eNeuro Accepted Manuscript

eNeuro

Research Article: New Research | Novel Tools and Methods

Blue light-induced gene expression alterations in cultured neurons are the result of phototoxic interactions with neuronal culture media

https://doi.org/10.1523/ENEURO.0386-19.2019

Cite as: eNeuro 2019; 10.1523/ENEURO.0386-19.2019

Received: 25 September 2019
Revised: 27 November 2019
Accepted: 13 December 2019

This Early Release article has been peer-reviewed and accepted, but has not been through the composition and copyediting processes. The final version may differ slightly in style or formatting and will contain links to any extended data.

Alerts: Sign up at www.eneuro.org/alerts to receive customized email alerts when the fully formatted version of this article is published.

Copyright © 2019 Duke et al.
This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license, which permits unrestricted use, distribution and reproduction in any medium provided that the original work is properly attributed.

eNeuro Accepted Manuscript

1. Blue light-induced gene expression alterations in cultured neurons are the result of phototoxic interactions with neuronal culture media
2. Light induced gene changes *in vitro* depend on culture media
3. Corey G. Duke[1], Katherine E. Savell[2], Jennifer J. Tuscher[1], Robert A. Phillips III[1], & Jeremy J. Day[1]*

[1]Department of Neurobiology and Evelyn F. McKnight Brain Institute, University of Alabama at Birmingham, Birmingham, AL 35294, USA

[2]Behavioral Neuroscience Research Branch, Intramural Research Program, National Institute on Drug Abuse, NIH/DHHS, Baltimore, MD 21224, USA

*Corresponding author
4. C.G.D., K.E.S., J.J.T., and J.J.D conceived of the experiments. C.G.D., K.E.S., and J.J.T. performed the experiments with assistance from R.A.P. C.G.D., K.E.S., J.J.T., and R.A.P. performed statistical and graphical analysis, and data interpretation. C.G.D. and J.J.D. wrote the manuscript. J.J.D. supervised all work. All authors have approved the final version of the manuscript.
5. Jeremy J. Day, jjday@uab.edu
6. 4
7. 1
8. 0
9. 246
10. 98
11. 564
12. 579
13. This work was supported by NIH grants DA039650, DA034681, and MH114990 (J.J.D.), NS061788 (C.G.D.), DA042514 (K.E.S.). Additional assistance to J.J.D. was provided by the UAB Pittman Scholars Program.
14. The authors declare no competing financial interests.
15. NIH grants DA039650, DA034681, and MH114990 (J.J.D.), NS061788 (C.G.D.), DA042514 (K.E.S.). Additional assistance to J.J.D. was provided by the UAB Pittman Scholars Program.

eNeuro Accepted Manuscript

Blue light-induced gene expression alterations in cultured neurons are the result of phototoxic interactions with neuronal culture media

Abstract

Blue wavelength light is used as an optical actuator in numerous optogenetic technologies employed in neuronal systems. However, the potential side effects of blue light in neurons has not been thoroughly explored, and recent reports suggest that neuronal exposure to blue light can induce transcriptional alterations *in vitro* and *in vivo*. Here, we examined the effects of blue wavelength light in cultured primary rat cortical cells. Exposure to blue light (470nm) resulted in upregulation of several immediate early genes (IEGs) traditionally used as markers of neuronal activity, including *Fos* and *Fosb*, but did not alter the expression of circadian clock genes *Bmal1*, *Cry1*, *Cry2*, *Clock*, or *Per2*. IEG expression was increased following 4 hours of 5% duty cycle light exposure, and IEG induction was not dependent on light pulse width. Elevated levels of blue light exposure induced a loss of cell viability *in vitro*, suggestive of overt phototoxicity. Induction of IEGs by blue light was maintained in cortical cultures treated with AraC to block glial proliferation, indicating that induction occurred selectively in post-mitotic neurons. Importantly, changes in gene expression induced by blue wavelength light were prevented when cultures were maintained in a photoinert media supplemented with a photostable neuronal supplement instead of commonly utilized neuronal culture media and supplements. Together, these findings suggest that light-induced gene expression alterations observed *in vitro* stem from a phototoxic interaction between commonly used media and neurons, and offer a solution to prevent this toxicity when using photoactivatable technology *in vitro*.

Significance Statement

Technology utilizing blue wavelength light is increasingly utilized in neuroscience, and recent reports have noted unintended gene expression alterations during light exposure *in vitro*. Here, we identify light-induced gene expression alterations in rat cortical cultures, illustrate that this induction coincides with a loss of cell viability, and show that light induced gene induction is dependent on the culture media utilized

eNeuro Accepted Manuscript

in these experiments. We demonstrate that these unintended effects can be prevented by using phototinert media during to light exposure *in vitro,* opening the door for extended light exposure experiments when utilizing powerful optical techniques in neuronal cultures.

Introduction

Optically-driven technology has been widely adopted in neuroscientific investigation over the past 15 years (Boyden et al., 2005; Kim et al., 2017), opening new avenues into experimental design by allowing unprecedented spatial and temporal control over neuronal firing, protein signaling, and gene regulation. Blue wavelength light (~470nm) is most often used as the actuator of these technologies. For instance, channelrhodopsin (Boyden et al., 2005) is a light-gated ion channel that responds to blue light to allow for experimental control over neuronal firing. Similarly, cryptochrome 2 (Cry2) (Kennedy et al., 2010; Konermann et al., 2013; Polstein and Gersbach, 2015) and light-oxygen sensitive protein (LOV) based systems (Möglich et al., 2009; Dietz et al., 2012; Quejada et al., 2017) utilize blue light to regulate protein binding and gene expression. Additionally, genetically-encoded calcium sensor technologies to visualize neuronal activity states are becoming more widely utilized both *in vivo* and *in vitro*, and these sensors often rely on prolonged or repeated blue light exposure (Lin and Schnitzer, 2016; Deo and Lavis, 2018; Wang et al., 2018). Together, these optically-driven technologies provide robust experimental control and have enabled new insights into neuronal functioning in healthy and diseased states. However, increased use of these technologies in neuroscience also warrants a more complete understanding of potential off-target effects of prolonged exposure to blue light.

While the phototoxic effects of both ambient and targeted light on cell viability *in vitro* has been noted for decades (Wang, 1976; Dixit and Cyr, 2003; Carlton et al., 2010), recent reports documenting blue light-induced gene expression alterations both *in vitro* and *in vivo* have emphasized deleterious effects of blue light on cellular function (Marek et al., 2019; Tyssowski and Gray, 2019). Multiple reports have documented robust effects of blue light exposure *in vitro*, including upregulation of genes such as *Fos* (aka *cFos*) that are often used as markers of neuronal activity but which can also be induced in response

eNeuro Accepted Manuscript

to cellular stress (Bahrami and Drabløs, 2016; Marek et al., 2019; Tyssowski and Gray, 2019). Others have noted that cellular phototoxicity is often the result of reactive oxygen species (ROS) generated in culture media during photostimulation, which can be prevented by utilizing a non-light-reactive media instead of the typical media utilized in neuronal cultures (Stockley et al., 2017). To our knowledge, it has not yet been determined if the blue light-induced expression alterations of activity-dependent genes observed *in vitro* are the result of a stress response stemming from the culture conditions.

In the present work, we characterized the effects of blue light on gene expression and cell viability *in vitro* using a rat primary neuronal culture model. As recent reports indicate that ROS are generated when culture media is exposed to blue wavelength light (Dixit and Cyr, 2003; Marek et al., 2019), we hypothesized that light-induced alterations in gene expression would be dependent on the neuronal cell culture media utilized in these experiments. We replicated and extended previous literature by demonstrating that blue light exposure induces multiple IEGs in neuronal cultures, and characterized the duration, frequency, and temporal properties of this effect. Notably, we found that replacing cell culture media with a photostable media supplemented with antioxidants prevented blue light-induced gene expression alterations. Together, these experiments provide insight into the mechanism underlying the unwanted “off-target” effects observed when using optically-driven technology, and offer a path forward to achieving a more precise level of experimental control *in vitro*.

Methods

Animals. All experiments were performed in accordance with the University of Alabama at Birmingham Institutional Animal Care and Use Committee. Sprague-Dawley timed pregnant rat dams were purchased from Charles River Laboratories. Dams were individually housed until embryonic day 18 for cell culture harvest in an AAALAC-approved animal care facility on a 12-hour light/dark cycle with *ad libitum* food and water.

eNeuro Accepted Manuscript

Cortical Cell Cultures. Primary rat cortical cultures were generated from embryonic day 18 (E18) rat cortical tissue, as described previously (Day et al., 2013; Savell et al., 2016; 2019). Briefly, cell culture plates (Denville Scientific Inc.) were coated overnight with poly-L-lysine (Sigma-Aldrich; 50 µg/ml) and rinsed with diH_2O. Dissected cortical tissue was incubated with papain (Worthington LK003178) for 25 min at 37°C. After rinsing in complete Neurobasal media (Neurobasal Medium (Gibco; #21103049), supplemented with B27 (Gibco; #17504044, 1X concentration) and L-glutamine (Gibco; # 25030149, 0.5mM), a single cell suspension was prepared by sequential trituration through large to small fire-polished Pasteur pipettes and filtered through a 100 µm cell strainer (Fisher Scientific). Cells were pelleted, re-suspended in fresh media, counted, and seeded to a density of 125,000 cells per well on 24-well culture plates (65,000 cells/cm^2). Cells were grown in complete Neurobasal media for 11 days *in vitro* (DIV 11) in a humidified CO_2 (5%) incubator at 37°C with half media changes at DIV 1 and 5. On DIV 10, cells received either a half or full change to complete Neurobasal media, or complete NEUMO media (Neumo Media (Cell Guidance Systems; M07-500) supplemented with SOS (Cell Guidance Systems; M09-50, 1x concentration) and Glutamax (Thermo Fisher; 35050061, 1x concentration)), as indicated above. In experiments comparing complete Neurobasal media to complete NEUMO media, Glutamax at a 1x concentration was utilized in place of L-glutamine for the complete Neurobasal media DIV10 media change, so that the effects of SOS/NEUMO and Neurobasal/B27 could be compared directly. To block glial proliferation, β-d-arabinofuranoside hydrochloride (AraC; Sigma-Aldrich) was added to complete Neurobasal media on DIV4 to achieve a final concentration of 5 µM, as previously described (Henderson et al., 2019). These culture wells received half media changes on DIV1, DIV7, and a full media change on DIV10 with complete Neurobasal media prior to light exposure on DIV11. Control wells received the same media changes with no AraC present on the DIV 4 media change.

Illumination. A custom built 12 LED array was used to illuminate cells, as previously described (Polstein and Gersbach, 2014). Three series of four blue LEDs (Luxeon Rebel Blue (470nm) LEDs; SP-05-B4) regulated by a 700mA BuckPuck (Luxeon STAR) were mounted and soldered onto a rectangular grid circuit board (Radioshack) and positioned inside a plastic enclosure (Radioshack) beneath transparent

eNeuro Accepted Manuscript

plexiglass (2cm thick). Primary cortical culture plates were positioned atop this enclosure and illuminated from below. Irradiance was determined through an empty culture plate placed atop the light box at 6 positions without a foil wrapping and at 2 positions while encased in foil using a spectrophotometer (Spectrascan PR-670; Photo Research). Irradiance ranged from 0.40 mW/cm^2 in the corner position (0.42 mW/cm^2 while under foil), to 0.84 mW/cm^2 in the center (0.91 mW/cm^2 while under foil). An Arduino Uno was used to control LED arrays, delivering light in 1 second pulses at the frequencies required to achieve specific duty cycles. In all experiments, duty cycle percentage was defined as light on time/total time*100. Aluminum foil was placed on top of the culture dish and enclosure during light delivery. No-light control culture plates were placed atop an identical LED enclosure and wrapped in foil. All handling of culture plates was performed under red light conditions after DIV 5.

RNA extraction and RT-qPCR. Total RNA was extracted (RNAeasy kit, Qiagen) and reverse-transcribed (iScript cDNA Synthesis Kit, Bio-Rad) following the manufacturers' instructions. cDNA was subject to RT-qPCR for genes of interest in duplicate using a CFX96 real-time PCR system (Bio-Rad) at 95 °C for 3 min, followed by 40 cycles of 95 °C for 10 s and 58 °C for 30 s, followed by real-time melt analysis to verify product specificity, as described previously (Savell et al., 2016; 2019). *Gapdh* was used for normalization via the ΔΔCt method (Livak and Schmittgen, 2001). A list of PCR primer sequences is provided in **Table 1**.

Calcein AM Viability Assay. Cell viability was assessed using a Calcein AM Cell Viability Assay Kit (Trevigen; 4892-010-K) according to manufacturer's instructions for adherent cells. Briefly, cell culture media was removed followed by a wash with 400µl of Calcein AM DW Buffer. 200ul of Calcein AM DW Buffer and 200ul of Calcein AM Working Solution were then added to the culture well and allowed to incubate at 37°C in a humidified CO_2 (5%) incubator for 30 min. Culture well florescence was then assessed under 470nm excitation in a standard plate imager (Azure Biosystems c600), and quantified in ImageJ by taking the background subtracted mean pixel value of identical regions of interest areas

eNeuro Accepted Manuscript

encompassing individual culture wells. Background was calculated for subtraction by taking the mean pixel value of 2 regions above and below the cell culture plate.

Immunocytochemistry. Immunostaining to assess the cell type composition of the primary cortical cultures was performed as described previously (Savell et al., 2016). After removal of neuronal culture media, cells were washed with PBS and incubated at room temperature for 20 min in freshly prepared 4% paraformaldehyde in PBS. After fixation, cells were washed twice with PBS and neuronal membranes were permeabilized with PBS containing 0.25% Triton X-100 for 15 min at room temperature. Cells were then washed three times in PBS, blocked for 1 h (10% Thermo Blocker bovine serum albumin (BSA) #37525, 0.05% Tween-20, and 300 mM glycine in PBS) and co-incubated with Anti-NeuN Antibody, clone A60, Alexa Fluor 555 Conjugate (1:100 in PBS with 10% Thermo Blocker BSA Millipore Sigma catalog #MAB377A5, RRID: AB_2814948) and Anti-Glial Fibrillary Acidic Protein Antibody, clone GA5, Alexa Fluor® 488 (1:250 in PBS with 10% Thermo Blocker BSA, Millipore Sigma catalog #MAB3402X, RRID: AB_11210273) overnight at 4°C. Cells were then washed twice with PBS containing 0.25% Triton X-100, followed by a final wash with PBS for 10 min. Slide covers slips with Prolong Gold anti-fade medium (Invitrogen) containing 4,6-diamidino-2-phenylindole (DAPI) stain were placed atop the culture wells. A Nikon TiS inverted fluorescent microscope was used to capture 10X magnification (1,888mm^2 field of view) images from 6 wells (2 images/well) from a 24-well culture plate. Total number of NeuN and GFAP positive cells were quantified for each image captured using Cell Counter in ImageJ v2.0.0. Values for each cell population are expressed as a percentage of the total combined (GFAP+NeuN) number of cells.

Statistical Analysis. Transcriptional differences from RT-qPCR experiments were compared with either an unpaired *t*-test or one-way ANOVA with Dunnett's or Tukey's *post-hoc* tests where appropriate. Statistical significance was designated at $\alpha = 0.05$ for all analyses. Statistical and graphical analyses were performed with Prism software (GraphPad). Statistical assumptions (e.g., normality and homogeneity for parametric tests) were formally tested and examined via boxplots.

eNeuro Accepted Manuscript

Data Availability. All relevant data that support the findings of this study are available by request from the corresponding author.

Results

Blue light induces immediate early gene expression in primary cortical cultures

To investigate the effects of blue light exposure on gene expression *in vitro*, we exposed DIV11 primary cortical cultures to 470nm light and monitored gene expression with reverse transcription quantitative PCR (RT-qPCR; **Fig. 1**). Cortical cells cultured in standard media conditions (complete Neurobasal supplemented with B27) were placed on top of a blue LED array light box (Polstein and Gersbach, 2014) inside of a standard cell culture incubator. Pulsed 470nm light was delivered across 7 duty cycle conditions for 0.5 to 8 hrs, followed by RT-qPCR to compare gene expression of light-exposed plates to control plates that were not exposed to light (**Fig. 1a**). First, neuronal cultures were exposed to 5% duty cycle (1 s pulses every 19 s) light for 8 hr, and RNA was extracted to examine the effects of blue light exposure on immediate early gene (IEG) expression. RT-qPCR revealed significant induction of *Fos*, *Fosb*, *Egr1*, and *Arc* mRNA, but not mRNA arising from *Bdnf-IV* (**Fig. 1b**). To determine if blue light exposure had an effect on the circadian clock, expression of circadian rhythm genes *Bmal1*, *Clock*, *Per2*, *Cry2*, and *Cry1* was measured under same light exposure conditions. In contrast to robust changes in IEGs, no significant light-induced changes were documented at these key circadian rhythm genes (**Fig. 1c**).

Optogenetic methods often rely on precise programs of light stimulation. Therefore, we sought to understand if the duty cycle, pulse width, or duration of blue light influenced the induction of IEGs, using *Fos* mRNA as a representative marker. First, we varied the duty cycle to determine whether IEG induction scaled with increased light exposure. *Fos* mRNA was significantly induced at duty cycles of 5% and 2.5%, but not at 1.67% or 0.33% (**Fig. 1d**). Next, while maintaining 5% duty cycle light exposure for

eNeuro Accepted Manuscript

8 hrs, we varied the light pulse width to determine if the same total light exposure at different frequencies would impact the induction of *Fos* mRNA. All light pulse variations induced expression of *Fos* mRNA to similar levels, indicating that this effect was not dependent on pulse frequency (**Fig. 1e**). Finally, we sought to identify the duration of light exposure necessary to induce *Fos* mRNA by varying the overall length of light exposure. We detected differences in *Fos mRNA* at 4hr after light exposure began, but not at earlier timepoints (**Fig. 1f**). Taken together, these results demonstrate that blue wavelength light can alter gene expression in cortical cultures at relatively low duty cycles, that this effect is insensitive to specific exposure frequencies, and that longer exposure times were required to observe transcriptional responses at a 5% duty cycle.

Blue light is phototoxic to primary cortical cultures

To understand if light-induced gene expression alterations corresponded with changes in cell health, we next examined the effects of blue light exposure on cell viability (**Fig. 2**). Primary cortical cultures were exposed to blue light (470nm) for 8 hr (at 1.67%, 3.33%, and 6.67% duty cycles) before assessing cell health using fluorescence measurements in a Calcein AM viability assay in which decreased fluorescence marks a loss in cell viability (**Fig. 2a-b**). We observed decreased fluorescence intensity at both 3.33% and 6.67% light exposure as compared to a no-light control, indicative of cell death at these duty cycles (**Fig. 2c**). These findings suggest that cellular health is significantly impacted during sustained light exposure, correlating IEG induction with a loss in cellular viability.

Glia depleted cortical cultures maintain blue light-induced gene expression alterations

Next, we investigated if these alterations were neuron specific, as E18 rat primary cortical cultures often contain trace amounts of glial growth (**Fig. 3**). Immunostaining of GFAP confirmed that glial cells were present in these primary cortical cultures (**Fig 3a**), but in small numbers relative to NeuN+ neuronal cells (3.10% of positively stained cells were GFAP+ against NeuN staining across 6 culture wells, **Fig. 3b**). To determine if the blue light-induced gene expression response was dependent on the presence of proliferating glial cells, cytosine arabinoside (AraC, an inhibitor of DNA synthesis) was applied to deplete

eNeuro Accepted Manuscript

the cultures of dividing glial cells prior to light exposure (**Fig. 3c**). The cultures were then exposed to blue light for 8 hr at a 5% duty cycle and *Fos* gene expression was monitored. *Fos* mRNA was significantly increased in the light exposure groups relative to light-off controls to similar levels in both AraC treated wells and in control wells receiving no AraC treatment, suggesting that these blue light-induced effects are not dependent on glial presence. Together, these results demonstrate that cortical cell cultures used here contain only a small fraction of glial cells and demonstrate that glia are not required for light-induced transcriptional alterations.

Photoinert media protects cortical cultures from blue light-induced gene expression alterations

Recent reports suggest light-induced cell viability losses can be overcome with photoinert media (Stockley et al., 2017), but it remains unclear if light-induced gene expression effects are also dependent on the culture media utilized in these experiments. To examine the contributions of culture media to light-induced gene expression changes, we explored the effects of light exposure in neurons cultured in photoinert media (**Fig. 4**). Culture media was replaced 12 hr before light exposure with a full or half media change to either Neumo + SOS or Neurobasal + B27 prior to blue light exposure (8 hr at 5% duty cycle) (**Fig. 4a**). Interestingly, both a full and a half media change to photoinert media completely blocked light-induced *Fos* mRNA increases observed when using standard neuronal culture media (**Fig. 4b**). To confirm that neurons cultured in photoinert media remained physiologically capable of *Fos* gene induction, we depolarized neurons for 1 hr with potassium chloride (KCl, 25mM) stimulation in this media and observed significant upregulation of *Fos* mRNA (**Fig. 4c**). Taken together, these results suggest that light-induced upregulation of IEGs in cultured neuron experiments are the result of an interaction with light and culture media, not the result of a direct cellular response to light.

Discussion

The increased adoption of optical techniques requiring prolonged light exposure in neuroscience highlights a pressing need to both characterize and overcome any off-target effects due to light exposure

eNeuro Accepted Manuscript

alone. To better understand the effects of blue light exposure in cultured neurons, we exposed primary cortical cultures to blue wavelength light and monitored gene expression alterations and cell viability changes. We observed significant elevation of multiple IEGs in primary cultures in response to blue light, noting that this induction is dependent on the amount of light delivered, and that alterations occur after 4 hr of photostimulation or more. The IEGs we characterized are downstream of the ERK/MAPK pathways and upregulated in response to robust synaptic activation during long term plasticity induction (Sheng and Greenberg, 1990; West and Greenberg, 2011; Chung, 2015). However, these genes are also triggered in response to cellular stress, including exposure to reactive oxygen species at timescales consistent with those used here (Janssen et al., 1997; Hughes et al., 1999; Chaum et al., 2009; Bahrami and Drabløs, 2016). In contrast, we observed no alterations in expression of circadian rhythm machinery genes, suggesting that this IEG response was not due to light-induced alterations of the circadian cycle. The role of IEG family members in survival and programmed cell death are well known, with IEG induction often preceding and playing critical functions in apoptosis programs (Smeyne et al., 1993; Haby et al., 1994; Morris, 1995; Janssen et al., 1997; Ameyar et al., 2003; Gazon et al., 2017). To determine if this transcriptional response is indicative of cellular stress, we examined cell viability across increasing light exposures, demonstrating a decrease in cell viability with increasing amounts of blue light. These results suggest that the gene expression changes we observed following blue light exposure are associated with a cellular stress response.

Previous reports have found that culture media and its supplements can react with light to generate ROS, and recent efforts to overcome this have resulted in the generation of photostable culture media which prevents a decay in cell health during sustained light exposure (Wang, 1976; Dixit and Cyr, 2003; Stockley et al., 2017; Marek et al., 2019). Importantly, we report that blue light-induced alterations in IEGs such as *Fos* are prevented when neuronal culture media is transitioned to photostable solution supplemented with antioxidants before light exposure. While in this photostable media, neurons maintain their ability to elicit IEG induction following strong depolarization, indicating that the light-induced gene response is dependent on culture media and can be readily overcome.

eNeuro Accepted Manuscript

With the rapid and widespread adoption of light-inducible technologies in neurobiology (Rost et al., 2017), these results provide a path forward when utilizing these techniques *in vitro*. Recent reports have documented light-induced gene expression alterations of *Fos in vivo* (Villaruel et al., 2018), which may be the result of a similar stress response from poor heat dissipation during extended exposure times *in vivo* (Owen et al., 2019). In sum, our study highlights the importance of experimental design when using photoactivatable and imaging technologies. Specifically, these results highlight the necessity of including a light exposure only control group when adapting these promising techniques to particular experimental conditions, and the utilization of photostable culture media wherever possible. Improving experimental precision and accuracy is of high priority given the remarkable experimental control and power these techniques provide. Together, the approach outlined here offers an easily implementable solution for the integration of photoactivatable technologies to neuroscientific inquiry *in vitro* that mitigates experimental confounds due to phototoxicity.

eNeuro Accepted Manuscript

References

Ameyar M, Wisniewska M, Weitzman JB (2003) A role for AP-1 in apoptosis: the case for and against. Biochimie 85:747–752.

Bahrami S, Drabløs F (2016) Gene regulation in the immediate-early response process. Adv Biol Regul 62:37–49.

Boyden ES, Zhang F, Bamberg E, Nagel G, Deisseroth K (2005) Millisecond-timescale, genetically targeted optical control of neural activity. Nature Neuroscience 8:1263–1268.

Carlton PM, Boulanger J, Kervrann C, Sibarita J-B, Salamero J, Gordon-Messer S, Bressan D, Haber JE, Haase S, Shao L, Winoto L, Matsuda A, Kner P, Uzawa S, Gustafsson M, Kam Z, Agard DA, Sedat JW (2010) Fast live simultaneous multiwavelength four-dimensional optical microscopy. Proc Natl Acad Sci USA 107:16016–16022.

Chaum E, Yin J, Yang H, Thomas F, Lang JC (2009) Quantitative AP-1 gene regulation by oxidative stress in the human retinal pigment epithelium. J Cell Biochem 108:1280–1291.

Chung L (2015) A Brief Introduction to the Transduction of Neural Activity into Fos Signal. Dev Reprod 19:61–67.

Day JJ, Childs D, Guzman-Karlsson MC, Kibe M, Moulden J, Song E, Tahir A, Sweatt JD (2013) DNA methylation regulates associative reward learning. Nature Neuroscience 16:1445–1452.

Deo C, Lavis LD (2018) Synthetic and genetically encoded fluorescent neural activity indicators. Curr Opin Neurobiol 50:101–108.

Dietz DM et al. (2012) Rac1 is essential in cocaine-induced structural plasticity of nucleus accumbens neurons. Nature Neuroscience 15:891–896.

eNeuro Accepted Manuscript

333 Dixit R, Cyr R (2003) Cell damage and reactive oxygen species production induced by fluorescence
334 microscopy: effect on mitosis and guidelines for non-invasive fluorescence microscopy. Plant J
335 36:280–290.

336 Gazon H, Barbeau B, Mesnard J-M, Peloponese J-M (2017) Hijacking of the AP-1 Signaling Pathway
337 during Development of ATL. Front Microbiol 8:2686.

338 Haby C, Lisovoski F, Aunis D, Zwiller J (1994) Stimulation of the cyclic GMP pathway by NO induces
339 expression of the immediate early genes c-fos and junB in PC12 cells. J Neurochem 62:496–501.

340 Henderson BW, Greathouse KM, Ramdas R, Walker CK, Rao TC, Bach SV, Curtis KA, Day JJ,
341 Mattheyses AL, Herskowitz JH (2019) Pharmacologic inhibition of LIMK1 provides dendritic spine
342 resilience against β-amyloid. Sci Signal 12:eaaw9318.

343 Hughes PE, Alexi T, Walton M, Williams CE, Dragunow M, Clark RG, Gluckman PD (1999) Activity and
344 injury-dependent expression of inducible transcription factors, growth factors and apoptosis-related
345 genes within the central nervous system. Prog Neurobiol 57:421–450.

346 Janssen YM, Matalon S, Mossman BT (1997) Differential induction of c-fos, c-jun, and apoptosis in lung
347 epithelial cells exposed to ROS or RNS. Am J Physiol 273:L789–L796.

348 Kennedy MJ, Hughes RM, Peteya LA, Schwartz JW, Ehlers MD, Tucker CL (2010) Rapid blue-light-
349 mediated induction of protein interactions in living cells. Nat Methods 7:973–975.

350 Kim CK, Adhikari A, Deisseroth K (2017) Integration of optogenetics with complementary methodologies
351 in systems neuroscience. Nat Rev Neurosci 18:222–235.

352 Konermann S, Brigham MD, Trevino A, Hsu PD, Heidenreich M, Cong L, Platt RJ, Scott DA, Church GM,
353 Zhang F (2013) Optical control of mammalian endogenous transcription and epigenetic states.
354 Nature 500:472–476.

eNeuro Accepted Manuscript

355 Lin MZ, Schnitzer MJ (2016) Genetically encoded indicators of neuronal activity. Nature Neuroscience
356 19:1142–1153.

357 Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative
358 PCR and the 2(-Delta Delta C(T)) Method. Methods 25:402–408.

359 Marek V, Potey A, Réaux-Le-Goazigo A, Reboussin E, Charbonnier A, Villette T, Baudouin C, Rostène
360 W, Denoyer A, Mélik Parsadaniantz S (2019) Blue light exposure in vitro causes toxicity to trigeminal
361 neurons and glia through increased superoxide and hydrogen peroxide generation. Free Radic Biol
362 Med 131:27–39.

363 Morris BJ (1995) Stimulation of immediate early gene expression in striatal neurons by nitric oxide.
364 Journal of Biological Chemistry 270:24740–24744.

365 Möglich A, Ayers RA, Moffat K (2009) Design and signaling mechanism of light-regulated histidine
366 kinases. J Mol Biol 385:1433–1444.

367 Owen SF, Liu MH, Kreitzer AC (2019) Thermal constraints on in vivo optogenetic manipulations. Nature
368 Neuroscience 22:1061–1065.

369 Polstein LR, Gersbach CA (2014) Light-inducible gene regulation with engineered zinc finger proteins.
370 Methods Mol Biol 1148:89–107.

371 Polstein LR, Gersbach CA (2015) A light-inducible CRISPR-Cas9 system for control of endogenous gene
372 activation. Nat Chem Biol 11:198–200.

373 Quejada JR, Park S-HE, Awari DW, Shi F, Yamamoto HE, Kawano F, Jung JC, Yazawa M (2017)
374 Optimized light-inducible transcription in mammalian cells using Flavin Kelch-repeat F-
375 box1/GIGANTEA and CRY2/CIB1. Nucleic Acids Res 45:e172–e172.

376 Rost BR, Schneider-Warme F, Schmitz D, Hegemann P (2017) Optogenetic Tools for Subcellular
377 Applications in Neuroscience. Neuron 96:572–603.

eNeuro Accepted Manuscript

Savell KE, Bach SV, Zipperly ME, Revanna JS, Goska NA, Tuscher JJ, Duke CG, Sultan FA, Burke JN, Williams D, Ianov L, Day JJ (2019) A Neuron-Optimized CRISPR/dCas9 Activation System for Robust and Specific Gene Regulation. eNeuro 6:ENEURO.0495–18.2019.

Savell KE, Gallus NVN, Simon RC, Brown JA, Revanna JS, Osborn MK, Song EY, O'Malley JJ, Stackhouse CT, Norvil A, Gowher H, Sweatt JD, Day JJ (2016) Extra-coding RNAs regulate neuronal DNA methylation dynamics. Nature Communications 7:12091.

Sheng M, Greenberg ME (1990) The regulation and function of c-fos and other immediate early genes in the nervous system. Neuron 4:477–485.

Smeyne RJ, Vendrell M, Hayward M, Baker SJ, Miao GG, Schilling K, Robertson LM, Curran T, Morgan JI (1993) Continuous c-fos expression precedes programmed cell death in vivo. Nature 363:166–169.

Stockley JH, Evans K, Matthey M, Volbracht K, Agathou S, Mukanowa J, Burrone J, Káradóttir RT (2017) Surpassing light-induced cell damage in vitro with novel cell culture media. Sci Rep 7:849.

Tyssowski KM, Gray JM (2019) Blue light increases neuronal-activity-regulated gene expression in the absence of optogenetic proteins. eNeuro:ENEURO.0085–19.2019.

Villaruel FR, Lacroix F, Sanio C, Sparks DW, Chapman CA, Chaudhri N (2018) Optogenetic Activation of the Infralimbic Cortex Suppresses the Return of Appetitive Pavlovian-Conditioned Responding Following Extinction. Cereb Cortex 28:4210–4221.

Wang H, Jing M, Li Y (2018) Lighting up the brain: genetically encoded fluorescent sensors for imaging neurotransmitters and neuromodulators. Curr Opin Neurobiol 50:171–178.

Wang RJ (1976) Effect of room fluorescent light on the deterioration of tissue culture medium. In Vitro 12:19–22.

eNeuro Accepted Manuscript

West AE, Greenberg ME (2011) Neuronal activity-regulated gene transcription in synapse development and cognitive function. Cold Spring Harb Perspect Biol 3:a005744–a005744.

eNeuro Accepted Manuscript

Figure 1. Blue light induces immediate early gene expression in primary cortical cultures. (**a**) Illustration of the experimental design. Primary rat cortical cultures were placed on top of a light box and exposed to blue (470nm) light prior to measurement of gene expression with RT-qPCR. (**b**) Blue light induces gene expression alterations at multiple immediate early genes (n = 5, unpaired t-test; *Fos* $t_{(8)}$ = 6.301, P = 0.0002; *Fosb* $t_{(8)}$ = 6.384, P = 0.0002; *Egr1* $t_{(8)}$ = 7.613, P < 0.0001; *Arc* $t_{(8)}$ = 10.54, P < 0.0001; *Bdnf-IV* $t_{(8)}$ = 1.563, P = 0.1566). (**c**). Circadian rhythm genes were not altered by this blue light exposure (n = 4, unpaired t-test; *Bmal1* $t_{(6)}$ = 1.772, P = 0.1268; *Clock* $t_{(6)}$ = 1.499, P = 0.1845 *Per2* $t_{(6)}$ = 1.910, P = 0.1048; *Cry2* $t_{(6)}$ = 1.491, P = 0.1865; *Cry1* $t_{(6)}$ = 0.7978, P = .4554). (**d**) *Fos* gene expression alterations are dependent on the amount of light exposure received (n = 4, One-Way ANOVA; $F_{(4, 15)}$ = 215.1, P < 0.0001). (**e**) Gene induction is not dependent on pulse width when duty cycle is held constant (n = 4, one-Way ANOVA; $F_{(3, 12)}$ = 32.96, P < 0.0001). (**f**) Gene expression is altered as early as 4 hr after light exposure (n = 4, One-Way ANOVA; $F_{(4, 15)}$ = 9.075, P = 0.0006). All data are expressed as mean ± s.e.m. Individual comparisons, **P < 0.01, ***P < 0.001, ****P < 0.0001. D.C. = Duty Cycle.

Figure 2. Blue light is phototoxic to primary cortical cultures. (**a**) Illustration of the experimental design. Primary rat cortical cultures were exposed to blue wavelength light before cell viability was assessed with a Calcein AM assay. (**b**). Blue light causes a loss in cell viability with increased light exposure. (**c**) Quantified effects of blue light exposure on cell viability at different duty cycles (n = 2, One-Way ANOVA; $F_{(3, 4)}$ = 10.20, P = 0.0241). All data are expressed as mean ± s.e.m. Individual comparisons, *P < 0.05.

Figure 3. Glia depleted cortical cultures maintain blue light-induced alterations in *Fos* mRNA expression. (**a**) Immunocytochemistry for NeuN and GFAP in primary rat cortical cultures. (**b**) Quantification of NeuN+ and GFAP+ cells revealed that 96.9% of positively stained cells were NeuN+ across 6 culture wells (**c**). Depletion of glial cells using AraC (5μM) supplemented culture media did not prevent blue light-induced gene expression changes (n = 12, unpaired t-test; *Neurobasal/B27* $t_{(22)}$ =

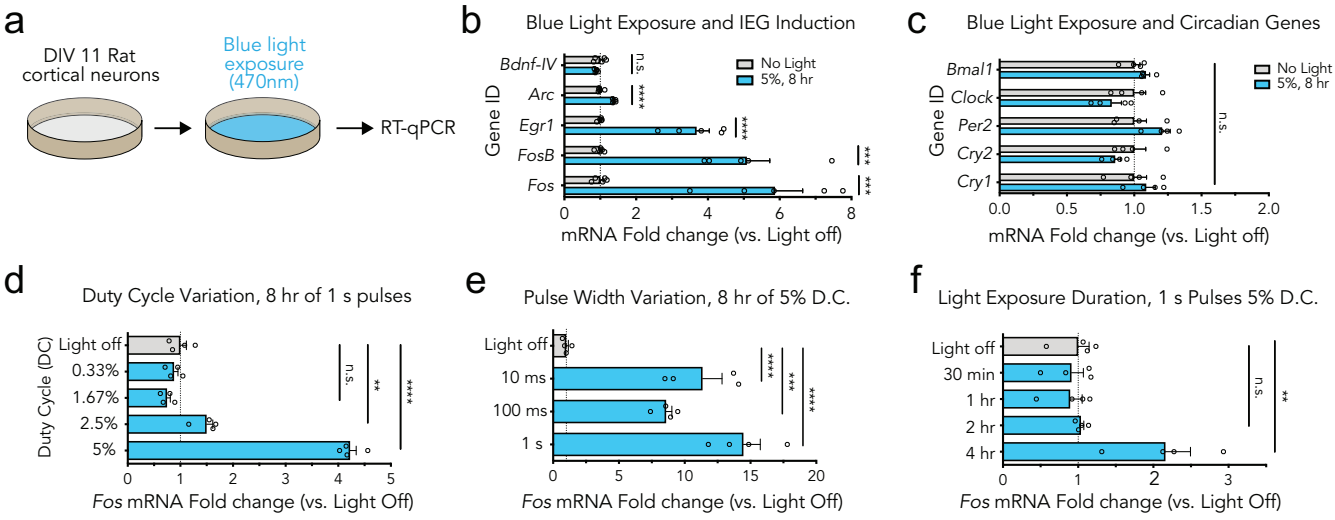
eNeuro Accepted Manuscript

11.19, P = <0.000001; *AraC + Neurobasal/B27* $t_{(22)}$ = 13.82, P = <0.000001). All data are expressed as mean ± s.e.m. Individual comparisons, ****P < 0.0001. D.C. = Duty Cycle.

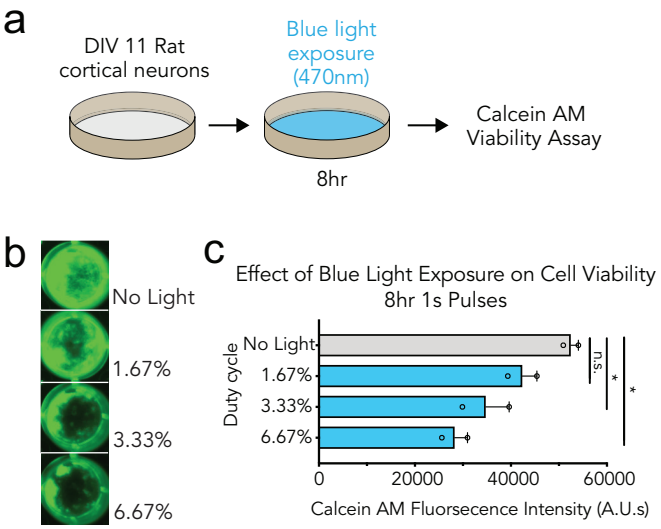
Figure 4. Photoinert media protects cortical cultures from blue light-induced gene expression alterations. (**a**) Illustration of the experimental design. Primary rat cortical cultures were exposed to blue wavelength light 12 hr following a media change and then gene expression was assessed by RT-qPCR. (**b**). Blue light exposure does not induce *Fos* mRNA changes in photoprotective culture media, even if only a half media change is performed (n = 3-9, unpaired t-test; Neurobasal $t_{(14)}$ = 6.012, P = 0.000032; Neumo (1/2) $t_{(4)}$ = 0.4099, P = 0.708249; Neumo (Full) $t_{(16)}$ = 0.02414, P = 0.981036). (**c**) *Fos* mRNA can be induced by a 1 hr 25mM KCl stimulation in photoprotective media, indicating that the cultures are still capable of induced gene expression alterations (n = 4, unpaired t-test, two-tailed; $t_{(6)}$ = 5.221, P = .0020). All data are expressed as mean ± s.e.m. Individual comparisons, **P < 0.01, ****P < 0.0001. D.C. = Duty Cycle.

Table 1. RT-qPCR Primer Sets. RT-qPCR primer sets utilized in the experiments detailed in this manuscript.

eNeuro Accepted Manuscript

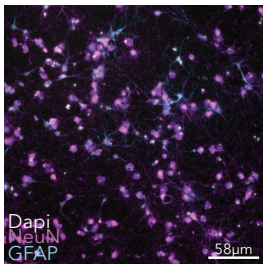


eNeuro Accepted Manuscript

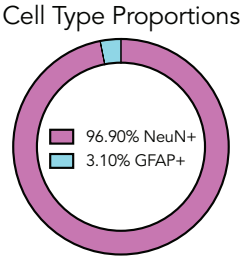


eNeuro Accepted Manuscript

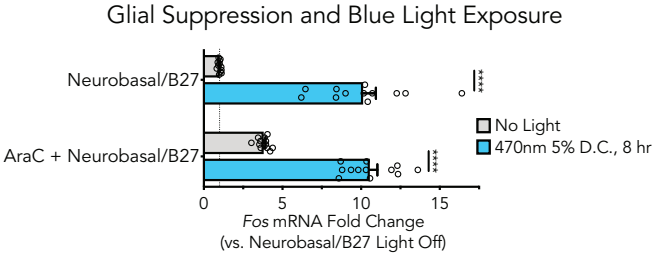
a



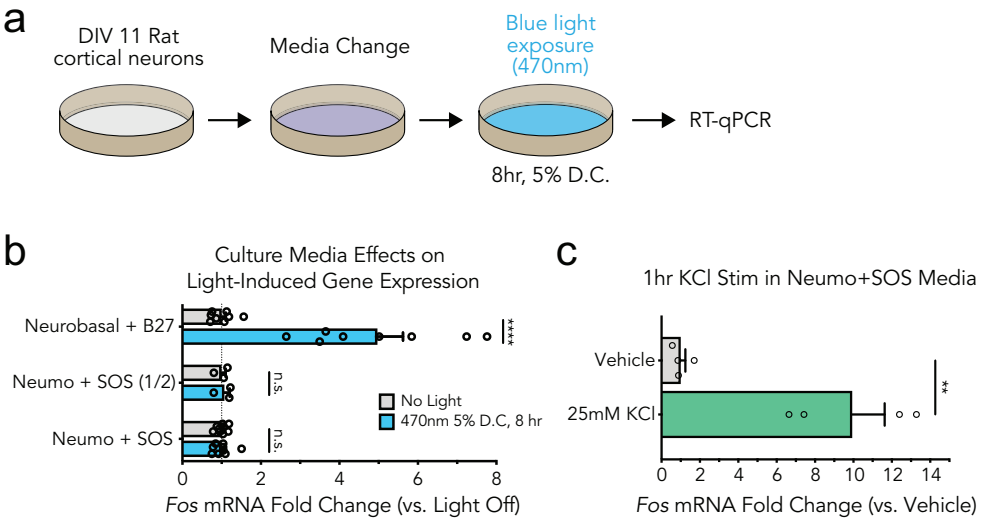
b



c



eNeuro Accepted Manuscript



eNeuro Accepted Manuscript

Table 1. RT-qPCR Primer Sets Utilized in this Study.

Gene	Forward Primer	Reverse Primer
Gapdh	ACCTTTGATGCTGGGGCTGGC	GGGCTGAGTTGGGATGGGGACT
Fos	CAGCCTTTCCTACTACCATTCC	ACAGATCTGCGCAAAAGTCC
Egr1	TCCTCAAGGGGAGCCGAGCG	GGTGATGGGAGGCAACCGGG
Fosb	TGCAGCTAAATGCAGAAACC	CTCTTCGAGCTGATCCGTTT
Arc	GCTGAAGCAGCAGACCTGA	TTCACTGGTATGAATCACTGCT
Bdnf IV	GCTGCCTTGATGTTTACTTTGA	GCAACCGAAGTATGAAATAACC
Per2	CACCCTGAAAAGAAAGTGCGA	CAACGCCAAGGAGCTCAAGT
Cry1	AAGTCATCGTGCGCATTTCA	TCATCATGGTCGTCGGACAGA
Cry2	GGATAAGCACTTGGAACGGAA	ACAAGTCCCACAGGCGGT
Clock	TCTCTTCCAAACCAGACGCC	TGCGGCATACTGGATGGAAT
Bmal1	CCGATGACGAACTGAAACACCT	TGCAGTGTCCGAGGAAGATAGC