Title
Hot topic in optogenetics: new implications of in vivo tissue heating

Journal
Nature Neuroscience, 22(7)

ISSN
1097-6256

Authors
Cardozo Pinto, Daniel F
Lammel, Stephan

Publication Date
2019-07-01

DOI
10.1038/s41593-019-0426-z

Peer reviewed

HHS Public Access

Author manuscript

Nat Neurosci. Author manuscript; available in PMC 2020 July 01.

Published in final edited form as:

Nat Neurosci. 2019 July ; 22(7): 1039–1041. doi:10.1038/s41593-019-0426-z.

Hot topic in optogenetics: new implications of *in vivo* tissue heating

Daniel F. Cardozo Pinto[1], **Stephan Lammel**[2,3,*]

[1]Department of Psychiatry and Behavioral Sciences, Stanford University, Stanford, CA 94305, USA

[2]Department of Molecular and Cell Biology and Helen Wills Neuroscience Institute, University of California Berkeley, Berkeley, CA 94720, USA

[3]Lead Contact

Abstract

In this issue of Nature Neuroscience, Owen et al. show that widely used optogenetic light delivery can heat brain tissue and produce changes in neural activity and behavior in the absence of opsins. How will this finding influence experimental design in the optical age of neuroscience?

MAIN TEXT

Optogenetic technologies have wrenched the idea of shooting lasers into the brain away from the domain of science fiction and established it as standard practice in the neurobiology laboratory. Today, the confluence of light-sensitive ion channels, viral vector technologies, and transgenic mouse lines enables investigators to study the biological and behavioral consequences of toggling neural activity on or off with the flip of a switch; but what unintended effects might we be causing when we blast neurons with photons? A new study by Owen et al.[1] demonstrates that tissue heating caused by illumination of dorsal striatum suppresses neural activity in medium spiny neurons (MSNs) and affects locomotor behavior. These effects were more pronounced with continuous light delivery, as commonly used in opto-inhibition experiments.

Optogenetic control of neural activity *in vivo* involves a variety of manipulations including viral infection, expression of exogenous proteins, and implantation of an optical fiber with concomitant lesion of structures above the targeted brain area. All of these manipulations are controlled for with the widely used practice of comparing an experimental group expressing an opsin coupled to a fluorophore with an otherwise identical group expressing only the fluorophore. However, in these experiments both cohorts receive light.

*Correspondence: Stephan Lammel, Ph.D., Department of Molecular and Cell Biology and Helen Wills Neuroscience Institute 142 Life Science Addition #3200 University of California Berkeley Berkeley, CA 94720, USA Phone: 510-664-7821 lammel@berkeley.edu.

COMPETING INTERESTS

The authors declare no competing interests.

Owen et al. asked whether the light delivery itself could be changing neural activity. Indeed, there was reason to suspect that this might be the case. Like pavement on a sunny day, the brain absorbs and is warmed by light, and a variety of biological processes are known to be temperature sensitive[2]. This sparked concerns about tissue heating dating back to the early days of optogenetics and spurred the development of models to estimate the distribution of light and heat in optogenetic experiments[2–5]. However, there was only limited evidence to suggest that the modest heating predicted by these models could affect neural activity and the mechanisms that could underlie such an effect were not well understood.

Owen et al. set out to address these questions by implanting recording electrodes below an optical fiber in the striatum of wildtype mice and compared the normalized firing rates of MSNs before and during light delivery (Fig. 1). Strikingly, continuous illumination suppressed firing in these neurons even though they did not express any optogenetic constructs. This effect could be reproduced in acute brain slices, and in both cases the decrease in cell activity scaled with increasing laser power.

To test the hypothesis that suppression of MSN activity was caused by light-induced tissue heating, Owen et al. measured the temperature of the striatum below an optical fiber in head-fixed mice. They found that the time course of light-induced heating closely matched the time course of the decrease in MSN firing they had previously observed. In a key experiment, the authors then recorded from MSNs in voltage clamp mode and found that locally manipulating the temperature of a brain slice independently of laser stimulation evoked an outward current in MSNs that scaled with the magnitude of the temperature change. Tantalizingly, a similar experiment provided the first clue about the identity of the ion channel responsible for this mysterious light-evoked current: plotting its I-V relationship revealed an electrophysiological signature that resembled the I-V curve of an inwardly rectifying potassium channel (K_{ir}). This current was abolished in recordings made with cesium-based internal solution, confirming that light delivery was activating a potassium conductance. Then, additional experiments showed that a light-induced current was present in multiple cell populations known to express K_{ir} channels (e.g., striatum, dentate gyrus, cortex), and was absent in one cell population known to lack (or minimally express) K_{ir} channels (CA1 pyramidal neurons). While future work may strengthen this body of evidence, Owen et al.'s results argue in favor of light-induced thermal modulation of K_{ir} channels suppressing neural activity.

With a likely molecular culprit behind the light and heating-induced current in mind, Owen et al. finally examined whether illumination delivered in commonly used optogenetic protocols could affect animal behavior. They implanted a cohort of wildtype mice with optical fibers above dorsal striatum and analyzed changes in the mice's locomotor behavior in response to unilateral light delivery in an open field. Strikingly, light delivery was sufficient to bias the mice's rotational behavior in favor of the illuminated hemisphere, as would be expected from suppression of MSN activity in the motor circuits of dorsal striatum.

In view of these important findings, some mechanistic questions remain. For example, given that the authors' evidence implicating K_{ir} channels as the source of a light-induced current is

indirect in nature, additional experiments to confirm this result may be informative. One potentially fruitful approach may be to test whether the light-induced current disappears in knockout mice lacking one or more subunits of the K_{ir} channel. Absent this information, contributions from other potassium channels that may exhibit thermal modulation cannot be entirely excluded. Moreover, because glia express K_{ir} channels it is likely that their physiology is also affected by light delivery. Further work will be necessary to characterize network-level effects on synaptic plasticity or clearance of transmitters from the synaptic cleft due to optically-induced changes in glial functions. Nevertheless, Owen et al. make a compelling case that light-induced thermal modulation of a potassium conductance can change neural activity in the absence of opsins to exert an effect on physiology and behavior. What are some of the practical implications of this result, and how should it inform the design of future optogenetic experiments?

Tissue heating depends on a variety of light delivery parameters. Using a computer model[5], Owen et al. demonstrate that heating can be minimized by using illumination protocols that favor shorter pulse durations, lower laser power, longer wavelength light, and higher pulse frequencies for a fixed duty cycle. Of these, pulse duration and laser power appear to be the most important variables affecting temperature changes; such that high laser powers (up to 30 mW) are expected to produce negligible heating (~0.1 ºC) for pulses below ~100 ms, and long pulses (up to 20 min) are predicted to produce heating of less than ~0.2 ºC for laser powers below ~0.5 mW. Because most optogenetic stimulation patterns employ pulse durations less than 100 ms, and calcium imaging techniques (e.g., fiber photometry, microendoscopy) typically use light power in the microwatt range, thermal constraints on experimental design will be most relevant in the context of optogenetic inhibition, which typically involves delivery of continuous light[6]. Below, we evaluate the array of available tools for opto-inhibition to identify which opsins are best suited for inhibition experiments while minimizing tissue heating.

Inhibitory opsins are used to induce temporally specific loss-of-function manipulations[2,6]. The chloride pump halorhodopsin (eNpHR3.0) stands out for its widespread use and has been shown to produce hyperpolarization of up to ~100 mV in response to amber light (593 nm) at a power of ~3 mW[7]. Together with the modeling results reported by Owen et al., this suggests that halorhodopsin may be used for inhibition bouts of ~1 second with minimal heating. The related tool Jaws offers similar kinetics but with greater sensitivity to red (i.e., long wavelength) light, and thus it may be less likely to produce heating when pulse durations >1 sec are required[8]. Though less commonly used, the light activated anion channels GtACR1 and GtACR2 outperform all chloride pumps in terms of light sensitivity and produce large photocurrents in response to light power below 0.1 mW[9]. This makes GtACRs attractive tools for long term photoinhibition without tissue heating, along with SwiChR++ – a bistable anion channel that can produce a long-lasting photocurrent in response to short pulses of light[10]. However, GtACRs, SwiChR++, and other anion channels (e.g., iC++[10]) inhibit neurons via chloride-mediated shunting, so effects on cell activity depend on the local chloride gradient. Thus, where the reversal potential of chloride is depolarizing, these tools can produce paradoxically excitatory effects[11].. Similarly, while the commonly used proton pump ArchT offers improved light sensitivity compared to halorhodopsin (in the 1–10 mW/mm^2 range, and likely appropriate for inhibition bouts of a

few seconds), its use is complicated by a reported pH-dependent increase in spontaneous transmitter release during inhibition with the related proton pump eArch3.0[6,11] Thus, halorhodopsin and Jaws remain the most effective tools available for reliable photoinhibition in circuits where chloride gradients are unknown, though anion-channels may prove useful when experiments require photoinhibition on timescales that make tissue heating a serious concern with halorhodopsin or Jaws.

Beyond opsin choice, the data presented by Owen et al. motivate further considerations for experimental design. For example, the authors argue convincingly in favor of an amendment to the classical opsin-fluorophore versus fluorophore-only design of opto-inhibition experiments to include an additional cohort of fluorophore-only controls that are treated with 0 mW laser power. Optogenetic silencing experiments that include this kind of light-off control, especially within laser off-on-off behavioral paradigms, will enable us to more clearly separate opsin-mediated and light-induced effects in physiological and behavioral data. Alternatively, where the temporal precision afforded by optogenetics is less important, complementary inhibitory strategies could obviate concerns of tissue heating altogether (for a more comprehensive review, see[6]). These include use of the inhibitory DREADD hM4Di, which activates a signaling cascade that activates inwardly rectifying potassium channels following administration of clozapine-N-oxide (CNO), as well as the genetically targeted expression of toxins that will permanently silence neurons (e.g., tetanus toxin light chain, TeNT[6]). These silencing alternatives are chronic in nature compared to acute photoinhibition and can affect network dynamics in distinct ways[12]; however, whenever the results of chronic and acute inhibition experiments agree, these complementary strategies may help to confirm that the observed effect is not caused by artifacts such as light-induced heating.

Overall, Owen et al. demonstrate that light-induced tissue heating can be a confounding factor that should be controlled for in optogenetic silencing experiments. Together with other recent reports about germline recombination and non-cell-type-specific expression in Cre-driver mouse lines[13,14], paradoxical excitation effects of inhibitory opsins[11], and off-target effects of metabolites from the CNO ligand used in DREADD experiments[15], the work by Owen et al. is an important reminder that as our technology grows increasingly complex to enable ever defter control over neural activity, our application of these resources must also evolve to be more sophisticated so that we may account for the inevitable caveats and limitations that will always be associated with our tools.

ACKNOWLEDGEMENTS

D.C.P. is supported by a Graduate Research Fellowship from the National Science Foundation. S.L. is a John P. Stock Faculty Fellow and Rita Allen Scholar. This work was supported by grants from NIDA (R01-DA042889), NIMH (R01MH112721), TRDRP (26IP-0035), the Rita Allen Foundation, the Wayne and Gladys Valley Foundation and the Shurl and Kay Curci Foundation.

REFERENCES

1. Owen SF, Liu MH, and Kreitzer AC. Nat. Neurosci. https://dx.doi.org/xxx (2019)
2. Yizhar O, Fenno LE, Davidson TJ, Mogri M & Deisseroth K Optogenetics in neural systems. Neuron 71, 9–34 (2011). [PubMed: 21745635]

3. Gysbrechts B et al. Light distribution and thermal effects in the rat brain under optogenetic stimulation. J. Biophotonics 9, 576–585 (2016). [PubMed: 26192551]

4. Arias-Gil G, Ohl FW, Takagaki K & Lippert MT Measurement, modeling, and prediction of temperature rise due to optogenetic brain stimulation. Neurophotonics 3, 045007 (2016).

5. Stujenske JM, Spellman T & Gordon JA Modeling the Spatiotemporal Dynamics of Light and Heat Propagation for InVivo Optogenetics. Cell Rep. 12, 525–534 (2015). [PubMed: 26166563]

6. Wiegert JS, Mahn M, Prigge M, Printz Y & Yizhar O Silencing Neurons: Tools, Applications, and Experimental Constraints. Neuron 95, 504–529 (2017). [PubMed: 28772120]

7. Gradinaru V et al. Molecular and Cellular Approaches for Diversifying and Extending Optogenetics. Cell 141, 154–165 (2010). [PubMed: 20303157]

8. Chuong AS et al. Noninvasive optical inhibition with a red-shifted microbial rhodopsin. Nat. Neurosci. 17, 1123–1129 (2014). [PubMed: 24997763]

9. Govorunova EG, Sineshchekov OA, Janz R, Liu X & Spudich JL Natural lightgated anion channels: A family of microbial rhodopsins for advanced optogenetics. Science 349, 647–650 (2015). [PubMed: 26113638]

10. Berndt A et al. Structural foundations of optogenetics: Determinants of channelrhodopsin ion selectivity. Proc. Natl. Acad. Sci. 113, 822–829 (2015). [PubMed: 26699459]

11. Mahn M, Prigge M, Ron S, Levy R & Yizhar O Biophysical constraints of optogenetic inhibition at presynaptic terminals. Nat. Neurosci. 19, 554–556 (2016). [PubMed: 26950004]

12. Wolff SB & Ölveczky BP The promise and perils of causal circuit manipulations. Curr. Opin. Neurobiol. 49, 84–94 (2018). [PubMed: 29414070]

13. Song AJ & Palmiter RD Detecting and Avoiding Problems When Using the Cre–lox System. Trends Genet. 34, 333–340 (2018). [PubMed: 29336844]

14. Lammel S et al. Diversity of transgenic mouse models for selective targeting of midbrain dopamine neurons. Neuron 85, 429–438 (2015). [PubMed: 25611513]

15. Rodriguez LA et al. Chemogenetics revealed: DREADD occupancy and activation via converted clozapine. Science 357, 503–507 (2017). [PubMed: 28774929]

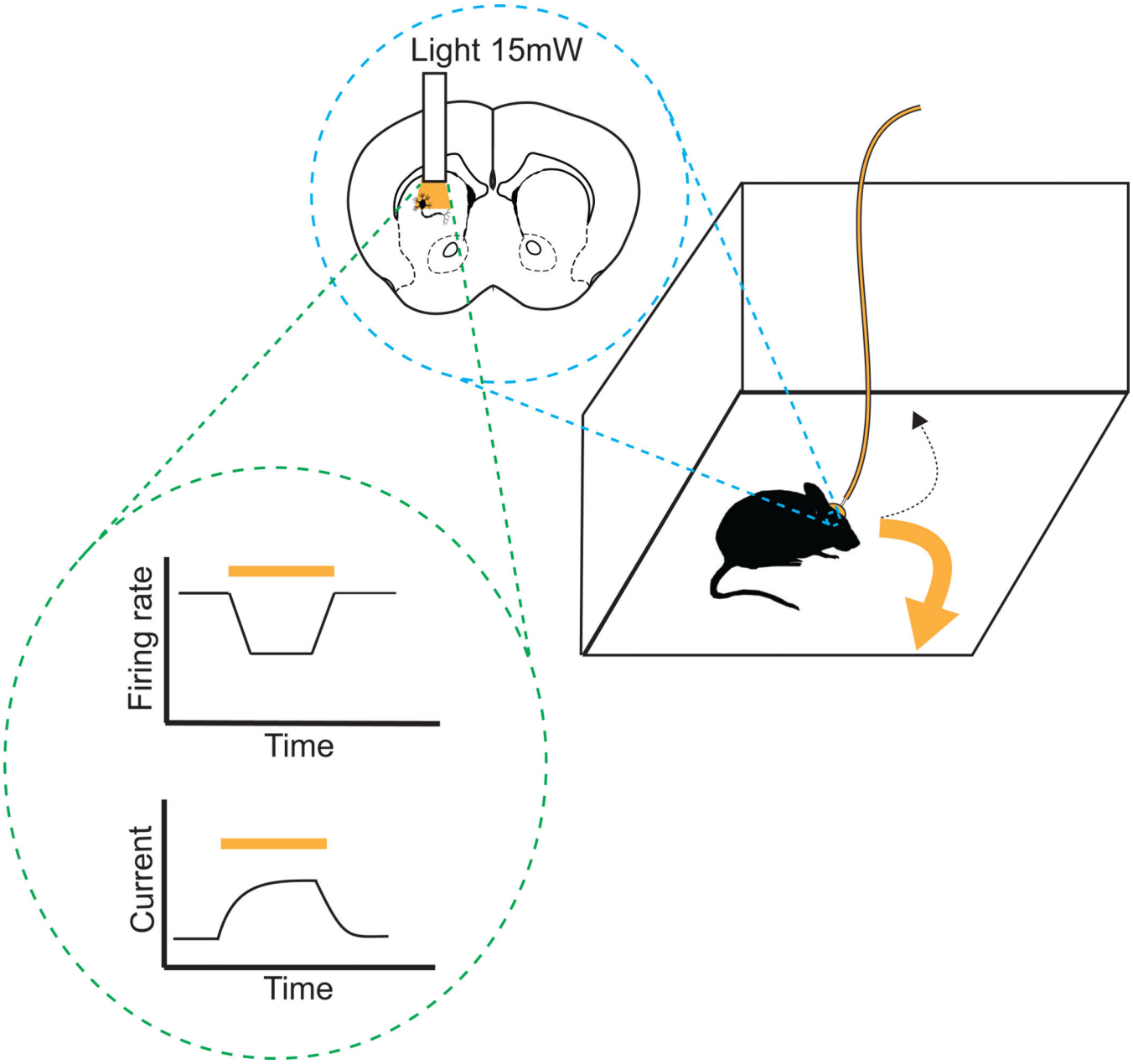

Figure 1. Physiological and behavioral consequences of high intensity light delivery into the striatum.

In the striatum of wildtype mice, *in vivo* light delivery at powers commonly used for optogenetic experiments (7–15 mW) promotes biased rotational behavior even if no optogenetic constructs are expressed in MSNs. This behavioral effect is likely caused by heating, which suppresses the firing of MSNs through activation of an inwardly-rectifying potassium current.