy for modulating neuronal excitability, with possible translational relevance. Future studies, guided by the framework outlined here, will be critical to validate these effects in human systems, optimize stimulation paradigms, and establish the conditions under which this approach may be developed into a therapeutic intervention for brain disorders.

Data 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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Competing interests

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

Supplementary material

Supplementary material would be available at Brain online

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