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## Fiber-Optic Implantation for Chronic Optogenetic Stimulation of Brain Tissue

--Manuscript Draft--

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<b>Abstract:</b>	<p>Elucidating patterns of neuronal connectivity has been a challenge for both clinical and basic neuroscience. Electrophysiology has been the gold standard for analyzing patterns of synaptic connectivity, but paired electrophysiological recordings can be both cumbersome and experimentally limiting. The development of optogenetics has introduced an elegant method to stimulate neurons and circuits, both in vitro and in vivo. By exploiting cell-type specific promoter activity to drive opsin expression in discrete neuronal populations, one can precisely stimulate genetically defined neuronal subtypes in distinct circuits. Well described methods to stimulate neurons, including electrical stimulation and/or pharmacological manipulations, are often cell-type indiscriminate, invasive, and can damage surrounding tissues. These limitations could alter normal synapse and/or circuit function. In addition, due to the nature of the manipulation, current methods are often acute and terminal. Optogenetics affords the ability to stimulate neurons in a relatively innocuous manner, and in genetically targeted neurons. The majority of studies involving in vivo optogenetics currently use an optical fiber guided through an implanted cannula; however, limitations of this method can include damaged brain tissue with repeated insertion of an optical fiber, and potential breakage of the fiber inside the cannula. Given the burgeoning field of optogenetics, a more reliable method of chronic stimulation is necessary to facilitate long-term studies with minimal collateral tissue damage. Here we provide a video article to complement the method previously described by Sparta et al. showing the fabrication of fiber optic implants, their permanent fixation onto the craniums of anesthetized mice, and assembly of the fiber optic coupler connecting the implant to a light source. The implant, connected with optical fibers to a solid-state laser through small detachable tethers, allows for an efficient method to chronically photostimulate functional neuronal circuitry in awake, behaving mice with minimal tissue damage.</p>
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May 17, 2012

Claire Standen, Ph.D.  
Editorial  
Journal of Visualized Experiments

Dear Dr. Standen,

Thank you for handling our manuscript titled **Fiber-Optic Implantation for Chronic Optogenetic Stimulation of Brain Tissue**. In it we describe a new approach that describes step-by-step methods for fabricating and implanting silica-based fiber optics into the mouse brain for photostimulation of Channelrhodopsin expressing neurons.

We have read the Reviewer comments and revised our manuscript to address all points raised by the reviewers. These changes are highlighted in an accompanying 'Response to Reviewers'. In addition, we made sure to address your editorial and production requests.

We hope that by incorporating these changes our manuscript is suitable for production and publication in the Journal of Visualized Experimentation.

Thank you in advance for your consideration, and please let me know if I can provide any additional information or material.

Best regards,

Benjamin R. Arenkiel, Ph.D.

# Fiber-Optic Implantation for Chronic Optogenetic Stimulation of Brain Tissue

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Keywords: Neuroscience, optogenetics, fiber optics, implantation, neuronal circuitry, chronic stimulation

## **Short Abstract**

The development of optogenetics now provides the means to precisely stimulate genetically defined neurons and circuits, both *in vitro* and *in vivo*. Here we describe the assembly and implantation of a fiber optic for chronic photostimulation of brain tissue.

## **Long Abstract**

Elucidating patterns of neuronal connectivity has been a challenge for both clinical and basic neuroscience. Electrophysiology has been the gold standard for analyzing patterns of synaptic connectivity, but paired electrophysiological recordings can be both cumbersome and experimentally limiting. The development of optogenetics has introduced an elegant method to stimulate neurons and circuits, both *in vitro*<sup>1</sup> and *in vivo*<sup>2,3</sup>. By exploiting cell-type specific promoter activity to drive opsin expression in discrete neuronal populations, one can precisely stimulate genetically defined neuronal subtypes in distinct circuits<sup>4-6</sup>. Well described methods to stimulate neurons, including electrical stimulation and/or pharmacological manipulations, are often cell-type indiscriminate, invasive, and can damage surrounding tissues. These limitations could alter normal synapse and/or circuit function. In addition, due to the nature of the manipulation, current methods are often acute and terminal. Optogenetics affords the ability to stimulate neurons in a relatively innocuous manner, and in genetically targeted neurons. The majority of studies involving *in vivo* optogenetics currently use a optical fiber guided through an implanted cannula<sup>6,7</sup>; however, limitations of this method can include damaged brain tissue with repeated insertion of an optical fiber, and potential breakage of the fiber inside the cannula. Given the burgeoning field of optogenetics, a more reliable method of chronic stimulation is necessary to facilitate long-term studies with minimal collateral tissue damage. Here we provide a video article to complement the method previously described by Sparta et al.<sup>8</sup> showing the fabrication of fiber optic implants, their permanent fixation onto the craniums of anesthetized mice, and assembly of the fiber optic coupler connecting the implant to a light source. The implant, connected with optical fibers to a solid-state laser through small detachable tethers, allows for an efficient method to chronically photostimulate functional neuronal circuitry in awake, behaving mice<sup>10</sup> with minimal tissue damage<sup>9</sup>.

## **Protocol**

\*All materials along with respective manufacturers and/or vendors are listed below the protocol.

### **1. Assembly of implant**

- 1.1) Prepare a mixture of heat-curable fiber optic epoxy by adding 100 mg of hardener to 1 g of resin.
- 1.2) Measure and cut approximately 35 mm of 125  $\mu\text{m}$  diameter fiber optic with 100  $\mu\text{m}$  core by scoring it with a wedge-tip carbide scribe. Position the scribe perpendicular to the fiber optic and score in a single, unidirectional motion, followed by breaking at a right angle. Cutting the fiber completely will damage the fiber core.
- 1.3) Insert a LC ceramic ferrule with a 127 $\mu\text{m}$  inside diameter bore into a vise, convex side pointed down.
- 1.4) Insert the fiber optic into the ferrule. The fiber optic should slide in smoothly and marginally protrude beyond the convex end of the ferrule (Fig. 1a).
- 1.5) Apply one drop of heat-curable fiber optic epoxy to the flat end and heat with heat gun until epoxy turns black. The epoxy should fill the ferrule as it is heated and before curing. The epoxy should cure within ~1 minute of constant heat application.
- 1.6) Clean off any epoxy along the sides of the ferrule, as it will obstruct interfacing with the coupler.
- 1.7) Polish the convex end of the ferrule using a LC fiber optic polishing disc (FOPD) on aluminum oxide polishing sheets on the polishing pad (Fig. 1b). Make circular rotation patterns and polish on four decreasing grades in the following order: 5, 3, 1 0.3  $\mu\text{m}$  grit.
- 1.8) Cut the fiber optic at the flat end to the appropriate length such that it targets the region of interest. The length can be determined using the stereotaxic atlas.
- 1.9) Test the implant by connecting it to the laser via the coupler cord described below. The polished end of the implant is inserted into sleeve of the coupler and should make direct contact with the opposing ferrule. The implant should be able to maintain constant light output, measured at the tip of the implant fiber. A bad implant will have a weak focal point near the tip of the fiber optic, whereas a good implant will transmit a smooth concentric circle of light that with output powers to 10 mW.
- 1.10) Store the finished implants (Fig. 1c) in foam until use.

### **2. Assembly of fiber optic coupler cord**

- 2.1) Prepare a mixture of heat-curable fiber optic epoxy as above.
- 2.2) Measure and cut an appropriate length of 220  $\mu\text{m}$  diameter fiber optic with 200  $\mu\text{m}$  core by scoring it with a wedge-tip carbide scribe. The length of the fiber should allow the mouse to move freely around the housing but not allow the mouse to chew through the fiber.
- 2.3) Insert the fiber optic into a length of furcation tubing slightly longer than the fiber optic length. The tubing should have an inner diameter slightly larger than the fiber optic.

- 2.4) Strip ~25mm at one end of the fiber optic and insert it into the metal end of a Multimode FC MM Ferrule Assembly with 230  $\mu\text{m}$  inside diameter bore until it stops. The fiber optic should stick out through the ferrule end (Fig. 2a).
- 2.5) Secure the connection with cyanoacrylate (super glue) at the metal end. Cover the connection with a Connector Boot and polish the ferrule end with a FC FOPD. Make circular rotation patterns and polish on four grades in the following order: 5, 3, 1, 0.3  $\mu\text{m}$  grit (Fig. 2b).
- 2.6) Strip and insert the other end of the fiber optic into a LC ceramic ferrule (230  $\mu\text{m}$  i.d. bore) with the convex end distal. Apply a drop of epoxy to the flat end and heat until cured.
- 2.7) Polish the convex end of the ferrule using a FC FOPD on aluminum oxide polishing sheets as described above.
- 2.8) Slide a LC ferrule sleeve over the convex end of the ferrule until the midpoint of the sleeve.
- 2.9) Place heat-shrink tubing over the furcation tubing and sleeve and heat to secure and protect the connection (Fig. 2c).
- 2.10) Test the coupler by connecting it to the laser source and measuring the light output through the coupler with a spectrophotometer. The light loss between the laser output and the measured coupler output should not exceed 30%.

### **3. Surgical Implantation**

- 3.1) Anesthetize the mouse with an intraperitoneal injection Ketamine/Xylazine mixture 100 and 10 mg/kg respectively using a 30-gauge needle.
- 3.2) Shave the scalp with clippers. Wipe the scalp with 70% isopropyl alcohol followed by Betasept wipe (4% chlorohexadine solution) to disinfect surgical area.
- 3.3) Place the mouse in the stereotaxic rig and secure the head, ensuring that the skull is level. Apply ophthalmic ointment to eyes to prevent dryness and postoperative pain. Maintain anesthesia using volatized isoflurane (1-3% diluted with oxygen depending on the physiological state of the mouse, which should be continuously monitored by response to a tail pinch).
- 3.4) Make an incision through the midline of the scalp, exposing the cranium from the eye orbits to the lambda suture. Push aside connective tissue as necessary.
- 3.5) Use Serafin clamps to hold back the skin and maintain an access to the cranium (Fig. 3a).
- 3.6) Etch a checkered pattern throughout the surface of the cranium with a dental pick or bent 30 gauge needle. Wash debris away with sterile saline. Dry thoroughly.
- 3.7) Apply hydrogen peroxide (3%) to the exposed cranium with a cotton swab for ~2-3 seconds to create micropores. Wash multiple times and dry thoroughly. Alternatively, anchor screws can be inserted into the cranium, as described in Sparta et al. (2012).
- 3.8) Again, etch a checkered pattern throughout the cranium with a dental pick and wash away debris with saline. Dry thoroughly.

- 3.9) Using a rotary dental drill, make a small bur hole craniotomy (<1 mm in diameter) above the region of interest, determined by the stereotaxic atlas calibrated to bregma and lambda. Be careful not to break the dura or damage any tissue. Wash away debris and dry thoroughly.
- 3.10) Insert the fiber optic ferrule (implant) into the probe holder and connect to the stereotaxic arm.
- 3.11) Position the implant in place directly above the region of interest using the stereotaxic arm (Fig. 3b). If inserting the optical fiber in the brain tissue, the fiber should be advanced slowly at a rate of ~2 mm/min. The ferrule should rest on the surrounding cranial tissue.
- 3.12) Prepare a mixture of dental cement. The mixture should have a low enough viscosity to easily apply across the cranium. The mixture will be usable for 2-4 minutes.
- 3.13) Using a sterile pipette tip, apply a thin, even layer of dental cement across the cranium and onto the lower portion of the implant. The base layer of dental cement should cover as much surface area on the cranium as possible. Do not let the dental cement come into contact with the skin of the mouse. This will lead to increased difficulty in suturing as well as irritation to the mouse.
- 3.14) Allow it to dry completely.
- 3.15) Apply even layers of dental cement to form a small mound on top of the cranium and around the implant, allowing each layer to dry completely (Fig. 3c). Leave ~3-5 mm of the convex end of the ferrule clean of cement to allow for a smooth, unobstructed connection.
- 3.16) Suture the scalp over the mound of dental cement and around the implant. Optional: Use VetBond adhesive for additional binding after suturing.
- 3.17) Apply topical analgesics and antibiotics to the sutured skin and around the base of the implant.
- 3.18) Place the mouse in a cage over a heating blanket for post-operative recovery.
- 3.19) Monitor during recovery for signs of distress.

### **Representative Results**

Proper assembly of the fiber optic implant and coupler results in minimal photon loss between the light source and the end of the fiber optic in the region of interest. Well-polished fiber optics should transmit light in a uniform, concentric circle (Fig. 2d). With careful implantation and suturing, the implant causes no visible irritation to the mouse and can remain in place for long-term studies (Fig. 3d, >1month, unpublished observations) without any significant degradation of the fiber optic or the amount of light transmitted. Improper implantation or suturing can cause irritation and can result in the mouse scratching the scalp, exposure of dental cement, or breakage of the ferrule from the dental cement due to persistent manipulation. A schematic diagram of the assembled system can be seen in Figure 4.

## **Discussion**

Optogenetics is a powerful new technique that allows unprecedented control over specific neuronal subtypes. This can be exploited to modulate neural circuits with anatomic and temporal precision, while avoiding the cell-type indiscriminate and invasive effects of electrical stimulation through an electrode. Implantation of fiber optics allows for consistent, chronic stimulation of neural circuits over multiple sessions in awake, behaving mice with minimal damage to tissue. This system, originally pioneered by Sparta et al.<sup>8</sup> and modified to fit our purposes, goes one step beyond the implanted cannula and fixes the fiber optic in place in the region of interest to ensure consistent targeting between sessions in long-term studies. The implants can be adapted to stimulate different regions of the brain.

Various steps within this method require precision and attention to detail. Each junction of fiber optic coupling is necessarily polished to ensure minimal light loss. After polishing, the ends should be examined under a microscope to verify that there is no damage to the fiber core. If light loss between the source and the measured output exceeds 30%, each part should be repolished to allow maximum photon flux, or the part should be discarded and remade. If the ferrule does not slide into the sleeve, there is likely debris inside the sleeve obstructing the ferrule. When attaching and removing the coupler cord to the implant, force should be applied directly parallel to the axis of the implant. Due to the fact that mammalian tissue scatters light heavily and the relatively low energy of blue light, the implant should be positioned such that the tip of the fiber is within 500  $\mu\text{m}$  of the region of interest, where >10% of initial light power density persists<sup>6</sup>. During implantation, the base layer of dental cement is the critical step, as it is this layer that fixes the implant to the cranium. The subsequent layers secure the implant to the base layer and provide protection. The base layer will not adhere well if the cranium is not completely dry; if any section is not adhered well, it is likely that manipulation from the mouse will dislodge the entire implant. Alternatively, anchors for the dental cement can be screwed into the cranium for a more secure fixture.

In behavioral studies, external light leak may provide an unintended cue to the mouse. External light leak is most likely to occur at the connection between the implant and coupler cord directly over the mouse. In order to minimize light leakage, the heat-shrink tubing can be further extended such that it



completely covers the ferrule sleeve to provide extra shielding against leakage. If this option is pursued, the heat-shrink tubing will cover the window in the sleeve that provides visual feedback for direct contact between ferrules and contact should be determined with tactile feedback. Alternatively, or in addition, black nail polish can be applied to the ceramic ferrule ends to occlude further light transmission.

Towards further development of this technique, it is possible to implant multiple fiber optics onto a single mouse using additional stereotaxic arms, as described in Sparta et al<sup>8</sup>. This would enable more complex studies through differential wavelength stimulation in the same region in a temporally specific manner, or simultaneous stimulation of different regions. Additionally, fiber optics can be coupled with electrodes (optrode) for *in vivo* electrophysiology for local stimulation and recording.

**Table of specific reagents and equipment:**

<b>Name of the Reagent or Equipment</b>	<b>Company</b>	<b>Catalogue #</b>	<b>Comments</b>
LC Ferrule Sleeve	Precision Fiber Products (PFP)	SM-CS125S	1.25mm ID
FC MM Pre-Assembled Connector	PFP	MM-CON2004-2300	230 µm Ferrule
Miller FOPD-LC Disc	PFP	M1-80754	For LC ferrules 900µm o.d., 250µm i.d.
Furcation tubing	PFP	FF9-250	
MM LC Stick Ferrule 1.25mm	PFP	MM-FER2007C-1270	127µm ID Bore
MM LC Stick Ferrule 1.25 mm	PFP	MM-FER2007C-2300	230 µm ID Bore
Heat-curable epoxy, hardener and resin	PFP	ET-353ND-16OZ	
FC/PC and SC/PC Connector Polishing Disk	ThorLabs	D50-FC	For FC ferrules
Digital optical power and Energy Meter	ThorLabs	PM100D	Spectrophotometer 9 "x 13" 50
Polishing Pad	ThorLabs	NRS913	Durometer
Aluminum oxide Lapping (Polishing) Sheets: 0.3, 1, 3, 5 µm grits	ThorLabs	LFG03P, LFG1P, LFG3P, LFG5P	
Standard Hard Cladding Multimode Fiber	ThorLabs	BFL37-200	Low OH, 200µm Core, 0.37 NA Clad/Coat: 200µm / 300µm
Fiber Stripping Tool	ThorLabs	T10S13	High -OH, UV Enhanced, 0.22 NA
SILICA/SILICA Optical Fiber	Polymicro Technologies	FVP100110125	
1x1 Fiberoptic Rotary Joint	doric lenses	FRJ_FC-FC	
Mono Fiberoptic	doric lenses	MFP_200/230/900-	

Patchcord		0.37_2m_FC-FC	
Heat shrink tubing, 1/8 inch	Allied Electronics	689-0267	
Heat gun	Allied Electronics	972-6966	250W; 750-800°F
	Puritan Medical		
Cotton tipped applicators	Products Company	806-WC	
VetBond tissue adhesive	Fischer Scientific	19-027136	
Flash denture base acrylic	Yates Motloid	ColdPourPowder+Liq	
BONN Miniature Iris Scissors	Integra Miltex	18-1392	3-1/2" (8.9cm), straight, 15mm blades
Johns Hopkins Bulldog Clamp	Integra Miltex	7-290	1-1/2" (3.8cm), curved
MEGA-Torque Electric Lab Motor	Vector Clarkson	EL-S	
Panther Burs-Ball #1	Laboratory	77.1006	
Violet Blue Laser System	CrystaLaser	CK473-050-O	Wavelength: 473 nm
Laser Power Supply	CrystaLaser	CL-2005	
Dumont #2 Laminectomy Forceps	Fine Science Tools	11223-20	
Probe	Fine Science Tools	10140-02	
5" Straight Hemostat	Excelta	35-PH	
Vise with weighted base	Altex Electronics	PAN381	

## **Acknowledgements**

We would like to acknowledge that this technique was originally described by Sparta et al., 2012 and has been easily adapted for use in our lab. This work was funded through the McNair Foundation, and NIH grants R00NS064171 and R01NS078294 to B.R.A.

## **Disclosures**

We have no official disclosures.

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## **Figures**

Figure 1. Assembly of implantable fiber optics. (a) The fiber optic is inserted into the ferrule, marginally protruding beyond the convex end indicated by the arrowhead. (b) The convex end of the ferrule is polished using a FOPD on progressively finer grades of polishing sheets. (c) The finished implantable fiber optic.

Figure 2. Assembly of fiber optic coupler used to tether the fiber optic rotary joint to the implant. (a) Fiber optic sticking through the ferrule assembly. (b) The ferrule side of the assembly is inserted into the FOPD and polished using progressively finer grades of polishing paper. (c) The ferrule sleeve is fitted over the ferrule and secured with heat shrink tubing. (d) The finished fiber optic coupler should produce a concentric light with minimal photon loss.

Figure 3. Surgical implantation the fiber optics. (a) The entire surface of the cranium is exposed and connective tissue is cleared. (b) The fiber optic implant is held in position with the stereotaxic arm. (c) Dental cement is applied fixing the fiber optic implant to the cranium. (d) >1 month after implantation, the skin has healed around the implant and there are no signs of irritation.

Figure 4. Schematic diagram of the functional system

## Fiber-Optic Implantation for Chronic Optogenetic Stimulation of Brain Tissue

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The development of optogenetics now provides the means to precisely stimulate genetically defined neurons and circuits, both *in vitro* and *in vivo*. Here we describe the assembly and implantation of a fiber optic for chronic photostimulation of brain tissue.

## Long Abstract

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## **Protocol**

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### **1. Assembly of implant**

- 1.1) Prepare a mixture of heat-curable fiber optic epoxy by adding 100 mg of hardener to 1 g of resin.
- 1.2) Measure and cut approximately 35 mm of 125  $\mu\text{m}$  diameter fiber optic with 100  $\mu\text{m}$  core by scoring it with a wedge-tip carbide scribe. Position the scribe perpendicular to the fiber optic and score in a single, unidirectional motion, followed by breaking at a right angle. Cutting the fiber completely will damage the fiber core.
- 1.3) Insert a LC ceramic ferrule with a 127 $\mu\text{m}$  inside diameter bore into at the vise, convex side pointed down.
- 1.4) Insert the fiber optic into the ferrule. The fiber optic should slide in smoothly and marginally protrude beyond the convex end of the ferrule (Fig. 1a).
- 1.5) Apply one drop of heat-curable fiber optic epoxy to the flat end and heat with heat gun until epoxy turns black. The epoxy should fill the ferrule as it is heated and before curing. The epoxy should cure within ~1 minute of constant heat application.
- 1.6) Clean off any epoxy along the sides of the ferrule, as it will obstruct interfacing with the coupler.
- 1.7) Polish the convex end of the ferrule using a LC fiber optic polishing disc (FOPD) on aluminum oxide polishing sheets on the polishing pad (Fig. 1b). Make circular rotation patterns and polish on four decreasing grades in the following order: 5, 3, 1 0.3  $\mu\text{m}$  grit.
- 1.8) Cut the fiber optic at the flat end to the appropriate length such that it targets the region of interest. The length can be determined using the stereotaxic atlas.

1.9) Test the implant by connecting it to the laser via the coupler cord described below. The polished end of the implant is inserted into sleeve of the coupler and should make direct contact with the opposing ferrule. The implant should be able to maintain constant light output, measured at the tip of the implant fiber. A bad implant will have a weak focal point near the tip of the fiber optic, whereas a good implant will transmit a smooth concentric circle of light that with output powers to 10 mW.

1.9)1.10) Store the finished implants (Fig. 1c) in foam until use.

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- 2.3) Insert the fiber optic into a length of furcation tubing slightly longer than the fiber optic length. The tubing should have an inner diameter slightly larger than the fiber optic.
- 2.4) Strip ~25mm at one end of the fiber optic and insert it into the metal end of a Multimode FC MM Ferrule Assembly with 230  $\mu\text{m}$  inside diameter bore until it stops. The fiber optic should stick out through the ferrule end (Fig. 2a).
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- 2.6) Strip and insert the other end of the fiber optic into a LC ceramic ferrule (230  $\mu\text{m}$  i.d. bore) with the convex end distal. Apply a drop of epoxy to the flat end and heat until cured.
- 2.7) Polish the convex end of the ferrule using a FC FOPD on aluminum oxide polishing sheets as described above.
- 2.8) Slide a LC ferrule sleeve over the convex end of the ferrule until the midpoint of the sleeve.
- 2.9) Place heat-shrink tubing over the furcation tubing and sleeve and heat to secure and protect the connection (Fig. 2c).

2.10) Test the coupler by connecting it to the laser source and measuring the light output through the coupler with a spectrophotometer. The light loss between the laser output and the measured coupler output should not exceed 30%.

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### 3. Surgical Implantation

- 3.1) Anesthetize the mouse with an intraperitoneal injection of 100  $\text{mg kg}^{-1}$  Ketamine/Xylazine mixture 100 and 10  $\text{mg/kg}$  respectively using a 30-gauge needle.
- 3.2) Shave the scalp with clippers. Wipe the scalp with 70% isopropyl alcohol followed by Betasept wipe (4% chlorohexadine solution) to disinfect surgical area.
- 3.3) Place the mouse in the stereotaxic rig and secure the head, ensuring that the skull is level. Apply ophthalmic ointment to eyes to prevent drying and postoperative pain. Maintain anesthesia through isoflurane inhalation using volatized isoflurane (1-3% diluted with oxygen depending on the physiological state of the mouse, which should be continuously monitored by response to a tail pinch).
- 3.4) Make an incision through the midline of the scalp, exposing the cranium from the eye orbits to the lambda suture. Push aside connective tissue as necessary.
- 3.5) Use Serafin clamps to hold back the skin and maintain an access to the cranium (Fig. 3a).
- 3.6) Etch a checkered pattern throughout the surface of the cranium with a dental pick or bent 30 gauge needle. Wash debris away with sterile saline. Dry thoroughly.
- 3.7) Apply hydrogen peroxide (3%) to the exposed cranium with a cotton swab for ~2-3 seconds to create micropores. Wash multiple times and dry thoroughly. Alternatively, anchor screws can be screwed-in into the cranium, as described in Sparta et al. (2012).

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3.8) Again, etch a checkered pattern throughout the cranium with a probe-dental pick and wash away debris with saline. Dry thoroughly.

3.9) Using a rotary dental drilltool, make a pinhole-sizedsmall bur hole craniotomy (<1 mm in diameter) above the region of interest, determined by the stereotaxic atlas calibrated to bregma and lambda. Be careful not to break the dura or damage any tissue. Wash away debris and dry thoroughly.

3.10) Insert the fiber optic ferrule (implant) into the probe holder and connect to the stereotaxic arm.

3.11) Position the implant in place directly above the region of interest using the stereotaxic arm (Fig. 3b). If inserting the optical fiber in the brain tissue, the fiber should be advanced slowly at a rate of ~2 mm/min. The ferrule should rest on the remaining surrounding cranial tissue.

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3.12) Prepare a mixture of dental cement. The mixture should have a low enough viscosity to easily apply across the cranium. The mMixture will be usable for 2-4 minutes.

3.13) Using a sterile pipette tip, aApply a thin, even layer of dental cement across the cranium and onto the lower portion of the implant. The base layer of dental cement should cover as much surface area on the cranium as possible. Do not let the dental cement come into contact with the skin of the mouse. This will lead to increased difficulty in suturing as well as irritation to the mouse.

3.14) Allow it to dry completely.

3.15) Apply even layers of dental cement to form a small mound on top of over-the cranium and around the implant, allowing each layer to dry completely (Fig. 3c). Leave ~3-5 mm of the convex end of the ferrule clean of cement to allow for a smooth, unobstructed connection.

3.16) Suture the scalp over the mound of dental cement and around the implant. Optional: Use VetBond adhesive for additional binding after suturing.

3.17) Apply topical analgesics and antibiotics to the sutured skin and around the base of the implant.

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3.17)3.18) Place the mouse in a cage over a heating blanket for post-operative recovery from anesthesia.

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3.17)3.19) Monitor during recovery for signs of distress.

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## Representative Results

Proper assembly of the fiber optic implant and coupler results in minimal photon loss between the light source and the end of the fiber optic in the region of interest. Well-polished fiber optics should transmit light in a uniform, concentric circle (Fig. 2d). With careful implantation and suturing, the implant causes no visible irritation to the mouse and can remain in place for long-term studies (Fig. 3d, >1month, unpublished observations) without any significant degradation of the fiber optic or the amount of light transmitted. Improper implantation or suturing can cause irritation and can result in the mouse scratching through its scalp, exposureing ofthe dental cement, or breakage of the ferrule from the dental

cement due to persistent manipulation. A schematic diagram of the assembled system can be seen in Figure 4.

## **Discussion**

Optogenetics is a powerful new technique that allows unprecedented control over specific neuronal subtypes. This can be exploited to modulate neural circuits with anatomic and temporal precision, while avoiding the cell-type indiscriminate and invasive effects of electrical stimulation through an electrode. Implantation of fiber optics allows for consistent, chronic stimulation of neural circuits over multiple sessions in awake, behaving mice with minimal damage to tissue. This system, originally pioneered by Sparta et al.<sup>8</sup> and modified to fit our purposes, goes one step beyond the implanted cannula and fixes the fiber optic in place in the region of interest to ensure consistent targeting between sessions in long-term studies. The implants can be adapted to stimulate different regions of the brain.

Various steps within this method require precision and attention to detail. Each junction of fiber optic coupling is necessarily polished to ensure minimal light loss. After polishing, the ends should be examined under a microscope to verify that there is no damage to the fiber core. **If light loss between the source and the measured output exceeds 30%, each part should be repolished to allow maximum photon flux, or the part should be discarded and remade. If the ferrule does not slide into the sleeve, there is likely debris inside the sleeve obstructing the ferrule. When attaching and removing the coupler cord to the implant, force should be applied directly parallel to the axis of the implant. -Light transmission can be further evaluated using a small photometer (not shown here). -Due to the fact that mammalian tissue scatters light heavily and the relatively low energy of blue light, the implant should be positioned such that the tip of the fiber is within 500  $\mu\text{m}$  of the region of interest, where >10% of initial light power density persists<sup>6</sup>.** During implantation, the base layer of dental cement is the critical step, as it is this layer that fixes the implant to the cranium. The subsequent layers secure the implant to the base layer and provide protection. The base layer will not adhere well if the cranium is not completely dry; if any section is not adhered well, it is likely that manipulation from the mouse will dislodge the entire implant. Alternatively, anchors for the dental cement can be screwed into the cranium for a more secure fixture.

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In behavioral studies, external light leak may provide an unintended cue to the mouse. External light leak is most likely to occur at the connection between the implant and coupler cord directly over the mouse. In order to minimize light leakage, the heat-shrink tubing can be further extended such that it completely covers the ferrule sleeve to provide extra shielding against leakage. If this option is pursued, the heat-shrink tubing will cover the window in the sleeve that provides visual feedback for direct contact between ferrules and contact should be determined with tactile feedback. Alternatively, or in addition, black nail polish can be applied to the ceramic ferrule ends to occlude further light transmission.

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Towards further development of this technique, it is possible to implant multiple fiber optics onto a single mouse using additional stereotaxic arms, as described in Sparta et al<sup>8</sup>. This would enable more complex studies through differential wavelength stimulation in the same region in a temporally specific manner, or simultaneous stimulation of different regions. Additionally, fiber optics can be coupled with electrodes (optrode) for *in vivo* electrophysiology for local stimulation and recording.

#### Table of specific reagents and equipment:

Name of the Reagent or Equipment	Company	Catalogue #	Comments
LC Ferrule Sleeve	Precision Fiber Products (PFP)	SM-CS125S	1.25mm ID
FC MM Pre-Assembled Connector	PFP	MM-CON2004-2300	230 µm Ferrule
Miller FOPD-LC Disc	PFP	M1-80754	For LC ferrules 900µm o.d., 250µm i.d.
Furcation tubing	PFP	FF9-250	
MM LC Stick Ferrule 1.25mm	PFP	MM-FER2007C-1270	127µm ID Bore
MM LC Stick Ferrule 1.25 mm	PFP	MM-FER2007C-2300	230 µm ID Bore
Heat-curable epoxy, hardener and resin	PFP	ET-353ND-16OZ	
FC/PC and SC/PC Connector Polishing Disk	ThorLabs	D50-FC	For FC ferrules
Digital optical power and Energy Meter	ThorLabs	PM100D	Spectrophotometer 9 "x 13" 50 Durometer
Polishing Pad	ThorLabs	NRS913	
Aluminum oxide Lapping (Polishing) Sheets: 0.3, 1, 3, 5 µm grits	ThorLabs	LFG03P, LFG1P, LFG3P, LFG5P	
Standard Hard Cladding Multimode Fiber	ThorLabs	BFL37-200	Low OH, 200µm Core, 0.37 NA
Fiber Stripping Tool	ThorLabs	T10S13	Clad/Coat: 200µm /

SILICA/SILICA Optical Fiber	Polymicro Technologies	FVP100110125	300µm High -OH, UV Enhanced, 0.22 NA
1x1 Fiberoptic Rotary Joint	doric lenses	FRJ_FC-FC	
Mono Fiberoptic Patchcord	doric lenses	MFP_200/230/900-0.37_2m_FC-FC	
Heat shrink tubing, 1/8 inch	Allied Electronics	689-0267	
Heat gun	Allied Electronics	972-6966	250W; 750-800°F
Cotton tipped applicators	Puritan Medical Products Company	806-WC	
VetBond tissue adhesive	Fischer Scientific	19-027136	
Flash denture base acrylic	Yates Motloid	ColdPourPowder+Liq	
BONN Miniature Iris Scissors	Integra Miltex	18-1392	3-1/2" (8.9cm), straight, 15mm blades
Johns Hopkins Bulldog Clamp	Integra Miltex	7-290	1-1/2" (3.8cm), curved
MEGA-Torque Electric Lab Motor	Vector Clarkson Laboratory	EL-S	
Panther Burs-Ball #1		77.1006	
Violet Blue Laser System	CrystaLaser	CK473-050-O	Wavelength: 473 nm
Laser Power Supply	CrystaLaser	CL-2005	
Dumont #2 Laminectomy Forceps	Fine Science Tools	11223-20	
Probe	Fine Science Tools	10140-02	
5" Straight Hemostat	Excelta	35-PH	
Vise with weighted base	Altex Electronics	PAN381	

### **Acknowledgements**

We would like to acknowledge that this technique was originally described by Sparta et al., 2012 and has been easily adapted for use in our lab. This work was funded trough the McNair Foundation, and NIH

grants R00NS064171 and R01NS078294 to B.R.A.

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### **Disclosures**

We have no official disclosures.

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## **Figures**

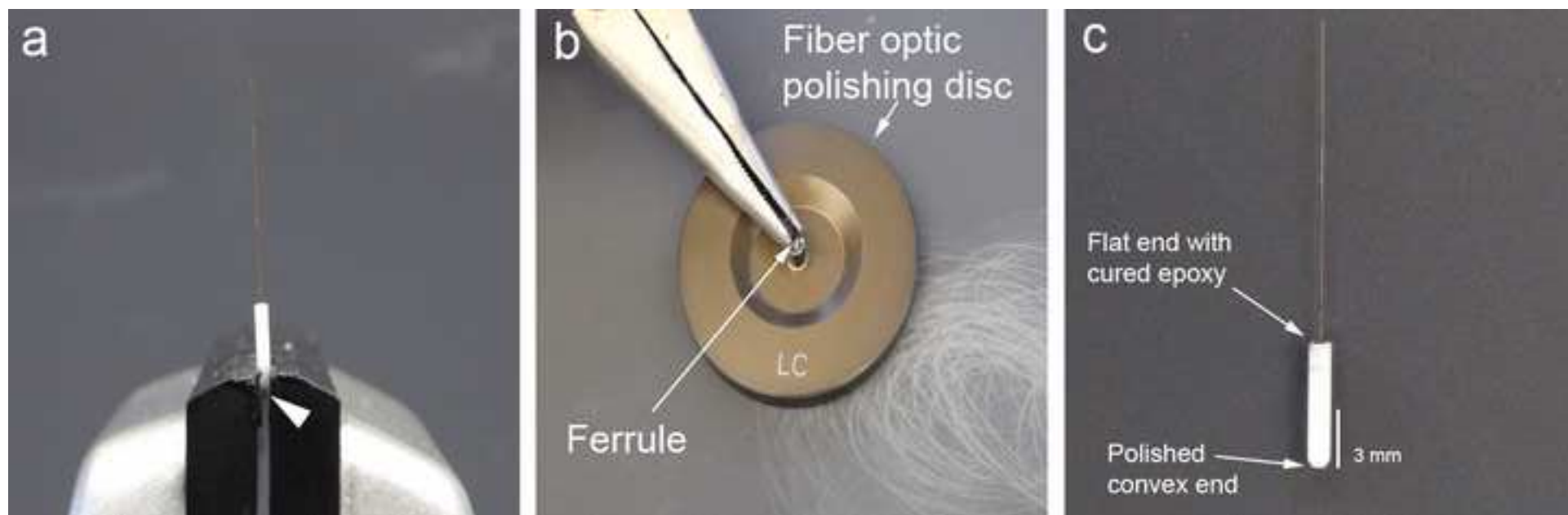
Figure 1. Assembly of implantable fiber optics. (a) The fiber optic is inserted into the ferrule, marginally protruding beyond the convex end indicated by the arrowhead. (b) The convex end of the ferrule is polished using a FOPD on progressively finer grades of polishing sheets. (c) The finished implantable fiber optic.

Figure 2. Assembly of fiber optic coupler used to tether the fiber optic rotary joint to the implant. (a) Fiber optic sticking through the ferrule assembly. (b) The ferrule side of the assembly is inserted into the FOPD and polished using progressively finer grades of polishing paper. (c) The ferrule sleeve is fitted over the ferrule and secured with heat shrink tubing. (d) The finished fiber optic coupler should produce a concentric light with minimal photon loss.

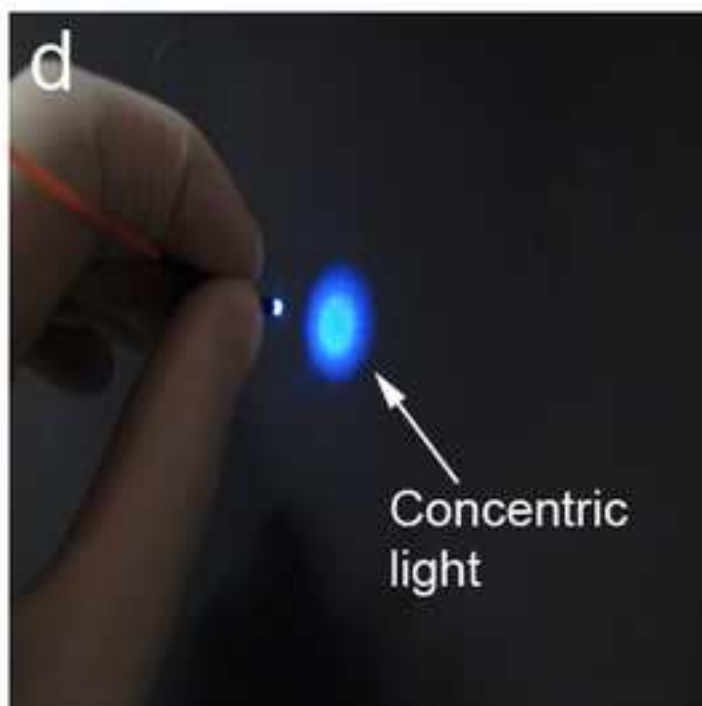
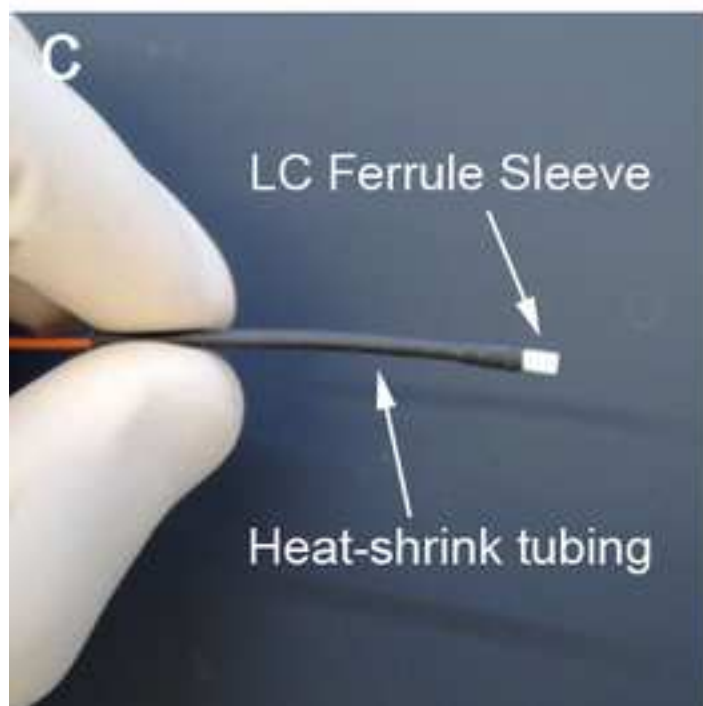
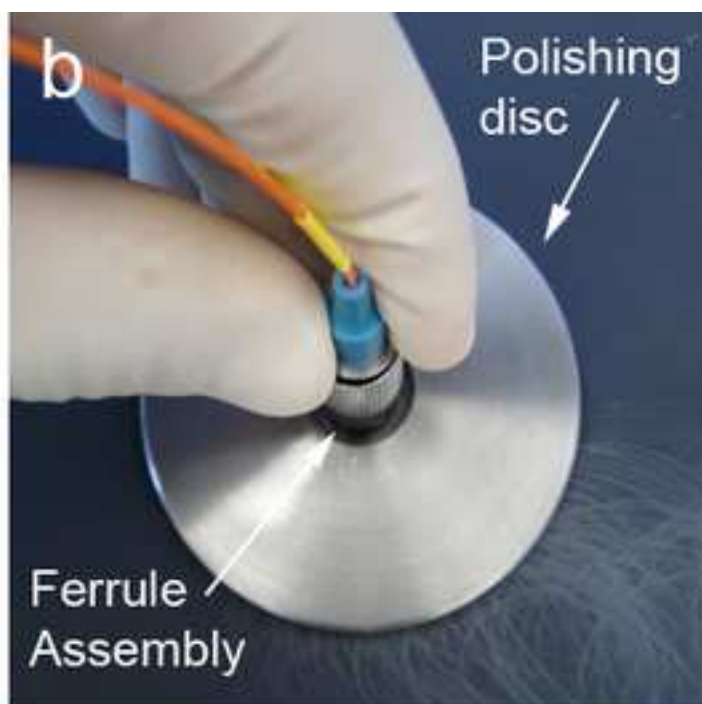
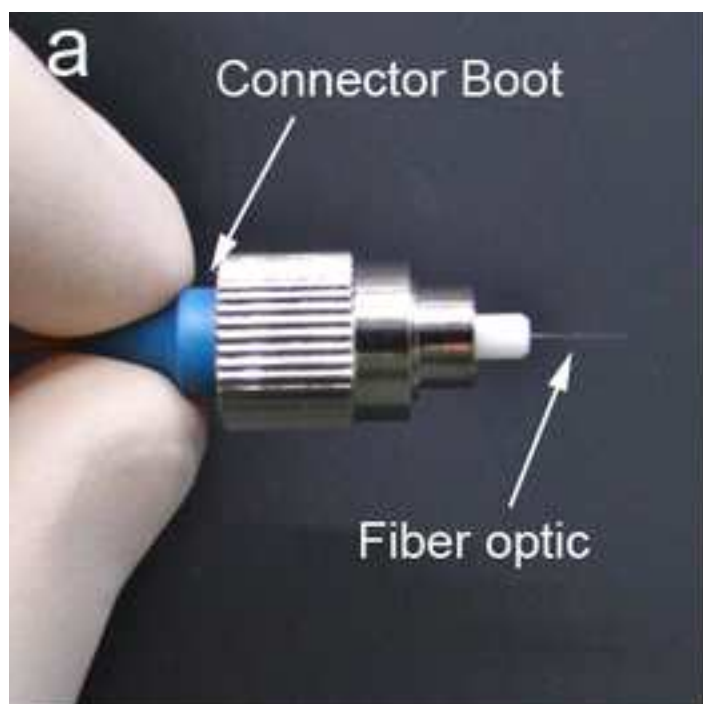
Figure 3. Surgical implantation the fiber optics. (a) The entire surface of the cranium is exposed and connective tissue is cleared. (b) The fiber optic implant is held in position with the stereotaxic arm. (c) Dental cement is applied fixing the fiber optic implant to the cranium. (d) >1 month after implantation, the skin has healed around the implant and there are no signs of irritation.

Figure 4. Schematic diagram of the functional system

\*Figure 1  
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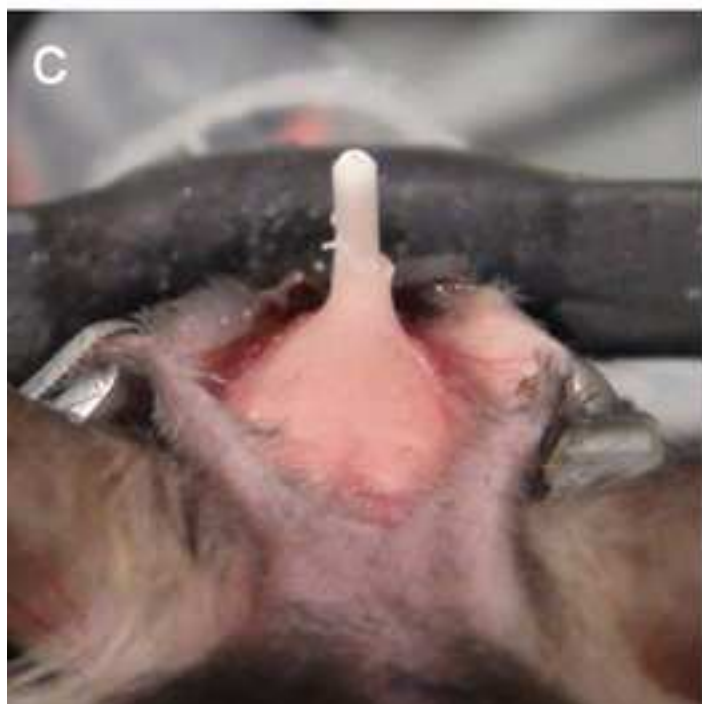
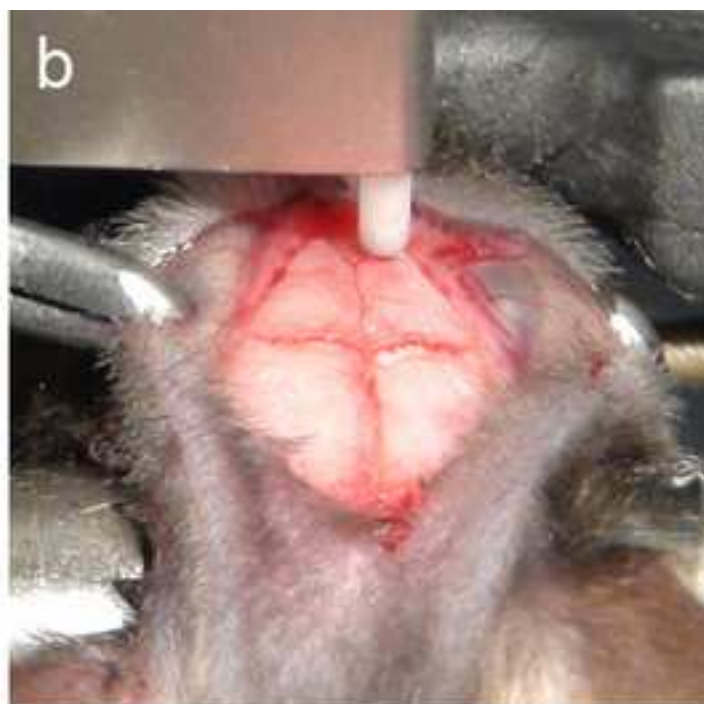


\*Figure 2  
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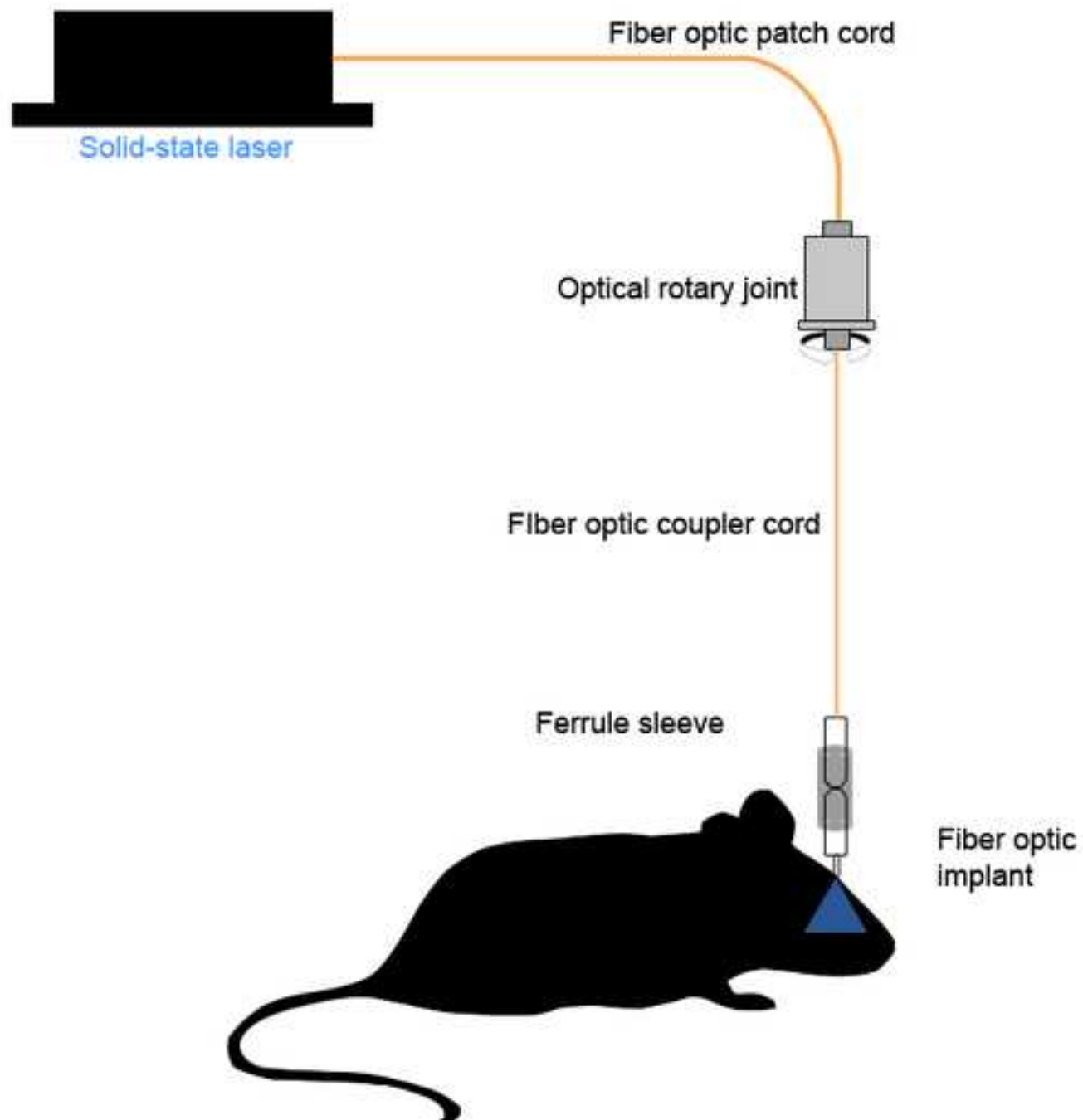




\*Figure 3  
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\*Figure 4  
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Miller FOPD-LC Disc	PFP	M1-80754	For LC ferrules 900µm o.d., 250µm i.d.
Furcation tubing	PFP	FF9-250	
MM LC Stick Ferrule 1.25mm	PFP	MM-FER2007C-1270	127µm ID Bore
MM LC Stick Ferrule 1.25 mm	PFP	MM-FER2007C-2300	230 µm ID Bore
Heat-curable epoxy, hardener and resin	PFP	ET-353ND-16OZ	
FC/PC and SC/PC Connector Polishing Disk	ThorLabs	D50-FC	For FC ferrules 9 "x 13" 50 Durometer
Polishing Pad	ThorLabs	NRS913	
Aluminum oxide Lapping (Polishing) Sheets: 0.3, 1, 3, 5 µm grits	ThorLabs	LFG03P, LFG1P, LFG3P, LFG5P	
Standard Hard Cladding Multimode Fiber	ThorLabs	BFL37-200	Low OH, 200µm Core, 0.37 NA Clad/Coat: 200µm / 300µm
Fiber Stripping Tool	ThorLabs	T10S13	High -OH, UV Enhanced, 0.22 NA
SILICA/SILICA Optical Fiber	Polymicro Technologies	FVP100110125	
1x1 Fiberoptic Rotary Joint	doric lenses	FRJ_FC-FC	
Mono Fiberoptic Patchcord	doric lenses	MFP_200/230/900-0.37_2m_FC-FC	
Heat shrink tubing, 1/8 inch	Allied Electronics	689-0267	
Heat gun	Allied Electronics	972-6966	250W; 750-800°F
Cotton tipped applicators	Puritan Medical Products Company	806-WC	
VetBond tissue adhesive	Fischer Scientific	19-027136	
Flash denture base acrylic	Yates Motloid	ColdPourPowder+Liq	
BONN Miniature Iris Scissors	Integra Miltex	18-1392	3-1/2" (8.9cm), straight, 15mm blades
Johns Hopkins Bulldog Clamp	Integra Miltex	7-290	1-1/2" (3.8cm), curved
MEGA-Torque Electric Lab Motor	Vector Clarkson	EL-S	
Panther Burs-Ball #1	Laboratory	77.1006	
Violet Blue Laser System	CrystaLaser	CK473-050-O	Wavelength: 473 nm

Laser Power Supply	CrystaLaser	CL-2005
Dumont #2 Laminectomy		
Forceps	Fine Science Tools	11223-20
Probe	Fine Science Tools	10140-02
5" Straight Hemostat	Excelta	35-PH
Vise with weighted base	Altex Electronics	PAN381

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## Response to Reviewers

Manuscript ID: JoVE50004 **Fiber-Optic Implantation for Chronic Optogenetic Stimulation of Brain Tissue**

Corresponding Author: Benjamin R. Arenkiel

We thank the reviewers for their helpful comments and suggestions to improve the manuscript. To address concerns raised by the reviewers, we have modified our manuscript as per the reviewers' input.

These changes and our response to the reviewers is itemized here and described in further detail in a point-by-point response below.

- We include more details about the surgical procedure.
- We add further details on stereotaxic implantation pertaining to the mouse and fiber optic implant.
- We describe a bad implant and the determining factor for a good implant, stated as the light output of the implant.
- We add in the discussion the possibility of light leak providing an unintended cue in behavioral studies and suggest a solution.
- We address some common problems and troubleshooting in the 'discussion' section.
- We clarify that the purpose of this article is to provide a visual complement to the method described in (Sparta et al., 2012).

### Reviewer #1

**Q1: Reviewer 1 requested more details about the surgical procedure, including protocols involving anesthesia and other typical IACUC standards, stating: "Some of the details of the surgical procedure are a little thin. For instance, ketamine anesthesia is typically combined with xylazine or a similar agent; no dosage is given for isoflurane, etc. This will be familiar to rodent surgery labs but a published protocol should meet typical IACUC standards."**

We included dosages for ketamine, xylazine and volatized isoflurane, as well as standard surgical practices such as disinfection of the surgical site and postoperative treatment with analgesics and antibiotics as per IACUC standards.

**Q2: Reviewer 1 requested more detail on the stereotaxic implantation.**

**"Some more detail on stereotaxic implantation would be useful – the skull should be leveled before inserting the fiber, approximate speeds for inserting to minimize damage, etc. The fiber is placed 'directly above the region of interest' – how close does it need to be for effective activation?"**

We add details about positioning the mouse in the stereotaxic rig and the rate of advance for fiber optic implant placement to minimize damage. We also added

information about measuring the light output of the fiber optic parts in the protocol, and information about the proximity of the implant to the region of interest in the “Discussion” section.

**Q3: “How is a good working implant distinguished from a bad one, what is a rough range for acceptable power or throughput of the fiber for a working device?”**

We describe a bad implant. We also include details of testing the power output of the fiber optic implant, ensuring it can maintain 10 mW of light output as measured by a spectrophotometer.

**Q4: “When using an implant fiber during behavior, are there any issues with external light leak that the animal could use as an unintended cue, any strategies for reducing this (shielding etc)?**

We added a section in the “Discussion” section to address the issue of an unintended visual cue to the mouse in behavioral tests and suggest a possible solution, including the possibility of ‘blacking out’ of light using paint.

**Q5: “Some more information on typical problems and troubleshooting would also increase the usefulness of the protocol.**

More details about typical problems have been included in the ‘discussion’ section.

## **Reviewer #2**

**Q1: “The one major concern with this submission is that it does not provide any obvious advance in approach or in detail as compared to the methodology described in (Sparta et al., 2012). While the authors acknowledge that the Sparta paper serves as the basis for the current manuscript, they do not make clear in what way their approach is different.”**

Although the existing text describes in detail a very elegant method, a visual demonstration can provide a very powerful complement to the text. We believe that a visual demonstration will enlighten some intricacies in the protocol that may not be obvious for labs just starting out with these procedures. We clarify in the ‘long abstract’ section that the purpose of the submission is to provide a video complement to the previously described method in (Sparta et al., 2012). Our main purpose is in fact to highlight this previously published method, and provide a visual demonstration to those less familiar with the parts and/or techniques.