A key assumption of optogenetics is that light only affects opsin-expressing neurons. However, illumination invariably heats tissue, and many physiological processes are temperature-sensitive. Commonly used illumination protocols increased the temperature by 0.2–2 °C and suppressed spiking in multiple brain regions. In the striatum, light delivery activated an inwardly rectifying potassium conductance and biased rotational behavior. Thus, careful consideration of light-delivery parameters is required, as even modest intracranial heating can confound interpretation of optogenetic experiments.

Light delivery into living brain tissue is critical for many neuroscience assays, including optogenetic manipulations and fluorescence imaging. These approaches assume that the light does not directly affect neuronal physiology. However, experimental measurements and modeling calculations have described heating of 0.2–2 °C following sustained illumination of brain tissue using commonly used light powers (3–30 mW). Many neuronal circuit processes are temperature-dependent, including ion channel conductance and synaptic transmission. Notably, changes in temperature have been used to alter neuronal physiology in the cortex of rodents' and in the HVC area in songbirds. In ex vivo brain-slice recordings, illumination-induced heating is reported to generate changes in spiking across various brain regions, which raises the possibility that temperature changes occurring during in vivo optogenetic manipulations could have both electrophysiological and behavioral consequences.

Here we explicitly test this assumption through ex vivo recordings of mouse striatum, hippocampus, and cortex slices, as well as through in vivo recordings in the mouse striatum. Our results indicate that continuous illumination at powers commonly used for optogenetic experiments (10–15 mW) markedly suppresses the firing rates of specific cell types across a range of brain regions, even in the absence of opsin expression. Experiments in medium spiny neurons (MSNs), the principal neurons in the striatum, demonstrate that this light-induced suppression of spiking can be reproduced by direct heating of brain tissue and results from the activation of an inwardly rectifying potassium conductance. Finally, illumination of the striatum in freely moving mice produces a rotational bias, which demonstrates that light alone may be sufficient to affect behavior in the absence of opsin expression.

To test how light delivery affects the activity of striatal MSNs in vivo, we performed acute single-unit recordings in the dorsal striatum of awake, head-fixed, wild-type mice (Fig. 1a,b). Putative MSNs were distinguished from other cell types based on waveform (Supplementary Fig. 1a,b). Despite the lack of opsin or fluorophore expression, light delivery through an optical fiber reversibly suppressed spiking activity in MSNs. This suppression was statistically significant at the population level for both the lower (3 mW) and higher (15 mW) light powers, but was most evident at the higher light power (Fig. 1c,d; Supplementary Fig. 1c–f).

We considered several explanations for this suppression of activity, including physiological responses to local heating of brain tissue and sensory responses to light detection at the back of the retina. To rule out the latter possibility, we tested whether equivalent physiological responses could be detected in an acute-slice preparation. We obtained whole-cell recordings from MSNs in acute slices from wild-type mice using a potassium-based internal solution (Fig. 1e). A current injection through the recording pipette elicited spiking in MSNs, which are otherwise quiescent (Fig. 1f). In agreement with the in vivo results, light delivery through an optical fiber at low power (3 mW) caused a modest drop in firing rate, while higher power (15 mW) more markedly reduced spiking (Fig. 1g,h; Supplementary Fig. 1g–i).

To investigate whether this suppression of activity is specific to MSNs, we repeated this experiment in other cell types in the hippocampus and cortex. Sparing activity in CA1 pyramidal neurons was unaffected by light, while dentate gyrus granule cells and cortical layer 5 (L5) pyramidal neurons were modestly suppressed (Fig. 1i–l; Supplementary Fig. 1j–o). The suppression of cortical pyramidal neurons was surprising, as previous reports have indicated that light-driven temperature changes increase cortical activity in vivo. To reconcile these results, we recorded fast-spiking interneurons (FSIs) from L5 of cortex, which were identified by narrow action-potential waveform (Fig. 1k,l; Supplementary Fig. 1m–o), which suggests that the light-driven elevation of cortical neuron activity reported in vivo may arise from disinhibition through the suppression of neighboring inhibitory interneurons.

We hypothesized that these responses to light delivery could arise from local heating of brain tissue. We therefore acutely inserted a miniaturized thermocouple probe in place of the recording electrode in vivo. As predicted by measurement and modeling in other brain regions, light delivery caused a transient increase in temperature of up to 2 °C (Fig. 2a,b; Supplementary Fig. 2a). The time-course of this temperature change, when fitted with a single exponential (Fig. 2b), closely matched with the decrease in MSN firing (Fig. 2c). Temperature changes in the slice were also graded by light power (0.2–1 °C), and this closely matched with the outward currents measured in MSNs held at −50 mV using a potassium-based internal solution (Fig. 2d–g).

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To test whether a temperature change was sufficient to elicit this outward current, we established a system to locally and rapidly change the temperature in acute slices. We used a small copper tube perfused with hot, warm, or cold water (Fig. 2h), and the measured temperature changes were $+1.92\, ^\circ\text{C}$ (hot), $+0.89\, ^\circ\text{C}$ (warm), or $-2.82\, ^\circ\text{C}$ (cold) (Fig. 2i). Whole-cell recordings measured the physiological consequences of this temperature change in MSNs voltage-clamped at $-50\, \text{mV}$ with a potassium-based internal solution. Warming the slice elicited a graded outward current with a time-course that tracked the temperature change, while cooling of the slice reduced this outward current (Fig. 2j). The relationship between temperature and current was strikingly linear, which suggests that there is temperature-dependent modulation of a tonically active current (Fig. 2k).

To better understand the relationship between light delivery and heating, we used a recently published model\(^1\) to predict the local temperature change as a function of light power and pulse duration (Fig. 2l). This plot includes a ‘threshold’ line at a temperature change of approximately 0.1 °C, which corresponds to the intensity and duration at which we began to observe physiological effects in wild-type MSNs (Fig. 1; 3 mW for 1 s). High light powers ($\geq 30\, \text{mW}$) caused minimal heating if pulse durations were shorter than 100 ms. However, long periods of continuous light, as are commonly used for optogenetic inhibition, caused significant heating even at modest powers ($3–5\, \text{mW}$). This model efficiently describes how changes in duty cycle, pulse frequency, and wavelength affect temperature during single light pulses and during pulse trains (Supplementary Figs. 2d,e and 3).

To characterize the physiology of this light-evoked current, we performed whole-cell voltage-clamp recordings in which we varied the holding potential of MSNs to between $-93\, \text{mV}$ and $-50\, \text{mV}$ (Fig. 2m–o). Consistent with activation of a potassium conductance, the current reversed at $-93\, \text{mV}$ and showed a striking inward rectification (Fig. 2o). This light-induced current was almost entirely abolished in MSNs recorded using a cesium-based internal solution (Fig. 2p). Furthermore, in MSNs recorded with a potassium-based internal solution, the light-induced current was sensitive to the application of BaCl\(_2\) ($250\, \mu\text{M}$) in the bath solution.
Light-induced heating increases an inwardly rectifying potassium conductance in MSNs. a, Configuration for in vivo head-fixed temperature measurements and physiology. The temperature increase in vivo fitted to a single exponential (broken line; n = 1 mouse). c, Time-course of the decrease in mean MSN firing rate (in vivo) fitted to a single exponential (broken line). Same data as in Fig. 1c (n = 3 mice, 99 MSNs). d, Configuration for whole-cell recordings or temperature measurements in acute slices. e,f, Exemplar temperature change (e) and average whole-cell current (f) in exemplar MSNs held at −50 mV with potassium-based internal solution. Light delivery was at 3 mW or 15 mW, 532 nm. g, Group data for light-evoked current in MSNs (n = 2 mice, 9 cells for 3 mW; n = 2 mice, 10 cells for 15 mW; two-sided rank-sum test, \( P < 0.001 \)). h, Configuration for whole-cell recordings with temperature modulation. i,j, Temperature changes (i) and exemplar whole-cell currents (j) in wild-type MSNs recorded in voltage-clamp mode with potassium-based internal solution. k, Linear relationship between temperature change and current (n = 2 mice, 7 cells for −2.82 °C; n = 2 mice, 7 cells for +0.89 °C; n = 2 mice, 10 cells for +1.92 °C). l, Modeled temperature changes predicted for this recording configuration. m,n, Exemplar light-activated current recorded at −50 mV with cesium-based internal solution (n = 2 mice, 10 cells). Light off in black, light on in green. o, Exemplar light-activated current recorded at −50 mV with potassium-based internal solution before (green) and after (purple) application of 250 μM BaCl₂ in the bath solution. p, Group data for BaCl₂ sensitivity of light-activated conductance (n = 2 mice, 7 cells for sham-treated mice; n = 2 mice, 6 cells for BaCl₂-treated mice; two-sided rank sum test, \( P = 0.001 \)). **P < 0.01, ***P < 0.001. All error bars and shaded regions represent the s.e.m.

(Fig. 2g,r). These results are most consistent with activation of an inwardly rectifying potassium (Kᵢ) channel (Fig. 2d–r). Together, our data indicate that light delivery in the striatum using commonly applied experimental parameters can heat brain tissue sufficiently to alter MSN activity through activation of an inwardly rectifying potassium conductance, leading to the suppression of firing in the absence of opsin expression.

In contrast to the brief pulses of high-intensity illumination used in optogenetics, imaging experiments often require lower intensity illumination for longer durations, spanning minutes to hours. Cortical heating during high-intensity multiphoton illumination has been described in some detail. Therefore, we explored the temperature changes resulting from long-duration, low-intensity illumination through optical fibers, such as those used for fiber photometry experiments. This revealed a modest temperature increase that rapidly reached an intensity-dependent plateau (Supplementary Fig. 2f). These data suggest that the illumination used for photometry experiments should be maintained at less than 0.25 mW average power to avoid temperature-dependent changes in physiological activity.

Finally, we tested whether this light-driven suppression of MSNs can affect animal behavior (Fig. 3; Supplementary Fig. 4). We implanted optical fibers into the dorsal striatum of wild-type mice and delivered light unilaterally, using automated post hoc tracking to monitor body position. Light delivery reversibly biased rotations in a direction that was ipsilateral to the illumination, which is consistent with the suppression of striatal network activity (Fig. 3; Supplementary Fig. 4). Light-driven, opsin-independent suppression of the striatum can therefore affect behavioral as well as physiological results.
Several factors can minimize light delivery while preserving the activation of opsins. Excitatory opsins such as channelrhodopsin-2 express well within ~2 weeks of virus injection, whereas inhibitory opsins such as eNPHR3.0 or eArchT can take as long as 6–8 weeks to develop significant photocurrents in response to modest illumination because of limited membrane trafficking. Any apparent physiological responses to light that occur before opsin expression is fully developed should be clearly treated with caution.

Careful consideration of the time-course of light-driven responses can help to differentiate opsin-driven effects from opsin-independent experimental artifacts. The time constants with which optogenetic proteins alter neuronal activity are on the order of ones to tens of milliseconds. This is true for direct optogenetic excitation of neuronal populations and for indirect synaptic disinhibition arising from optogenetic silencing of inhibitory neuronal populations. By contrast, the time constant for the heating of neuronal tissue with light is markedly slower (Fig. 2a–c). Physiological responses that develop slowly, over hundreds of milliseconds or seconds, should therefore be suspected of arising from local heating of tissue rather than a direct opsin-dependent physiological process. In these cases, we suggest that a higher bar ought to be met by the experimenter to describe a mechanism to reconcile the time-course of the optogenetic manipulation with the time-course of the physiological response and to establish the rigor of opsin-independent control experiments. The continuously growing toolbox of opsins with altered spectral sensitivity or kinetics opens the door for use of lower light powers or longer wavelengths that generate less heat (Supplementary Fig. 2e). New technologies, including tapered fibers, waveguides, and micro light-emitting diodes (LEDs) that spread light more evenly across the tissue, also support more efficient and homogeneous opsin activation across larger brain structures, which should help mitigate artifacts arising from tissue heating.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, statements of code and data availability and associated accession codes are available at https://doi.org/10.1038/s41593-019-0422-3.

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References
1. Stujenske, J. M., Spellman, T. & Gordon, J. A. Cell Rep. 12, 525–534 (2015).
2. Arias-Gil, G., Ohl, F. W., Takagaki, K. & Lippert, M. T. Neurophotonics 3, 045007 (2016).
3. Yizhar, O., Fenno, L. E., Davidson, T. J., Mogri, M. & Deisseroth, K. Neuron 71, 9–34 (2011).
4. Yang, F. & Zheng, J. eLife 3, e03255 (2014).
5. Moser, E., Mathiesen, I. & Andersen, P. Science 259, 1324–1326 (1993).
6. Sabatini, B. L. & Regehr, W. G. Nature 384, 170–172 (1996).
7. Long, M. A. & Fee, M. S. Nature 456, 189–194 (2008).
8. Aioua, K., Beurrier, C., Canepari, M., Laverne, G. & Kuczewski, N. Eur. J. Neurosci. 49, 6–26 (2019).
9. Podgorski, K. & Ranganathan, G. J. Neurophysiol. 110, 1012–1023 (2016).
10. Yang, E., Cui, Y., Wang, K. & Zheng, J. Proc. Natl Acad. Sci. USA 107, 7083–7088 (2010).
11. Prüss, H., Dersi, C., Lommel, R. & Veh, R. W. Brain Res. Mol. Brain Res. 139, 63–79 (2005).
12. Allen, B. D., Singer, A. C. & Boyden, E. S. Learn. Mem. 22, 232–238 (2015).
13. Gradinaru, V. et al. Cell 141, 154–165 (2010).
14. Pi, H.-J. et al. Nature 503, 521–524 (2013).
15. Mattis, J. et al. Nat. Methods 9, 159–172 (2012).
16. Owen, S. F., Berke, J. D. & Kreitzer, A. C. Cell 172, 683–695.e15 (2018).
17. Lin, Y. J., Knutsen, P. M., Muller, A., Kleinfeld, D. & Tsien, R. Y. Nat. Neurosci. 16, 1499–1508 (2013).
18. Berruda, A., Yizhar, O., Gouyarden, L. A., Hegemann, P. & Deisseroth, K. Nat. Neurosci. 12, 229–234 (2009).
19. Pisanello, F. et al. Nat. Neurosci. 20, 1180–1188 (2017).
20. Steude, A., Witts, E. C., Miles, G. B. & Gather, M. C. Sci. Adv. 2, e1600061 (2016).

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Author contributions
S.F.O., M.H.L., and A.C.K. designed the experiments. S.F.O. and M.H.L. performed the experiments and analyzed the data, and all authors wrote the manuscript.

Competing interests
The authors declare no competing interests.

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Methods
Experimental model and subject details. Adult mice (n = 52) on a C57BL/6 background, aged 45–250 days, of both sexes were used in experiments. All animals were housed and maintained on a 12 h light–12 h dark cycle and fed ad libitum. Wild-type mice (2 female, 1 male; Jackson stock no. 000664) were used for the in vivo electrophysiology experiments. Wild-type mice (2 female, 2 male; Jackson stock no. 000664) were used for the in vivo temperature recording experiments. Wild-type mice (12 males, 17 females; Jackson stock no. 000664) were used in the slice electrophysiology experiments. PV-2A-Cre mice (5 female; Jackson stock no. 012258) were used for the in vivo optogenetic suppression of FSl. Wild-type mice (4 female, 7 male; Jackson stock no. 000664) were used for the open-field behavioral experiments.

Sample sizes for physiological recordings were determined based on previously published studies\(^\text{1,2,16}\), and statistical significance was calculated using post hoc tests. Recordings were performed in a standardized way to minimize the possibility of experimenter bias. The age and sex of the mice were balanced across cohorts, and littermates were used for fluorophore control experiments.

Stereotaxic surgery. All procedures were carried out in accordance with protocols approved by the UCSF Institutional Animal Care and Use Committee. Experiments were carried out during the light cycle. All surgeries were carried out under aseptic conditions while mice were anesthetized with isoflurane (3% for induction, 0.5–1.5% for maintenance) in a manual stereotactic frame (Kopf). Buprenorphine HCl (0.1 mg per kg, intraperitoneal injection) and ketoprofen (5 mg per kg, subcutaneous injection), were used for postoperative analgesia.

Headbar implantation. Wild-type mice were prepared for in vivo, awake head-fixed recordings by the surgical implantation of a headbar. Animals were mounted on a stereotax and anesthesia was induced as described above. The overlying scalp was removed, and the skull was cleaned and exposed. A stainless steel headbar (eMachineShops, custom design) was then secured with a combination of dental adhesives (C&B Metabond, Parkell, Lang). After the dental adhesive had set, mice were allowed to recover for at least 7 days before head fixation or recordings.

Head-fixed in vivo electrophysiology recordings. At least 3 days before recordings, animals were acclimated to a head-fixed device to which a combination of a custom three-dimensional printed cylindrical running wheel (Evan Feinberg, custom design). The night before a recording session, animals were anesthetized and mounted on a stereotax as described above. Cranietomy procedures were performed above the targeted recording area in the dorsal striatum (+1.0 anterior–posterior (AP), +2.0 medio-lateral (ML) and +2.5 dorsal–ventral (DV) from the brain surface). The probe was then allowed to settle for 10–20 s. The current amplitude was measured across the following three separate periods: Pre (0–1 s), Light (1–2 s), and Post (2–3 s after the start of the light pulse). The light-induced current was calculated by subtracting the mean current over 250 ms before the light stimulus from the mean current during the light stimulus. All data were analyzed for spiking activity. Single units were manually isolated, using peak amplitude and principal components as variables, using the script MatClust (https://github.com/rafaeldias/MatClust).

Acute slice physiology. Adult mice (4–12 weeks of age) were killed by a lethal dose of ketamine and xylazine. The brain was rapidly dissected, and a 500 μm thick coronal slice containing the dorsal striatum, primary sensory cortex, or dorsal hippocampus was then prepared using a vibratome (Leica) in the same solution before incubation at 33 °C in ACSF containing (in mM): NaCl (125), NaHCO3 (26), MgCl2 (1), CaCl2 (2.3), NaH2PO4 (2.3), sucrose (68), NaH2PO4 (11), MgCl2 (6), n-glucone (12), and CaCl2 (0.5). Coronal slices (250–μm thick) containing the dorsal striatum, primary sensory cortex, or dorsal hippocampus were then prepared using a vibratome (Leica) in the same solution before incubation at 33 °C in ACSF containing (in mM): NaCl (125), NaHCO3 (26), MgCl2 (1.25), CaCl2 (2), and n-glucone (12.5). Physiological activity was recorded using a silicon electrode (0.6 mm). Spiking activity was driven by injection of current steps of 3 s in duration through the recording pipette with an inter-sweep interval of 15–20 s. The injection amplitude was chosen to drive spiking that was maintained throughout the 3-s current step (250–600 pA).

Whole-cell voltage-clamp recordings were obtained with an internal solution containing (in mM): k-glucuronate (135), NaCl (10), MgCl2 (2), ethylene glycol tetraacetate acid (EGTA; 0.5), HEPES buffer (10), Mg-ATP (2), and Na-GTP (0.3). The physiological activity was recorded using a silicon electrode (0.6 mm). Spiking activity was driven by injection of current steps of 3 s in duration through the recording pipette with an inter-sweep interval of 15–20 s. The injection amplitude was chosen to drive spiking that was maintained throughout the 3-s current step (250–600 pA). On interleaved sweeps, green laser light (532 nm) was delivered through a 200-μm optical fiber placed 500–800 μm from the recorded neuron at a power of either 3 mW or 15 mW. Light pulses were 1 s in duration, spanning the middle 1 s of the 3-s current step injection. To calculate group data for the spike rate modulation by light, the average spike rate for each neuron during 'light on' trials was calculated as the full-width half-maximum value of the action potential peak relative to the pre-spike baseline membrane potential. A clear bimodal distribution emerged to distinguish putative pyramidal neurons (width > 0.7 ms) from putative interneurons (width < 0.7 ms).

Whole-cell voltage-clamp recordings of light-activated currents were performed using the same potassium-based internal solution in a set of neurons that partially overlapped with the current-clamp recordings. For whole-cell voltage-clamp recordings using a cesium-based internal solution, the current recording solution contained (in mM): CsMeSO3 (135), NaCl (8), EGTA (0.5), HEPES (10), Mg-ATP (2), Na-GTP (0.3), and QX-314 (5). For all voltage-clamp recordings, MSNs were held at a resting potential of −85 mV between sweeps. Membrane potentials were not corrected for junction potential. Each sweep, the voltage was stepped to −50 mV for 3 s, and the light pulse (1 s in duration) was delivered for the middle 1 s of the 3-s voltage step. The inter-sweep interval was 10–20 s. The current amplitude was measured across the following three separate time windows: Pre (0.75–1 s), Light (1.5–2 s), and Post (2.5–2.75 s after the start of the voltage step). The light-induced current was calculated by subtracting the average current amplitude over the Pre and Post periods from the average current over the Light period. This average was calculated separately for light-on and light-off sweeps, and the final light-induced current for each cell was determined by subtracting the light-off value from the light-on value.

An equivalent protocol was performed for 3× as many trials to calculate the I–V curve and rectification of the light-activated current. The MSN was held at a resting potential of −85 mV with a potassium-based internal recording solution, and the voltage was stepped to varying holding potentials over a range from −140 mV to −50 mV for 3 s with the light pulse (1 s in duration) delivered over the middle 1 s of the 3-s voltage step. For the light-off experiments, the light pulse was set to 0.6 s, and the light-induced current was calculated by subtracting the light-off value from the light-on value.

On average, 3× as many trials were used for 2× as many trials to calculate the I–V curve and rectification of the light-activated current.
Temperature control in acute slices. A custom-designed system was constructed to locally control the temperature in acute slices. A short length of copper tubing was bent into a U-shape, and an additional copper extension was soldered to the end of the U to contact the slice. A peristaltic pump introduced a circulating flow while solenoids were used to gate the source of temperature-controlled water inside the copper tubing. Controlled warming of the tubing was accomplished by rapidly switching the flow from an in-line solution heater (Warner Instruments) to pass while solenoids were used to gate the source of temperature-controlled water inside.

A custom-designed system was constructed switching the flow from an in-line solution heater (Warner Instruments) to pass while solenoids were used to gate the source of temperature-controlled water inside a source that passed through a short –15 cm length of coiled copper tubing inside an ice bath immediately before entering the tubing that was in contact with the slice.

Optogenetic silencing of FSIs in vivo. Data in Supplementary Fig. 2c were re-analyzed from a previous publication to calculate the time-course of optogenetic responses using z-score firing rates. To record single-unit electrophysiological activity from MSNs and FSIs in vivo during optogenetic silencing of FSIs in awake freely moving mice, we implanted multielectrode arrays into the striatum of PV-2CaCre mice. Under anesthesia during stereotactic surgery, the scalp was opened and a hole was drilled in the skull (+0.5 to +1.5 mm AP, −2.5 to −1.5 mm ML from bregma). We implanted 1,000 μm of adeno-associated virus (AAV5-EF1α-DIO-eNhR3.0-YFP) using the coordinates +1.0 AP, ±2.2 ML, −2.5 DM from bregma in PV-2CaCre mice (Jackson, stock no. 012358). Two skull screws were implanted in the opposing hemisphere to secure the implant to the skull. Dental adhesive (C&B Metabond, Parkell) was used to fix the skull screws in place and coat the surface of the skull. Each microwire array (4 μm in diameter, 150-200-μm spacing between wires, 150–200-μm spacing between rows; Innovative Physiology) was combined with a 200-μm diameter optical fiber (Thorlabs, FT200UMT, flat cut) and lowered into the striatum (2.5 mm below the surface of the brain) and cemented in place with dental acrylic (Ortho-Jet, Lang Dental). After the cement dried, the scalp was sutured shut. Animals were allowed to recover for at least 7 days before stratal recordings were made.

Voltage signals from each site on a 32-channel microwire array were recorded in awake, freely moving mice in an open-field arena. Signals were band-pass filtered such that activity between 300 Hz and 6,000 Hz was analyzed as spiking activity. These data were amplified, processed, and digitally captured using commercial hardware and software (Plexon or SpikeGadgets). Single units were discriminated using principal component analysis (Plexon Offline Sorter or SpikeGadgets MatClust). The following two criteria were used to ensure the quality of recorded units: (1) recorded units smaller than 100 Hz (~3 times the noise band) were excluded from further analysis and (2) recorded units in which more than 1% of interspike intervals were shorter than 2 ms were excluded from further analysis. FSIs and MSNs were distinguished based on the waveform and firing rate, as previously described. Green light pulses 1 s in duration and 3 mW in brightness were delivered through a 200-μm optical fiber contained within the implanted microwire recording array with a duty cycle of 25% for 60 min (900 pulses for 1 s; 30 pulses for 30 s).

Temperature calibration. Temperature measurements in slices were performed using the same temperature setup of an Omega DAQ box and T-type thermocouple. In the slice rig, the tip of the sensor was placed at the approximate location of the recording site relative to the fiber or copper tubing with the bath perfusion system operating as usual for acute slice recordings. Temperature changes were measured during laser delivery or passage of hot, warm, or cold fluids through the copper tubing. Measurements for each power were repeated 30 times.

Open-field behavior tests. Wild-type animals were prepared for headbar surgery as described above, with the addition of bilateral 200-μm, 0.39 NA optical fibers (Thorlabs, FT200UMT, flat cut) chronically implanted into the brain and affixed to the skull during headbar surgery. The optical fibers were implanted at +1.0 AP, 1.5 ML, and −2.2 DM from bregma. Mice were allowed to recover for 2 weeks then acclimated to human handling. The open-field arena was a pair of 25-cm diameter circular areas lit from below and the sides with bright white LED strip lighting to minimize the visual silence of laser illumination. Videos recordings were made at 30 fps using an area scan camera (Basler acA1300-60 gm). Laser illumination was generated via two 532-nm diode-pumped solid-state lasers (Shanghai Laser) and coupled to commutators (Doric FRL_1x2i_FC-2FC). Power was tested at the end of the fiber patch cable at the beginning of each behavioral session, and between animals, and was corrected for the recorded efficiency of the implanted fibers for each animal. Mice were run twice at a time, one in each behavioral arena.

Fifteen minutes before each behavioral session, animals received an intraperitoneal injection of 5 mg per kg of amphetamine (d-amphetamine hemisulfate; Sigma Aldrich, A5880) and a saline injection. This limited the contamination to the open-field environment and ensured consistent locomotion throughout behavioral sessions (Fig. 3; Supplementary Fig. 4). At the start of each session, a fiber optic patch cable was attached unilaterally to one of the implanted optical fibers in the dorsal striatum. The mouse was placed in the open field for 30 min, during which time the laser was illuminated in a 10 s on, 30 s off cycle. The optical fiber was preset to the opposite hemisphere with the behavior video being recorded before returning the mouse to its home cage. Laser power was held constant for all sessions on each day. Laser powers were 3 mW (continuous), 7 mW (continuous), 15 mW (continuous), or 15 mW at 20 Hz (15 mW peak power, 10 ms pulse duration for a 20% duty cycle). Predicted temperature rises were based on a previously published computer model for 532-nm light delivered 200-μm below the tip of a 200-μm fiber. Continuous video recordings were acquired using a camera mounted above the arena, and were processed post hoc using DeepLabCut4.

Data analysis for in vivo physiology. Putative MSNs were identified based on waveform and regularity of firing (minimum interspike interval coefficient of variation = 1.1, minimum peak-trough spike width = 0.5 ms). The modulation index was calculated as follows:

\[ \text{Modulation index} = \frac{R_1 - R_2}{R_1 + R_2} \]

where \( R_1 \) represents the average firing rate during the entire duration of laser illumination, and \( R_2 \) represents the average firing rate for an equal period of time immediately before laser illumination.

Data analysis for open-field behavior tests. Videos of the open-field tests were first split into individual videos of each area using Fiji/Imag. Following this, they were processed using DeepLabCut, a machine-learning package that can perform tracking of animal features. Data in this paper were analyzed using a network trained on 200 frames of recorded behavior run for 1 million training iterations. The network was trained to detect the tip of the nose, the base of each ear, and the base of the tail. We then extracted the mean position of the two ears by averaging the x and y coordinates of the base of each ear, which we used as the center point of the mouse.

To determine total locomotion, we detected the change in position of the central point of the mouse across the entire session, and summed it by session.

To determine the change in mouse speed at laser onset, we extracted speed traces of the mouse center point aligned to laser onset. To determine the direction of the samples we generated a vector from the tail base to the center point. We then calculated the change in direction of the mouse for every laser trial. To determine the rotations of the mouse, we used custom MATLAB code (Mathworks) that counted 90° rotations whenever a mouse changed its direction in one direction for 90°. If the animal turned in the other direction for more than 45°, then rotation tracking was reset for the new direction. Rotation times were defined as the frame in which the animal completed 90° of rotation. Rotational analysis was performed blinded to the side of laser illumination. From these rotations, we then calculated rotational bias in the ipsilateral or contralateral direction during the analysis time periods by the formula:

\[ \text{Rotational bias} = \frac{CR - IR}{CR + IR} \]

where CR represents contralateral rotations and IR represents ipsilateral rotations.

Quantification and statistical analyses. All statistical analyses were performed using standard MATLAB functions or custom MATLAB scripts. The statistical tests used and \( P \) values are reported in the figure legends and the results section. Only nonparametric tests that did not include an assumption of distribution normality were used for data analysis. Statistical tests used and \( P \) values are reported in the figure legends and the results section. Statistical tests used and \( P \) values are reported in the figure legends.

All statistical analyses were performed quantifying and statistical tests. Where CR represents contralateral rotations and IR represents ipsilateral rotations. Data analysis for open-field behavior tests. Videos of the open-field tests were first split into individual videos of each area using Fiji/Imag. Following this, they were processed using DeepLabCut, a machine-learning package that can perform tracking of animal features. Data in this paper were analyzed using a network trained on 200 frames of recorded behavior run for 1 million training iterations. The network was trained to detect the tip of the nose, the base of each ear, and the base of the tail. We then extracted the mean position of the two ears by averaging the x and y coordinates of the base of each ear, which we used as the center point of the mouse.

To determine total locomotion, we detected the change in position of the central point of the mouse across the entire session, and summed it by session. To determine the change in mouse speed at laser onset, we extracted speed traces of the mouse center point aligned to laser onset. To determine the direction of the samples we generated a vector from the tail base to the center point. We then calculated the change in direction of the mouse for every laser trial. To determine the rotations of the mouse, we used custom MATLAB code (Mathworks) that counted 90° rotations whenever a mouse changed its direction in one direction for 90°. If the animal turned in the other direction for more than 45°, then rotation tracking was reset for the new direction. Rotation times were defined as the frame in which the animal completed 90° of rotation. Rotational analysis was performed blinded to the side of laser illumination. From these rotations, we then calculated rotational bias in the ipsilateral or contralateral direction during the analysis time periods by the formula:

\[ \text{Rotational bias} = \frac{CR - IR}{CR + IR} \]

where CR represents contralateral rotations and IR represents ipsilateral rotations.

Quantification and statistical analyses. All statistical analyses were performed using standard MATLAB functions or custom MATLAB scripts. The statistical tests used and \( P \) values are reported in the figure legends and the results section. Only nonparametric tests that did not include an assumption of distribution normality were used for data analysis. Statistical tests used and \( P \) values are reported in the figure legends.
## Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

### Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

| n/a | Confirmed |
|-----|-----------|
| ☑   |           |

- The **exact sample size** \((n)\) for each experimental group/condition, given as a discrete number and unit of measurement
- An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
  - *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. \(F, t, r\)) with confidence intervals, effect sizes, degrees of freedom and \(P\) value noted
  - *Give \(P\) values as exact values whenever suitable.*
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen's \(d\), Pearson's \(r\)), indicating how they were calculated
- Clearly defined error bars
  - *State explicitly what error bars represent (e.g. SD, SE, CI).*

Our web collection on [statistics for biologists](https://natureresearch.com/nature-research-statistics) may be useful.

### Software and code

Policy information about availability of computer code

| Data collection | Data were acquired using Trodes 1.7.3 (from SpikeGadgets), OMEGA DAQ Central 1.0.7, Plexon MAP v2.5, and Igor Pro 6.0. Spiking data was sorted with either Plexon Offline Sorter 3.0.1 or Matclust v1.4 (Spike Gadgets). Modeling was performed using Matlab 2014a to run a previously published, freely available model. |
| Data analysis   | Behavioral videos were processed with FFmpeg 4.1.3. All other data were analyzed and plotted using Matlab 2014a. Scripts are relatively simple for data averaging, and generation of peri-stimulus triggered histograms, but all scripts and code are freely available upon request. |

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [guidelines for submitting code & software](https://natureresearch.com/nature-research-guidelines) for further information.

### Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

All data and software are available upon request.
Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- Life sciences
- Behavioural & social sciences
- Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | Sample size for physiological recording and in vivo manipulations were determined based on previously published studies, and statistical significance was calculated using post-hoc tests. |
|-------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Data exclusions | For in vivo recordings, other cell types were excluded based on spike waveform (Figure 1C; see methods). For whole cell recordings, cell types were distinguished by location, access resistance, and capacitance immediately upon break-in. |
| Replication | Each experiment was performed multiple times across at least two animals, and at least two separate experimental sessions (days). Every effect described in this manuscript was replicated across animals and sessions. Data were then pooled for analysis and presentation. |
| Randomization | All experiments were performed on wild-type mice. No randomization procedures were implemented. |
| Blinding | Physiological protocols were designed to interleave trials and experimental conditions within individual animals and within individual recording sessions. Analysis was automated using Matlab scripts for event detection and processing to remove experimenter bias. No explicit blinding procedures were implemented. |

Reporting for specific materials, systems and methods

Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ✗   | Unique biological materials |
| ✗   | Antibodies |
| ✗   | Eukaryotic cell lines |
| ✗   | Palaeontology |
| ✗   | Animals and other organisms |
| ✓   | Human research participants |

Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ✗   | ChIP-seq |
| ✗   | Flow cytometry |
| ✗   | MRI-based neuroimaging |

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

| Laboratory animals | Mouse C57Bl/6, age 45-250 days, male and female |
|--------------------|------------------------------------------------|
| Wild animals       | no wild animals                                  |
| Field-collected samples | no samples collected from field                  |