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Supplementary information

Deep brain optogenetics without intracranial surgery

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Supplementary Note. Considerations for tissue heating from transcranial optogenetics.

Tissue heating through high-power illumination can have nonspecific effects on neurophysiology and behavior[1]. For example, delivery of constant 532 nm light at 7 mW into the dorsal striatum has been shown to heat tissue by ~0.7 °C, suppress striatal activity, and bias rotational behavior in mice[1]. To address this, we used established models that combine Monte Carlo simulations of photon propagation with Pennes's bio-heat equation to predict light-induced temperature changes in brain tissue based on our stimulation protocols[1,2] (**Supplementary Table 2**). We found that light parameters used to robustly drive behavior were associated with a predicted maximum temperature change from 0.07 °C (40 mW/mm^2, 5 Hz, 100 ms pulse width) to 0.31 °C (800 mW/mm^2, 20 Hz, 5 ms pulse width), which were below the temperatures associated with thermal modulation of behavior (~0.7 °C, 7 mW, 222 mW/mm^2, 10 s)[1]. Based on the estimated temperature profile and histology assessment for neuroinflammation, we recommend using the laser parameters established in this study (635 nm, up to 800 mW/mm^2, 20 Hz, 5 ms pulse width or up to 400 mW/mm^2, 1 Hz, 100 ms pulse width) to deliver sufficient photon density for transcranial optogenetics with minimal tissue heating (**Extended Data. 3b and c**). By contrast, previously reported approaches for transcranial optogenetics with the mutated step-function opsin SOUL (473 nm, 400 mW/mm^2) and near-infrared ChR2 excitation with injection of upconversion nanoparticles (980 nm, 9.5e4 mW/mm^2) can cause temperature changes exceeding 5 ºC[3,4] (**Supplementary Table 2**, **Extended Data. 3e**).

Supplementary Table 1. Properties of channelrhodopsins for transcranial optogenetics.

Opsin[a]	Action spectra[b] (nm)	EPD50[c] ($\mu W/mm^2$)	Peak photocurrent (pA)	τ_{Off}[d] (ms)
ChRmine[5]	390-650 [585]	~30	~4000	40
bReaChES[5]	390-650 [585]	~200	~2000	49
SOUL[3]	350-550 [473]; 525-625 [589]	~10	~400	$\sim 1.8*10^6$

[a] Presented values for ChRmine, bReaChES, and SOUL were obtained from whole-cell patch clamp recordings of murine primary hippocampal neurons from previous publications[3,5].

[b] Value in square bracket indicate stimulation wavelength for measurements.

[c] Effective power density for 50% activation (EPD50), a measure of opsin photosensitivity independent of expression level.

[d] The rate of channel closure upon light termination (τ_{Off}). Fast off-kinetics are important for precise temporal control.

Supplementary Table 2. Light parameters used for transcranial optogenetics.

In vivo Assay[a]	λ (nm)	Laser Power (mW)	Irradiance (mW/mm^2)	*f* (Hz)	Pulse width (ms)	Duty Cycle (%)	ΔT_{max}[b] (°C)	d_{source}[c] (mm)	Source
Recordings (mouse)	635	25-200; 0.5-50	200-1600; 4-400	5-40; 1	1-10; 100	1-20; 10	0.32; 0.37	4.5	1c-f, ED1, ED8
Recordings (rat)	635	0.5-50	4-400	1	100	10	0.37	7	ED2
Tissue compatibility	635	100-800	800-6400	20	5 ms	10	2.38	n/a	ED3
RTPP	635	10-100; 5	80-800; 40	20; 5	5; 100	5; 50	0.22; 0.07	4.2	1g, h, ED4, ED5
Lever-press	635	50	400	20	5	10	0.15	4.2	1i, j, ED5
SPP, NOP, OFT	635	100	800	20	5	10	0.31	2.8	2i, j, ED7
Closed-loop EEG	635	5	40	6.7	50	33.5	0.05	1.4	2c-g, ED6
Feeding inhibition (SOUL)	473; 589	50; 25	400; 200	60 s; 100 s		100	6.1	5	Ref. 2
Whisker protraction (ReaChR)	617	100	15	1	100	10	0.2	1	Ref. 3
VTA stimulation (UCNP /ChR2)	980	3000	9.5e4	20	15	30	9.4	4.2	Ref. 4

[a] Listed parameters for ChRmine experiments (unless otherwise noted). Presented values for alternative transcranial optogenetic approaches using ReaChR, SOUL, or ChR2/upconversion nanoparticle (UCNP) stimulation were obtained from previous publications[3,4,6].

[b] Estimated maximum temperature change in tissue associated with the light parameters used. For irradiance-dependent recordings at 10 Hz and 5 ms pulse width presented in **Fig. 1c-f**, the estimated maximum temperature at 1600 mW/mm^2 was 0.32 °C. The light source dimensions to estimate the temperature changes were based on: a 400-μm, 0.39 NA fiber (this study) a 3 mm diameter Luxeon 617 nm LED (ReaChR), a 400-μm 0.22 NA fiber (SOUL), and a 200-μm 0.37 NA fiber (ChR2 with UCNPs).

[c] Proximity of light source to apex of targeted brain structure.

References

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