LETTERS

Soft, stretchable, fully implantable miniaturized optoelectronic systems for wireless optogenetics

Sung Il Park1,2,8, Daniel S Brenner3,8, Gunchul Shin1,2,8, Clinton D Morgan3,8, Bryan A Copits3, Ha Uk Chung1,2, Melanie Y Pullen3, Kyung Nim Noh1,2, Steve Davidson3, Soong Ju Oh1,4, Jangyeol Yoon1,2,5, Kyung-In Jang1,2, Vijay K Samineni3, Megan Norman3, Jose G Grajales-Reyes3, Sherri K Vogt3, Saranya S Sundaram3, Kellie M Wilson3, Jeong Sook Ha5, Renxiao Xu6, Taisong Pan6, Tae-il Kim7, Yonggang Huang6, Michael C Montana3, Judith P Golden3, Michael R Bruchas3, Robert W Gereau IV3 & John A Rogers1,2

Optogenetics allows rapid, temporally specific control of neuronal activity by targeted expression and activation of light-sensitive proteins. Implementation typically requires remote light sources and fiber-optic delivery schemes that impose considerable physical constraints on natural behaviors. In this report we bypass these limitations using technologies that combine thin, mechanically soft neural interfaces with fully implantable, stretchable wireless radio power and control systems. The resulting devices achieve optogenetic modulation of the spinal cord and peripheral nervous system. This is demonstrated with two form factors; stretchable film appliqués that interface directly with peripheral nerves, and flexible filaments that insert into the narrow confines of the spinal epidural space. These soft, thin devices are minimally invasive, and histological tests suggest they can be used in chronic studies. We demonstrate the power of this technology by modulating peripheral and spinal pain circuitry, providing evidence for the potential widespread use of these devices in research and future clinical applications of optogenetics outside the brain.

The use of optogenetics in the brain has revolutionized the interrogation of neural circuitry by enabling temporal and spatial control of neuronal function. However, attempts to apply optogenetic studies to tissues beyond the brain have been stymied by the inability to target peripheral and spinal circuits in freely moving animals. Studies to date have primarily used cumbersome tethered fiber-optic cables or light emitting diode (LED) arrays to activate opsins that are expressed transgenically or delivered through gene therapy1–3. Although these experimental approaches have utility, physical tethers impede movement, which can alter behavior and the natural motion of animals in complex environments. Additionally, the fixation of fiber-optic cables requires physical bonding to a static skeletal feature such as the skull, and external fixtures can cause device loss due to damage by the animal, a cage mate, or by inadvertent damage from housing. These fibers can also damage the surrounding neural tissue during insertion or during fiber coupling owing to relative motion of the hard fiber against soft tissues4,5.

Thin, injectable polymer filaments with integrated, cellular-scale LEDs and externally mounted, wireless power-harvesting systems6–9 represent attractive alternatives, but cannot illuminate spatially challenging and highly mobile areas like peripheral nerves or the spinal cord, which are critical to the study of the extracranial circuits involved in sensory input and motor output. Recently developed fully implantable devices with radio frequency (RF)-powered LEDs achieve some capabilities in these contexts10; however, these devices use hard materials and geometrically thick designs, which limit their potential for chronic biocompatibility and integration with soft tissues of the nervous system.

Miniaturized, biocompatible devices that can safely interface with peripheral neural tissue and illuminate challenging areas are needed to advance the technology support for advanced optogenetic studies not only of the brain, but also of issues related to chronic pain, itch and other neurological disorders. The development of suitable devices requires management of heat generation and power delivery; robust remote activation with uniform, natural operation across cage configurations and animal species; power delivery over large areas; and miniaturization with thin geometries and low-modulus, elastic mechanics for chronic tissue compatibility8.

Here we present miniaturized, soft wireless optoelectronic systems with versatile layout options that are fully compatible with advanced methods for mass production in semiconductor device manufacturing and capable of complete, minimally invasive implantation over multiple neural interfaces. The low-modulus mechanics of these biocompatible devices allows their implantation as thin appliqués and/or soft injectable filaments, without the need for skeletal fixation, thereby permitting experiments in regions where it would be impossible to operate with other approaches. We demonstrate that these devices can specifically and reversibly activate both peripheral and spinal pain

1Department of Materials Science and Engineering, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA. 2Frederick Seitz Materials Research Laboratory, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA. 3Washington University Pain Center and Department of Anesthesiology, Washington University School of Medicine, St. Louis, Missouri, USA. 4Department of Materials Science and Engineering, Korea University, Seoul, Republic of Korea. 5Department of Chemical and Biological Engineering, Korea University, Seoul, Republic of Korea. 6Department of Mechanical Engineering, Northwestern University, Chicago, Illinois, USA. 7School of Chemical Engineering, Sungkyunkwan University (SKKU), Suwon, Republic of Korea. 8These authors contributed equally to this work. Correspondence should be addressed to J.A.R. (jrogers@illinois.edu) or R.W.G. (gereaur@wustl.edu).

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circuits in freely moving, untethered mice. Detailed chronic studies and histological evaluations show the essential benefits of soft, compliant and fully implantable device technologies of this type.

Our miniaturized wireless optoelectronic systems were composed of an RF harvesting unit that receives signals from a transmitter, rectifies them, multiplies the voltages and routes the resulting direct-current output to the LEDs (a turn-on voltage of 2.7 V, 470 nm wavelength). The antenna and LEDs are connected with serpentine Ti/Au electrical interconnects, and the circuit is encapsulated by polyimide (40 µm width, 3 µm thickness) and a low-modulus silicone elastomer (~0.5 MPa, 100 µm thickness). This process yields soft, system-level mechanics (effective modulus of ~1.7 MPa) capable of accommodating anatomical shapes and natural motions6–9 (Fig. 1a, Supplementary Note 1, Supplementary Figs. 1–3 and Supplementary Table 1).

The mechanical compliance, miniaturized geometry (0.7 mm × 3.8 mm × 6 mm) and lightweight construction (16 mg) of these devices enabled implantation into anatomical regions that were previously inaccessible owing to physical constraints. In comparison with previous technology, these devices are substantially thinner, softer and more flexible (Supplementary Note 1)8,10,11. We deployed these devices underneath muscle for optogenetic stimulation of a peripheral nerve (Fig. 1b), and in the epidural space for optogenetic control in the spinal cord (Fig. 1c). Peripheral nerve illumination was achieved with a soft appliqué that was implanted with the antenna in a subcutaneous pocket with an LED extension that traverses under the gluteus maximus to the sciatic nerve. The distal extension with the LEDs included wings (1 mm × 3 mm) that anchored the LED tip in the gluteal pocket once the muscular architecture had been repaired with suture (Fig. 1b,d,f). Spinal illumination was achieved by implantation under the vertebra in the epidural space, which was accessed by laminectomy of the T13 spinous process (Fig. 1c,e,g). This placement centered the narrow part of the device (380 µm × 8 mm) over the lumbar spinal cord.

The key to miniaturizing these devices is a stretchable antenna that harvests RF power through capacitive coupling between adjacent serpentine traces (Supplementary Fig. 4), thereby lowering the resonant frequency and therefore the dimensions of the antenna12. For operation at 2.34 GHz, this design required an area of only 3 × 3 mm, a 100-fold reduction in volume and weight compared to conventional rigid antennas8. This antenna also featured a wide bandwidth, which is essential for reliable activation of the devices as the center frequency of the receiving antenna must be similar to that of the transmitting antenna to efficiently harvest transmitted power. The center frequency describes the frequency range where an antenna absorbs energy most effectively and corresponds to the range of frequencies that minimize the scattering parameter (S11). A lower S11 indicates that less incident energy is reflected off the antenna and therefore more of that energy is absorbed. The wide bandwidth (200 MHz) of the stretchable antenna allows it to harvest RF power from a much wider range of transmitting frequencies than conventional patch antennae (bandwidth of 50 MHz) (Supplementary Fig. 4d). This characteristic reduces the likelihood that a mismatch between the receiver and transmitter will prevent device activation. The S11 and center frequency in these devices are also affected by the physiological environment (Supplementary Note 2 and Supplementary Fig. 5).

The capacitive coupling that powers these devices can be diminished if strain deforms the metallized traces and increases the sizes of the gaps between them. To assess the reliability of these antennae under

Figure 1  Miniaturized, fully implantable, soft optoelectronic systems for wireless optogenetics. (a) Exploded view schematic illustration of the energy harvester component of the system, with an integrated LED to illustrate operation. (b,c) The anatomy and location of the peripheral and epidural devices relative to the sciatic nerve (b) and spinal cord (c). (d) Picture of an active device resting on the tip of the index finger. The device is 0.7 mm thick, 3.8 mm wide and 6 mm long; its weight is 1.6 mg. (e) Picture of the epidural device, highlighting the soft, stretchable connection to an LED. The diameter of the epidural implant component is 380 µm, with cross sectional dimensions comparable to the epidural space. (f,g) Mice with wireless devices implanted near the sciatic nerve (f) and the spinal cord (g).
biological strain, we modeled and tested performance under worst-case scenarios (30% strain) (Fig. 2a, b and Supplementary Fig. 5). Simulations showed that although uniaxial strains of ~10% increased the gap size in the direction of the strain, they reduced the gap size in the orthogonal direction by up to 50% (Supplementary Fig. 6). As a result, the decrease in coupling owing to increased gap size was balanced by enhanced coupling in the orthogonal direction, such that the harvesting efficiency of the antenna was largely unaffected (Fig. 2a, b).

Although strain does not alter the efficiency of capacitive coupling, it does shift the center frequency of the antenna toward lower frequencies (Fig. 2a, b). However, the magnitudes of the strain-induced shifts in the center frequency were small compared to the large bandwidth (Supplementary Fig. 4d), such that the net result of supraphysiological strain application was a 12% decrease in coupling efficiency due to center frequency shifts (Fig. 2a, b middle). This translates to a modeled optical power output decrease of only a few percent, suggesting that physiological strain is unlikely to significantly impair device function (Fig. 2a, b, right). We confirmed this modeling by testing devices under deformation conditions that greatly exceeded anything expected to occur in animals; the devices functioned reliably (Fig. 2h, Supplementary Fig. 7). Additionally, a mouse with a device interfaced to the sciatic nerve ran without trouble on an exercise wheel (Supplementary Fig. 8 and Supplementary Movie 1) and 6 months after implantation a wireless device still functioned in another mouse (Fig. 1f), providing evidence that these devices function reliably under physiological strain.

In addition to physical strain, other concerns for long-term implantation of electrical devices in animals include heat generation and long-term durability. Infrared imaging of an anesthetized mouse during device operation revealed that an optical power density of 10 mW/mm² (40% duty cycle; 20 Hz period; 20 ms pulse width) does not cause detectable temperature changes (Fig. 2c). Studies using implantable thermal sensors showed similar trends (Supplementary Fig. 9 and Supplementary Note 3). Exposure to biological conditions did not greatly alter device operation or durability; devices retained full functionality for 2 months when immersed in 37 °C saline, and for 6 days in saline at supraphysiological temperatures (90 °C) (Fig. 2d). In terms of mechanical stability, these devices were cycled >10⁵ times without a detectable loss in optical power (Fig. 2e). The robustness suggested in these in vitro assays is reinforced by the fact that 76% (31/41) of devices that were implanted for use in this work were still functional in the animals after 1 week. Two sciatic nerve devices retained reliable activation at least every month for 6 months after implantation; additionally, and of five sciatic nerve devices where we attempted reimplantation in new host mice after initial removal,
three remained functional for 3 weeks after reimplantation. All of these observations suggest that heat generation, hydration effects and durability are not obstacles for the use of these devices in animals.

For the devices to be useful in behavior experiments, the RF transmission (TX) systems must enable continuous operation throughout a location of interest (e.g., the home cage or testing arena), at field strengths that lie below IEEE and Federal Communications Commission (FCC) guidelines. A configuration of four TX antennas connected to a common RF power supply (Fig. 2f) provided total average RF power that was sufficient for operation (~2 W) throughout the

Figure 3 Electrophysiological and anatomical characterization of ChR2 expression in Advillin-ChR2 mice. (a) Schematic of the Ai32 locus and Advillin-cre mouse locus where stop codons are inserted in all three reading frames and flanked byloxP sites upstream of the coding region for ChR2. The Advillin-cre mouse locus shows cre-recombinase driven by the sensory neuron–specific Avil promoter. Cre recombinase expression results in recombination betweenloxP sites and excision of the stop codons, leading to expression of ChR2. Electrophysiological recordings from DRG neurons cultured from Advillin-ChR2 mice. For all traces, 470-nm illumination is delivered at 10 mW/mm². (b) 1-second-long illumination induces inward currents (lower trace) in voltage clamp recordings, and in some cells produces sustained firing in current clamp recordings (upper trace). (c) Pulsed illumination at 20 Hz induces action potential firing with high fidelity (upper trace) resulting from the inward currents that are generated in voltage clamp (lower trace). Note that the first pulse produces larger amplitude inward currents relative to the second and all subsequent light pulses, consistent with the rapid desensitization to a steady-state current seen with prolonged illumination (b, lower). (d) Immunohistochemical analysis of tissue from adult Advillin-ChR2 mice demonstrates that ChR2 is expressed along the peripheral neuraxis, including termination in lamina I and lamina II of the spinal cord dorsal horn as evidenced by overlap with CGRP (purple) and IB4 (red), respectively. (e) Staining of DRG shows considerable overlap of ChR2 expression with the neuronal marker βIII tubulin (purple) and IB4 (red) within the soma. (f,g) Longitudinal (f) and cross-sections (g) of sciatic nerve demonstrate robust staining along the plasma membrane of the axons of both myelinated (marked with NF200, purple) and unmyelinated neurons, and some expression of ChR2 in the circumferential nonexcitable epineurial tissue. Scale bars, 100 µm for d, f, g, and 50 µm for e.
Figure 4 Wireless activation of ChR2 expressed in nociceptive pathways results in spontaneous pain behaviors and place aversion. (a) Representation of nociceptive pathways and illumination of nociceptive fibers with a sciatic LED stimulator. (b) Implantation of the sciatic LED stimulator has no effect on motor behavior vs. sham animals in the rotarod test ($P = 0.894$, $n = 5$ sham, $n = 8$ device). (c) Wireless activation of the sciatic LED stimulator causes increased nocifensive behaviors (flinching, hind paw licking, jumping) in Advillin-ChR2 mice but not in controls (17.5 vs. 1.2 flinches, $P < 0.0001$ vs. without illumination $n = 3$ per group). No other statistical comparisons reach significance. (d) Mice in a modified Y-maze. One arm is targeted with the RF antenna to operate the LED device (LED ON) and the other is not (LED OFF). Time spent in the center area (dashed lines) is not scored. (e) Heat maps from individual mice representing the time spent in each zone, red indicating more and blue indicating less time. In animals implanted with the sciatic LED device, aversion to the LED-ON zone is observed in TrpV1-ChR2 and Advillin-ChR2 mice, but not in controls. (f) Quantification of time spent in each zone of the Y-maze. TrpV1-ChR2 (420.5 vs. 644.5 s; $P = 0.011$, $n = 5$) and Advillin-ChR2 (491.2 vs. 656 s; $P = 0.001$, $n = 8$) mice display aversion to the LED-ON zone vs. the LED-OFF zone. No difference is observed in control mice (547.0 vs. 512.1 s; $P = 0.551$, $n = 10$). (g) Representation of ascending nociceptive pathways and illumination of primary afferent terminals innervating the spinal cord with a wireless epidural implant. (h) Wireless activation of the epidural LED implant increased nocifensive behaviors in SNS-ChR2 mice (64.2% vs. 0% of time; $P < 0.001$, $n = 3$). (i) Heat maps representing the time spent in each zone of the Y-maze. Red indicates areas where the animals spend a higher proportion of time. Aversion to the LED-ON zone is observed in SNS-ChR2 mice but not in controls. (j) Quantification of the time spent in each zone of the Y-maze. SNS-ChR2 mice display aversion to the LED-ON zone (73 vs. 251 s; $P = 0.006$, $n = 6$). No difference is observed in control mice ($n = 3$). Group data are presented as mean ± s.e.m. Statistical comparisons were made using two-tailed t-tests, except for b, which was a two-way ANOVA. *$P < 0.05$, **$P < 0.01$.

volume of the cage, and was capable of activating multiple devices in the same region (Fig. 2g). These devices could be activated reliably up to 20 cm from the transmitters, which is ten times the reported range of any previous systems.8–11 (Supplementary Fig. 10). Under these conditions, we calculated distributions of the specific absorption rate (SAR) for the body and found that the SAR fell well below safety guidelines13 (Fig. 2f). This configuration allowed consistent device activation even with rapid changes in receiver location and orientation (Fig. 2h–j). This is demonstrated using long-exposure images captured during motion of an operating device; continuous streaks of light illustrate activation of the devices regardless of device position or orientation (Fig. 2i–j). (See Supplementary Notes 4 and 5 and Supplementary Figs. 10–12 for further discussion of the transmission efficiency with moving animals.)

To determine the utility of these optoelectronic devices in studies of pain pathways, we tested whether they could modulate pain-related behaviors of mice expressing ChR2 in all sensory neurons or in subpopulations of sensory neurons responsible for detection of noxious stimuli (nociceptors) (see Supplementary Note 6 and Online Methods). Mice expressing ChR2 in all sensory neurons were generated using a cre recombinase–based transgenic approach where cre recombinase expression is driven by the promoter of the sensory neuron–specific gene Advillin (Advillin-ChR2; Fig. 3a).14,15 Electrophysiological studies show that Advillin-ChR2 sensory neurons were consistently activated by blue light (Fig. 3b,c and Supplementary Note 7), and immunohistochemical studies demonstrate that ChR2 was present in mid-axon, in the dorsal root ganglia (DRG) and in the central terminals of sensory neurons (Fig. 3d–f and Supplementary Note 8). Similar results were observed in mouse lines where ChR2 expression is restricted to nociceptor populations (TrpV1-ChR2, SNS-ChR2; Supplementary Notes 6–8, Supplementary Figs. 13–17 and Supplementary Table 2).

Previous studies have shown that illumination of peripheral nerve terminals using an external light source on the skin induces spontaneous pain behaviors and place aversion in mice expressing ChR2 in sensory neurons.1–3. For these implantable devices to be viable for in vivo pain studies, illumination of ChR2-expressing axons (Fig. 4a) must generate action potentials. Consistent with this hypothesis, fiber-optic laser illumination of the exposed sciatic nerve in TrpV1-ChR2 mice produced reflexive withdrawal behaviors (Supplementary Fig. 18). It is also critical that device implantation not induce nerve injury or impair function. Devices implanted over the sciatic nerve for 2 weeks produced no signs of injury and no infiltration of immune cells compared to the contralateral nerve (Supplementary Figs. 19 and 20), and produced no motor impairment, even when the mouse was running (Supplementary Movie 1). This was quantified using the accelerating rotarod and open field tests, both of which indicated that the devices did not alter balance, motor coordination or locomotor activity compared with sham controls (Fig. 4b and Supplementary Fig. 21).
Devices implanted over the sciatic nerve in Advillin-ChR2 mice generated robust nocifensive responses. Wireless powering (20 Hz, 2.34 GHz RF, 3–5 dBm) of these devices produced reversible nocifensive behaviors in Advillin-ChR2 mice, but not in cre-negative littermates (Fig. 4c). These spontaneous responses are consistent with nociceptor activation. To evaluate whether optogenetic peripheral neuron activation produced behavioral aversion consistent with the perception of ongoing pain (as opposed to representing reflex activation), we placed mice in a modified Y-maze apparatus where one arm was exposed to a curtain RF (LED-ON) and one arm (LED-OFF) was not (Fig. 4d). Pretesting of devices in this arena demonstrated that activation occurred only in the LED-ON arm. Advillin-ChR2 mice showed significant aversion to the LED-ON arm compared to the LED-OFF arm (Fig. 4e,f), whereas cre-negative littermates spent a similar amount of time in the two arms. Similarly, TrpV1-ChR2 mice, which express ChR2 only in nociceptors, demonstrated significant aversion to the LED-ON arm compared to the LED-OFF arm (Fig. 4e,f).

With the epidural devices, we demonstrate optogenetic modulation of the spinal terminals of peripheral nerves using LED devices that are inserted in the epidural space (Fig. 4g). Implantation of devices into the epidural space did not cause significant damage to the spinal cord, as demonstrated histologically (Supplementary Fig. 22). Epidural device implantation also produced no impairment in motor behavior, locomotion or coordination compared to sham controls in the accelerating rotarod and open field tests (Supplementary Fig. 21). Activation of these devices (20 Hz, 2.24 GHz RF, 3–5 dBm) in the epidural space of SNS-ChR2 mice generated robust and reversible nocifensive behaviors that was entirely absent in cre-negative littermates (Fig. 4h). A Y-Arm maze assay to quantify behavioral aversion using SNS-ChR2 mice with epidural implants showed these mice had robust aversion to the LED-ON arm compared to littermate cre-negative mice (Fig. 4i–j).

These miniaturized, fully implantable, thin and soft optoelectronic systems enable robust operation and large transmission range without the need for optimization around specific cages or animal body types. The platforms are thinner by a factor of 5, more stretchable by a factor of 10, softer by a factor of 10,000 and more flexible by a factor of 10,000,000 than alternative technologies, thereby providing unique features in a wide variety of optogenetic applications. The low modulus of these biocompatible devices permits experiments in more extensive regions of the body and in a chronic manner, bypassing constraints associated with the hard mechanics and thick volumetric layouts of the most recently reported fully implantable designs19.

Providing easy access to this technology to the broader scientific community is essential to facilitate improved studies of neuronal circuitry. Our devices can be fabricated with 10 h of effort in standard laboratory facilities using inexpensive commercially available components, and the external power transmission systems require less than 1 h of training, making it possible for independent laboratories to construct and operate their own variants. Whereas the hand-crafted approach to device fabrication needed for other approaches10 offers some advantage in customization, it has limited potential to take advantage of increasingly powerful manufacturing approaches and accelerating trends in size miniaturization that drive progress in conventional optoelectronics. In contrast, our fabrication process is compatible with established flexible printed circuit board technologies and manufacturing tools from the electronics industry, making it possible to construct large numbers of devices in a cost-effective manner.

Extension of our approach to multiple LEDs could enable optogenetic modulation of the whole brain or other whole organs. Laminated films with high thermal conductivity could improve the efficiency of heat dissipation, and optical diffusers could yield spatially uniform illumination. These advanced forms, as well as the present designs, have potential not only for basic research, but also as clinical tools. Gene therapy that could be used to deliver optogenetic channels to human cells is already in clinical trials16–20, and with the appropriate testing these optogenetic stimulators could be adapted for use in treating chronic intractable human conditions such as chronic pain.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

S.I.P. designed wireless optoelectronic systems, fabricated devices, tested devices, made wireless measurements, conducted simulations of wireless performance, designed experiments, generated figures, wrote and edited the manuscript. D.S.B. designed sciatric nerve devices, implanted devices, tested mouse behavior, designed experiments, performed immunostaining, generated figures, wrote and edited the manuscript. G.S. designed and fabricated spinal cord devices, tested devices, generated figures, wrote and edited the manuscript. C.D.M. designed spinal cord devices, implanted devices, tested mice in behavior, designed experiments, performed immunostaining, generated figures, wrote and edited the manuscript. B.A.C. performed immunostaining and quantification, electrophysiology experiments, generated figures. H.U.C. and K.N.N. fabricated devices and tested devices. M.Y.P. performed surgical procedures, behavioral studies and electrophysiology, generated figures and edited the manuscript. S.D. performed experiments, implanted devices, generated figures. S.I.O., J.V. and K.-I.J. made contributions to fabrication and testing of devices. V.K.S. performed experiments, immunostaining and generated figures. M.N. performed immunostaining and quantification of slides, as well as mouse breeding. J.G.-R. performed experiments and generated figures. S.K.V. performed immunostaining and mouse breeding. S.S.S. performed immunostaining and mouse breeding. K.M.W. performed immunostaining. J.S.H. made contributions to fabrication and testing of devices. R.X., T.P. and Y.H. performed mechanical simulations of device tolerance levels. T.K. designed and tested wireless optoelectronic systems for sciatic nerve. M.C.M. designed experiments and generated figures. J.P.G. performed immunostaining, generated figures, performed behavioral experiments, helped develop epidural implants and edited the manuscript. M.R.B. designed experiments. R.W.G. and J.A.R. oversaw all experiments and data analysis, designed experiments and devices, wrote and edited the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

For all mouse studies, institutionally approved protocols were followed for all aspects of this study.

Device design and fabrication. The harvesting unit receives signals from a transmitter, rectifies them, multiplies the voltages (3x) and routes the resulting direct-current output to the LEDs. The harvesting unit is an impedance matching circuit consisting of a ceramic chip capacitor (1 pF; 0.20 mm width, 0.4 mm length, 0.22 mm thickness; bonded by solder paste) and an inductor (2.7 nH; 0.20 mm width, 0.4 mm length, 0.22 mm thickness; bonded by solder paste) connected in series. The rectifier uses miniaturized Schottky diodes (1.7 mm width, 1.5 mm length, 0.5 mm thickness) and ceramic chip capacitors (5 pF; 0.20 mm width, 0.4 mm length, 0.22 mm thickness; bonded by solder paste). The multiplier includes three Schottky diodes identical to those in the rectifier, and boosts voltages provided by the rectifier (–0.9 V) to values sufficient to operate the LEDs (–2.7 V; 220 µm width, 270 µm length, and 50 µm thickness for spinal device; 1.6 mm length, 0.8 mm width, and 0.75 mm for peripheral devices).

Fabrication begins with a clean glass slide (75 mm long, 50 mm width, and 1 mm thickness), with a layer (200 nm thickness) of polymethyl methacrylate (PMMA, 495 PMMA A6, Microchem) and a 2 µm layer of polyimide (PI) formed by spin-casting at 3000 rpm for 60 s, cured at 250 °C for 2 h. Photolithography (AZ 4620, AZ Electronic Materials) defines the necessary conducting traces after e-beam deposition of Ti/Au (3 µm thickness). A second 2 µm PI layer serves as encapsulation for making a mechanically neutral plane. Photolithography and reactive ion etching then define the PI/metal/PI layers into serpentine-shaped structures. The LED and circuit chips are placed onto the exposed pads (Supplementary Fig. 1) with a small amount (5–20 particles) of solder paste (SM290SNI250TS, Chipquik). The substrate is then cured at 250 °C in a vacuum oven for 10 min to electrically bond the LEDs and the surface-mounted device components to the conductive traces. An encapsulating layer of polydimethylsiloxane (PDMS), spin-cast and cured at 70 °C for 1 h seals the device before its release from the substrate by dissol-ution of the PMMA in acetone. For the epidural device, the narrow serpentine area (~360 µm width) and LED are inserted into a Teflon tube (PTFE-28-25, SAI), with an inner diameter of 380 µm. PDMS is added to the tube. The devices are cast and cured in the tube, which is then removed to complete the fabrication. The timing and steps required for device fabrication are detailed in Supplementary Note 1.

Configuration: RF system for power transmission. The RF transmission system consists of a signal generator (N5181 MXF, Agilent), a power amplifier (1189/BBM3K5KCO, Richardson RF), a DC power supply (UR8031A, Keysight Technologies) with a heat sink (53M7972, Fischer Elektronik), and TX antennas (PE51019-3, Paterneck Enterprises) with a splitter (RFLT4W0727GN, RFLambda). The amplifier and the fan are powered by separate DC power supplies. The outputs (channels 1 & 2) connect to the J3 input of the amplifier, with VDD into Pins #6, 7 and GND into Pins #8, 9 and to the fan, respectively. The output of the signal generator connects to the input of the amplifier, which is connected to the splitter to output to all of the TX antennas.

Animals and genetic strategy. Adult mice (8–12 weeks of age) are used for this study. Mice are housed in the animal facilities of the Washington University School of Medicine on a 12 h light/dark cycle, with access ad libitum to food and water. Institutionally approved protocols are followed for all aspects of this study.

Three core-driver lines were used for this study including heterozygous SNS-cre mice from Rohini Kuner21, heterozygous TrpV1-cre mice from Mark Hoon22, and heterozygous Advillin-cre mice provided by Fan Wang23. Mice from each of these three lines were crossed to homzygous Ai32 mice from Jackson Laboratory. As previously described, Ai32 mice harbor ChR2 (H134R)-eYFP in the Gf(ROSA)26So3 locus24. To generate mice with conditional expression of ChR2 in specific populations of sensory neurons, mice with ChR2 in the Rosa locus (Ai32 mice) were crossed to mice expressing cre from various sensory neuron-specific driver gene loci (Advillin, TrpV1, or SNS). For the purposes of this study, the three lines generated were referred to as Advillin-Chr2, TrpV1-Chr2 and SNS-Chr2, respectively.

Surgical procedure: sciatic device implantation. The surgical procedure was modified from the Chronic Constriction Injury procedure24. Mice were anes-thetized with isoflurane and their eyes were covered with Altalube ointment (Altaire Pharmaceuticals, Riverhead, NY) to prevent corneal drying. A small skin incision was made over the greater trochanter of the femur on the left flank of the animals. The fascia connecting the biceps femoris and the glutaeus maximus was blunt dissected apart to open a plane between the muscles, in which the sciatic nerve was clearly accessible. The fascia connecting the underlying muscle in the area directly rostral to the incision was blunt dissected apart using needle driver forceps. The body of the device was inserted under the skin into the subcutaneous pocket generated by the blunt dissection. The glutaeus maximus was pulled caudally to expose the sciatic nerve, and the tip of the device containing the LED was folded under the glutaeus and placed over the nerve. The glutaeus maximus was pulled over the device and sutured into place with a resorbable Ethicon 6-0 vicryl suture (Corinella, GA) to restore the original muscle architecture, and to secure the device between the muscles and above the nerve. The left flank incision was sutured closed using Ethicon 6-0 nylon monofilament suture and the mouse was allowed to recover from anesthesia in a warmed chamber.

Surgical procedure: epidural device implantation. Under isoflurane anesthesia on an isothermal heating pad, a 2-cm midline incision was made on the back, exposing the thoracolumbar vertebral transition. The paraspinal muscles were separated, exposing the T13 spinous process and lamina. A partial laminectomy was made at the rostral end of this landmark level, allowing insertion of the epidural stimulator with the LEDs centered over the dorsal horn of the L4-L6 spinal cord segment25. The distal end of the epidural stimulator and proximal stretchable antenna were secured with 6-0 suture. The skin was closed using interrupted sutures and mice were allowed to recover on an isothermal pad with access to food and water ad libitum.

Surgical procedure: spinal nerve ligation (SNL). Mice were deeply anes-thetized with vaporized isoflurane, and the paraspinal muscles were bluntly dissected to expose the LS transverse process. The LS process was removed, the L4 spinal nerve was tightly ligated with silk suture (6-0, Ethicon; Cornelia, GA) and the nerve was transected distal to the ligation. The skin was closed with staples and the animal was allowed to recover on an isothermal heating pad.

Surgical procedure: chronic constriction injury (CCI). The procedure was performed as described previously24. In brief, mice were deeply anesthetized with vaporized isoflurane and a small incision was made over the left flank. The fascial layer between the biceps femoris and glutaeus maximus was bluntly dissected to expose the sciatic nerve. Two loose chronic gut sutures were tied around the nerve, which was then resected and the muscular architecture was re-aproximated on top of it. The skin was closed with interrupted sutures, and the animal was allowed to recover on an isothermal heating pad.

Direct laser activation of the sciatic nerve in an open preparation. Mice were anesthetized with 2% isoflurane. A small skin incision was made over the greater trochanter of the femur on the left flank of the animals. The fascia connecting the biceps femoris and the glutaeus maximus was blunt dissected apart to open a plane between the muscles, in which the sciatic nerve was clearly accessible. A small cutaneous incision over the lateral leg of the mouse was made, and two silver electrodes were implanted in the exposed quadriceps muscles to amplify and record electrical activity representing muscle response. After completion of the surgical preparation, the isoflurane anesthesia was gradually reduced over 2 h to ~0.875% until a flexion reflex response (evoked by pinching the paw) was present but spontaneous escape behavior and righting reflex were still absent. The animals were not restrained in any fashion. Body temperature was maintained using an overhead radiant light and monitored throughout the experiment. These conditions were optimized to establish a stable depth of anesthesia and consistent baseline sciatic muscular activity. A laser stimulus delivered through a fiber-optic cable was then used to stimulate the sciatic nerve while the EMG response was recorded in real-time using a Grass CP511 preamplifier connected to a PC via a WinDaq DI-720 module. The data were exported for analysis to Igor Pro 6.05 software (Wavemetrics, Portland, OR). Using a custom script, the EMG signals were...
subtracted from the baseline, rectified and integrated to quantify the area under the curve. The area under the curve for the motor response was presented in arbitrary units. The investigator quantifying the motor response was blinded to testing condition.

Behavioral analyses. For behavioral studies, a priori power analyses were performed to estimate necessary sample sizes. However, study results demonstrated effect magnitudes larger than anticipated, and therefore, increased animal numbers could not be justified. For all behavioral analyses, the experimenters were blind to genotype and treatment (implant vs. sham). Animals from each genotype were randomly selected for implant vs. sham.

Behavior: spontaneous behavior. Each mouse was placed in an individual Plexiglas behavioral chamber. Mice were allowed to acclimate for at least 30 min before testing in the presence of white noise generators to reduce the influence of external noise pollution on testing. To measure spontaneous behaviors, the wireless LED devices were activated using the RF signal generator antenna at 3–5 dBm and 2.0–2.5 GHz. Behavior was recorded through an HD video camera (Sony) for one minute. Nocifensive behaviors (defined as licking hind paws, vocalizations, or jumping) were quantified post-hoc from the video recordings while blinded to genotype.

Behavior: Y-maze. Place aversion was tested in two arms of a Y-maze constructed of plexiglas with a layer of corn cob bedding. Each arm of the maze was 10 cm wide x 100 cm long and was marked with either vertical or horizontal black stripes with a neutral area between the arms. To generate the RF signal, one antenna was located below an arm of the maze allowing for the control of LED devices through the maze floor and a second antenna was positioned on the side of the same arm to ensure complete local field coverage. To begin the experimental protocol, a mouse was placed in the neutral area of the maze and was continuously monitored and recorded through a video connection for 20 min. During this time an experimenter blinded to the genotype manually controlled the RF signal by watching the monitoring system. Upon entry of the mouse into the “ON” chamber, activation of the LED device through the RF antenna was initiated; likewise, upon departure from the “ON” chamber RF activation was terminated. Video data were collected and time-in-chamber was analyzed using Ethovision software (Noldus, Leesburg, VA.).

Behavior: Rotarod. The method for this technique has been described previously. Briefly, an accelerating Rotarod (Ugo Basile) was used to study motor coordination and balance after implantation of the epidural and sciatic stimulators. Five consecutive acceleration trials were performed with 5 min breaks separating each acceleration trial.

Behavior: open field. As described previously, locomotion was measured in a Versamask Animal Activity Monitoring System (AccuScan Instruments) Open Field Arena. Mice were initially habituated to the climate-controlled test room for 1 h before testing. Locomotor activity was assessed by recording the number of beam breaks in this 42 (length) x 42 (width) x 30 (height) cm chamber for 1 h. The total distance traveled during this time, time spent moving, and the number of horizontal beam breaks was calculated for the entire chamber.

DRG culture. Lumbar DRG were dissected from 6- to 8-week-old Advlinn-Chr2, TrpV1-Chr2 or SNS-Chr2 mice in HBSS + 10 mM HEPES on ice and digested in 45U papain (Worthington Biochemical) in HBSS+H for 20 min at 37 °C. The tissue was washed with HBSS+H and then further digested in collagenase (1.5 mg/ml; Sigma) for an additional 20 min at 37 °C. After washing, cells were dissociated in Neurobasal A media (Gibco) containing 5% FBS (Life Technologies), 1× B27 supplement (Gibco), 2 mM GlutaMAX (Life Technologies) and 100 U/ml penicillin/streptomycin (Life Technologies). The tissue suspension was then filtered using a 40 μm nylon cell strainer, and centrifuged at 1,000g for 3 min, resuspended, triturated and then centrifuged at 1,000g. Neurons were resuspended in DRG media and plated onto coverslips coated with collagen and poly-β-lysine (Sigma). Cells were cultured for 3–4 days before electrophysiology experiments.

Electrophysiology. Whole-cell patch clamp recordings were made from cultured DRG neurons using pipettes with resistance values ranging from 2–3 megaohms, filled with (in mM) 120 potassium gluconate, 5 NaCl, 2 MgCl₂, 0.1 CaCl₂, 10 HEPES, 1.1 EGTA, 4 Na₄ATP, 0.4 Na₃GTP, 15 sodium phospho-creatine; pH adjusted to 7.3 using KOH. osmolarity 291 mOsm. The extra-cellular solution consists of (in mM): 145 NaCl, 3 KCl, 2 CaCl₂, 1.2 MgCl₂, 10 HEPES, 7 glucose; pH adjusted to 7.3 with NaOH. Recordings and light stimulation were performed using Patchmaster software (HEKA Instruments, Bellmore, NY) controlling an EPC10 amplifier (HEKA Instruments). Neurons were voltage clamped at −60 mV and held at −60 mV for current clamp recording. Optical stimulation was delivered with collimated light through the microscope objective, using a custom set-up with a blue LED (M470L2; Thorslabs) coupled to the back fluorescent port of an Olympus BX-51 microscope. Light intensity at the focal plane (10 mW/mm²) was calculated using a photodiode (S120C, Thorslabs) and power meter (PM100D, Thorslabs).

Immunohistochemistry. Mice were deeply anesthetized with a ketamine, xylazine and acepromazine cocktail, then transcardially perfused with cold 4% paraformaldehyde in PBS. Lumbar DRG, spinal cord and sciatic nerves were dissected and placed in 30% sucrose in PBS for overnight cryoprotection, then frozen in OCT. Frozen tissue was sectioned in a −20 °C cryostat (Leica) at either 30 μm (spinal cord and cross section sciatic nerve), 18 μm (DRG), or 6 μm (longitudinal sciatic nerve) and collected directly onto frosted glass slides. Immunohistochemistry was conducted as described previously. Goat anti-CGRP (1:400, AbD Serotec Cat # 1720-9007), rabbit anti-GFP (1:1,000, Life Technologies Cat# A11122), mouse anti-NF200 (1:400, Millipore Cat# MAB2666), mouse anti-GFAP (1:500, Cell Signaling Technologies, rabbit anti-Iba1 (1:300, Wako Biochemicals cat# 019-19741), goat anti-choline acetyltransferase (1:100, EMD Millipore cat# AB144P) and mouse anti-BIII-tubulin (1:1,000, Covance Research Products Inc. Cat# PRB-435P-100) were used whereas IB4 labeling was performed using an Alexa Fluor 568-conjugated IB4 (1:400, Life Technologies Cat# D12412). Research Resource IDs were provided below to assist the reader. Fluorescent-conjugated secondary antibodies (Life Technologies) were used to visualize primary immunostaining: donkey-anti-goat AF647 (1:500), donkey-anti-rabbit AF488 (1:500), and goat-anti-mouse AF647 (1:500). Slides were sealed overnight with Prolong Gold Antifade Mountant with DAPI (Life Technologies). Images from sealed slides were obtained using a Leica SPE confocal microscope, with gain and exposure time constant throughout image groups.

Antibody, dilution, company catalog ID, research resource ID. Goat anti-CGRP 1:400, AbD Serotec, 1720-9007, AB_2290729. Rabbit anti-GFP, 1:1,000, Life Technologies, A11122, AB_22156. Rabbit anti-Iba1, 1:300, Wako Chemicals, 019-19741, AB-839504. Mouse anti-NF200, 1:400, Sigma-Aldrich, N0142, AB_2149763. Mouse anti-GFAP, 1:500, Cell Signaling Technologies, rabbit anti-BIII-tubulin, 1:1,000, EMD Millipore, 05-166, AB_291637. Goat anti-ChAT, 1:100, EMD Millipore, AB144P, AB_1124092. Guinea Pig anti-GFAP, 1:500, Dynaptic Systems, 173-004, AB_10641162. Mouse anti-GFAP, 1:500, Cell Signaling Technologies, 3670, AB_561049.