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Convection Enhanced Delivery of an Optogenetic Adeno-associated Viral Vector to the Rhesus Macaque Cortex with Online MRI Images --Manuscript Draft--

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BIOENGINEERING

UNIVERSITY of WASHINGTON

A Department of the College of Engineering & School of Medicine

Dear Dr. DSouza,

Please accept our revised manuscript "Convection Enhanced Delivery (CED) of Optogenetic Adeno-associated viral (AVV) vector to the Cortex of Rhesus Macaques under guidance of online MRI images".

We have addressed all of the concerns raised by the editorial board, as outlined in our Rebuttal letter. We hope that the revised version of the manuscript will be suitable for publication.

Thanks again for your assistance with this manuscript,

Sincerely,

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TITLE:

Convection Enhanced Delivery of an Optogenetic Adeno-associated Viral Vector to the Rhesus Macaque Cortex with Online MRI Images

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KEYWORDS:

Optogenetics, Non-human primates, Rhesus Macaques, Viral vector delivery, Opsin expression, Primary motor cortex, Primary somatosensory cortex

SHORT ABSTRACT:

Here, we demonstrate magnetic resonance (MR)-guided convection enhanced delivery (CED) of viral vectors into the cortex as an efficient and simplified approach for achieving optogenetic expression across large cortical areas in the macaque brain.

LONG ABSTRACT:

In non-human primate (NHP) optogenetics, infecting large cortical areas with viral vectors is often a difficult and time-consuming task. Here, we demonstrate the use of magnetic resonance (MR)-guided convection enhanced delivery (CED) of optogenetic viral vectors into primary somatosensory (S1) and motor (M1) cortices of macaques to obtain efficient, widespread cortical expression of light-sensitive ion channels. Adeno-associated viral (AAV) vectors encoding the red-shifted opsin C1V1 fused to yellow fluorescent protein (EYFP) were injected into the cortex of rhesus macaques under MR-guided CED. Three months post-infusion, epifluorescent imaging confirmed large regions of optogenetic expression ($>130 \text{ mm}^2$) in M1 and S1 in two macaques. Furthermore, we were able to record reliable light-evoked electrophysiology responses from the expressing areas using micro-electrocorticographic arrays. Later histological analysis and immunostaining against the reporter revealed widespread and dense optogenetic expression in M1 and S1 corresponding to the distribution indicated by epifluorescent imaging. This technique

enables us to obtain expression across large areas of the cortex within a shorter period of time with minimal damage compared to the traditional techniques and can be an optimal approach for optogenetic viral delivery in large animals such as NHPs. This approach demonstrates great potential for network-level manipulation of neural circuits with cell-type specificity in animal models evolutionarily close to humans.

INTRODUCTION:

Optogenetics is a powerful tool that allows for the manipulation of neural activity and the study of network connections in the brain. Implementing this technique in non-human primates (NHPs) has the potential to enhance our understanding of large-scale neural computation, cognition, and behavior in the primate brain. Although optogenetics has been successfully implemented in NHPs in recent years¹⁻⁷, a challenge that researchers face is achieving high levels of expression across large brain areas in these animals. Here, we are providing an efficient and simplified approach to achieve high levels of optogenetic expression across large areas of the cortex in macaques. This technique has great potential to improve current optogenetic studies in these animals in combination with state-of-the-art recording^{8,9} and optical stimulation¹⁰ technologies.

Convection enhanced delivery (CED) is an established method of delivery of pharmacological agents and other large molecules, including viral vectors, to the central nervous system¹¹⁻¹³. Whereas conventional delivery methods involve multiple low volume infusions distributed across small regions of the brain, CED can achieve broader and more even agent distribution with fewer infusions. Pressure-driven bulk fluid flow (convection) during infusion allows for more widely and uniformly distributed transduction of the target tissue when delivering viral vectors with CED. In recent studies, we demonstrated the transduction and subsequent optogenetic expression of large areas of primary motor (M1) and somatosensory (S1) cortices⁹ and thalamus¹⁴ using magnetic resonance (MR)-guided CED.

Here, we outline the use of CED to achieve optogenetic expression across large cortical areas with only a few cortical injections.

PROTOCOL:

All procedures have been approved by the University of California, San Francisco Institutional Animal Care and Use Committee (IACUC) and are compliant with the Guide for the Care and Use of Laboratory Animals. The following procedure was performed using two adult male rhesus macaques of 8 and 7 years of age, weighing 17.5 kg and 16.5 kg (monkey G and monkey J), respectively.

NOTE: Use standard aseptic techniques for all surgical procedures.

1. Baseline imaging

1.1. Sedate and intubate the animal and maintain general anesthesia under isoflurane (concentration changed between 0 to 5% as needed, depending on vital signs such as respiratory

rate and heart rate). Monitor the animal's temperature, heart rate, oxygen saturation, electrocardiographic responses, and end-tidal partial pressure of CO₂ throughout the procedure.

1.2. Place the animal in an MR-compatible stereotaxic frame (see the **Table of Materials**) in which it will remain throughout the procedure. Connect the animal to a portable MR-compatible isoflurane machine and transport it to the MRI scanner (3 T).

1.3. Acquire standard T1 (flip angle = 9°, repetition time/echo time = 668.6, matrix size = 192 x 192 x 80, slice thickness = 1 mm) and T2 (flip angle = 130°, repetition time/echo time = 52.5, matrix size = 256 x 256 x 45, slice thickness = 1 mm) anatomical MR images as baseline reference and for surgical planning.

1.4. Recover the animal from anesthesia and transport it to the animal housing area.

1.5. Use the acquired T1 and T2 images to determine the placement of the craniotomy. The location of the craniotomy can be precisely determined by matching the cortical area of interest from MR with the macaque brain atlas (Paxinos, Huang, Petride, Toga 2nd Edition). In addition, these baseline images can provide an estimate for the locations of viral infusion. The cortical regions of interest demonstrated here are M1 and S1.

2. MR-Compatible chamber implantation

2.1. Sedate and intubate the animal and maintain general anesthesia with standard anesthetic monitoring as in 1.1.

2.2. Place the animal in the MR-compatible stereotaxic frame in which it will remain throughout chamber implantation and viral vector delivery. Connect the animal to a portable MR-compatible isoflurane machine.

2.3. Create a sagittal incision approximately 2 cm from the midline with a length of about 5 cm with a scalpel.

2.4. Remove the underlying soft tissue from the skull using elevators (see **Table of Materials**).

2.5. Create a circular craniotomy (2.5 cm diameter) to cover the pre-planned trajectories for injections using a trephine (see **Table of Materials**).

2.5.1. Lower the centering point of the trephine past the edge of the trephine. Create an indentation at the center of the planned craniotomy sufficiently deep in the skull to anchor the trephine using the adjustable centering point of the trephine. Exercise caution to avoid completely penetrating through the depth of the skull as this could cause damage to the underlying neural tissue.

2.5.2. Periodically flush the area with saline as needed to maintain tissue moisture throughout

the craniotomy.

2.5.3. Once the center has been made, lower the trephine onto the skull and rotate the trephine clockwise and counterclockwise while applying downward pressure until the bone cap can be removed with forceps. Exercise caution to avoid damaging the underlying tissue with the trephine.

2.6. Thread a fine suture (size 6) to the dura in the center of the craniotomy and lift the dura by gently pulling the thread from the surface of the brain creating a tent in the center of the craniotomy.

2.7. Next, puncture the dura close to the center of the tent using fine ophthalmic scissors to avoid damaging the brain. Then cut the dura from the center to the edge of the craniotomy and continue along the edge with fine ophthalmic scissors.

2.8. Mount the cylindrical custom-made CED MR-compatible chamber (**Figure 1**; see section 4 for production instructions) to the skull on top of the craniotomy to provide cannula support during CED infusion such that the curvature of the chamber flange aligns well with the curvature of the skull.

2.9. Secure the implants to the skull using either plastic screws and dental acrylic or a few titanium screws.

3. Viral vector delivery

3.1. Soon after implanting the MR-compatible chamber and inserting the cannula injection grid (**Figure 1A-B, Supplementary Figure 1**) into the chamber, fill the grid with saline (0.9% NaCl) for visualizing the injection locations via MR anatomical images and fill the chamber cavities with wet sterile absorbable gelatin to maintain moisture of the brain (**Figure 1F**).

3.2. Cover the skin and the cylinder with a sterile antimicrobial incise drape to maintain the sterility of the cylinders during transport and MR infusions. Place a vitamin E capsule to mark the top of the injection grid for positive identification.

3.3. While the animal remains intubated, detach the endotracheal tube from the anesthesia circuit and reattach it to a portable MR-compatible isoflurane machine and transport the animal to the MRI scanner.

3.4. Acquire T1 images (flip angle = 9° , repetition time/echo time = 668.6, matrix size = 192×192 , slice thickness = 1 mm, 80 slices) to calculate the distance from the top of the injection grid and the cortical surface. The vitamin E capsule is clearly visible in T1 images and should be used as a marker for the top of the injection grid (**Figure 2A**). The MR imaging software provides a ruler which enables measuring distance in metrics from different planes.

3.5. Acquire T2 images (flip angle = 130°, repetition time/echo time = 52.5, matrix size = 256 x 256, slice thickness = 1 mm, 45 slices) to determine the optimal cannula guides for each site based on the targeted site of infusion (**Figure 2B-C**). As mentioned earlier, the cannula grid is filled with saline which is best visible in T2 images. Using the MR imaging software, scroll through the coronal and sagittal planes to find the target location of infusion.

3.6. After thawing the viral vector for a few hours at room temperature, mix the viral vector with the MR contrast agent gadoteridol (Ratio of 250:1; 2mM Gd-DTPA, see table of materials) by pipette or vortex mixing.

NOTE: The presented technique was tested for AAV vectors with a CamKIIa promoter driving expression of C1V1 fused to EYFP (AAV2.5-CamKII-C1V1-EYFP, titer: 2.5×10^{12} virus molecules/mL; see **Table of Materials**).

3.7. Load the mixed virus into a 0.2 mL high pressure IV tubing (see **Table of Materials**).

3.8. Establish a sterile field outside the MR scanner for preparing the viral infusion line.

3.9. Using high pressure IV tubing, connect a long extension line (approximately 3-5 meters, depending on the location of the infusion pump with respect to the MR bore) to an MR-compatible 3 mL syringe and prime with saline.

3.10. Connect the syringe to 0.2 mL IV tubing loaded with the virus and attach the reflux-resistant cannula with a 1 mm stepped tip (**Figure 2D**; see section 5 for cannula production instructions) to the distal end of this assembly with a clamp style catheter connector (see **Table of Materials**) (**Figure 2E**).

3.11. Lastly, set the syringe in an MR-compatible pump (see **Table of Materials**). Place the pump controller in the scanner control room as it is not MR-compatible.

3.12. Using the baseline anatomical MR images obtained before, select the cannula injection grid location and insertion depth needed to reach the target infusion site. Mark the insertion depth on the cannula using sterile tape.

3.13. Begin the infusion at a rate of 1 μ L/min and visually validate the flow of the fluid in the infusion line by observing the ejection of fluid from the cannula tip.

3.14. Insert the cannula manually through the injection grid to the target depth while maintaining flow in the infusion line, as this will prevent the penetrated tissue from clogging the cannula during insertion.

3.15. Acquire fast (2 minute) flash T1 weighted images (flip angle = 30°, repetition time/echo time = 3.05, matrix size = 128 x 128, slice thickness = 1 mm, 64 slices) for online monitoring of the viral vector infusion.

3.16. After infusing ~10 μL of the vector such that enough virus is infused to detect in the MR scanner, obtain MR images to verify correct cannula placement as evident by the observed spread of the virus. If the depth of the inserted cannula is incorrect, adjust the depth accordingly or slowly remove the cannula and re-attempt the insertion as in 3.12.

3.17. Monitor the infusion via guidance of online MR images (flash T1 weighted) and increase the infusion rate to 5 $\mu\text{L}/\text{min}$ by 1 $\mu\text{L}/\text{min}$ steps every few minutes (**Figure 3A**).

3.18. After infusing approximately 40 μL of the viral vector, begin reducing the infusion rate by 1 $\mu\text{L}/\text{min}$ steps and stop the infusion after ~50 μL is injected and leave the cannula in place for 10 minutes.

3.19. Slowly remove the cannula from the brain and move to the next location. Repeat steps 3.9 to 3.17 for each location.

3.20. Cover the cylinder with a sterile drape at the end of injections, before animal transport. Then transport the animal back to the operating room.

3.21. Either explant the MR-compatible chamber and close the surgical incision by replacing the bone flap and overlying muscle and suturing the skin using standard aseptic techniques or replace the chamber with a titanium cylinder and artificial dura (see Yazdan-Shahmorad et al. 2016⁹) for neural recording and stimulation.

4. MR-Compatible chamber production

4.1. Fabricate the nylon cannula injection grid (**Figure 1A-B**) by machining according to the specified dimensions (**Supplementary Figure 1**).

4.2. 3D print the MR-compatible cylinder out of acrylonitrile butadiene styrene (ABS0 plastic (**Figure 1C-E**) (see **Supplementary File 1–3**; see **Table of Materials** for 3D printer and materials).

NOTE: One design maintains a fixed cannula injection grid within the MR-compatible cylinder (**Figure 1C**), while another allows for rotation of the grid, extending the injection region (**Figure 1D-E**).

4.3. Thread the cannula injection grid and tap the corresponding cavity of the MR-compatible chamber such that both pieces exhibit the same threading.

NOTE: Any type of threading can be used provided that both components have the same threading.

4.4. Insert the nylon cannula injection grid by screwing into the tapped hole of the MR-compatible cylinder.

5. Reflux-resistant cannula production

5.1. Cut a 30 cm silica tubing with 0.32 mm ID and 0.43 mm OD for the inner silica tubing (see **Table of Materials**) using a razor blade.

5.2. Cut a 7.5 cm and a 5 cm silica tubing with 0.45 mm ID and 0.76 mm OD for the outer silica tubing (see **Table of Materials**).

5.3. Adhere the 7.5 cm outer tubing to the 30 cm inner tubing with cyanoacrylate such that the inner tube extends 1 mm beyond the outer tube, making the reflux-resistant step (**Figure 2D**). To ensure that the cyanoacrylate does not enter the inside of the inner tubing, place the cyanoacrylate on the outside of the inner tubing far from the cannula tip.

5.4. Paste the 5 cm silica tubing to the other end of the inner tubing for attachment to the clamp style catheter connector (see step 3.6).

REPRESENTATIVE RESULTS:

Convection Enhanced Delivery (CED) under MRI Guidance

The spread of the viral vector was monitored during CED infusion under the guidance of online MR images (**Figure 3A**). In this study, S1 and M1 of two monkeys were targeted (**Figure 3B**). The three-dimensional distribution volumes were estimated in a post-hoc analysis of the MR images (**Figure 3C**) as described by Yazdan-Shahmorad et al. 2016⁹, confirming coverage of large areas of cortex. The M1 infusions for monkey G were done without MR guidance due to time constraints.

Validation of Viral Expression

Large cortical optogenetic expression was confirmed by epifluorescent imaging, electrophysiological recordings, and histological analysis as described by Yazdan-Shahmorad et al. 2016⁹. We monitored optogenetic expression by surface epifluorescence imaging of the fluorescent reporter^{2,9} and estimated more than 130 mm² of expression along the cortical surface in M1 and S1 of two macaques from only three cortical injections^{9,15}. Light stimulation (488 nm, 20 mW power at the tip; see **Table of Materials**) yielded reliable electrophysiological responses in the expressing areas as measured by semi-transparent micro-electrocorticographic arrays^{8,9,16} (**Figure 4**).

Analysis of the MR images yielded an estimated 233 mm³ and 433 mm³ of vector spread in M1 and S1 of monkey J, respectively, and 317 mm³ in S1 of monkey G⁹. We performed histological analyses on serial coronal sections and observed large-scale optogenetic expression around the infusion sites in M1 and S1 (**Figure 5A-B**), with an estimated 70-80% of cells expressing the opsin. The expression observed from histology aligned with the estimated expression distribution from epifluorescent imaging (**Figure 5A-D**). One of the two infusions performed outside of the MR scanner in monkey G was unsuccessful, yielding no expression in the corresponding region (**Figure 5D**). The spread estimated from MR imaging was completely within the bounds of both

the epifluorescent and the histological measures of viral spread (**Figure 5E-G**). The expansion of the epifluorescent imaging beyond the MR estimate can be attributed to the diffusion of viral particles beyond the advection region. Moreover, the histological estimate extended beyond the epifluorescence estimate due to a lack of optical access beyond the craniotomy. The details of these techniques are included in our previous paper⁹.

FIGURE AND TABLE LEGENDS:

Figure 1. MR-compatible cylinder and cannula injection grid. **A,B)** custom-designed nylon injection grid. **C)** MR-compatible fixed cylinder. **D,E)** Rotating MR-compatible cylinder. **F)** MR-compatible infusion cylinder with fixed grid position. The arrows point to the cavities that are designed to be filled with wet gelfoam keeping the surface of the brain moist for the duration of injection. **G)** Cannula inserted in the grid. This figure has been modified from Yazdan-Shahmorad et al. 2016⁹.

Figure 2. Baseline MR imaging and reflux resistant cannula. **A)** T1-weighted image of vitamin E that was attached to the top of the injection grid that enables us to measure the distance (white arrow) to the surface of the brain. **B)** T2-weighted image of the brain helps to plan the location of injection from the cannula grid filled with saline. **C)** MR image of the infusion chamber and the saline-filled cannula grid. The orthogonal lines represent the sagittal (yellow) and coronal (purple) planes. **D)** Photo of the reflux resistant injection cannula tip with the reflux resistant step (black arrow). **E)** Infusion lines. This figure has been modified from Yazdan-Shahmorad et al. 2016⁹.

Figure 3. MR imaging during infusion and estimated distribution. **A)** Spread of 50 μ L of the viral vector in coronal sections of Monkey G for one injection site in S1 (shown with an arrow in **B**). **B)** MRI surface rendering of the cortical surface below the cylinder for Monkeys G and J, respectively. The S1 infusion locations are shown in blue, M1 locations in red. **C)** MR Volume reconstruction of the spread of viral vector during CED infusion. Brain is shown in light gray; S1 and M1 infusion volumes are shown in blue and red, respectively. No MR volume reconstruction is available for the M1 infusions for monkey G since they were not done in the MR scanner. This figure has been modified from Yazdan-Shahmorad et al. 2016⁹.

Figure 4. Electrophysiological recordings of light-evoked activity. Micro-Electrocorticography (μ ECoG) recordings occurred during pulsed optical stimulation. Recording traces were from the closest electrode to the site of stimulation for examples of M1 (red) and S1 (green) stimulation locations. Shaded areas around the traces represent standard error across 25 trials. The blue squares on the traces show the timing of stimulation (1 ms). The full set of stimulus-triggered waveforms for both sample pairs of stimulation and recording sites are superimposed on the mean waveform as shown on the left side of the panel. This figure has been modified from Yazdan-Shahmorad et al. 2016⁹.

Figure 5. Histological analysis. **A)** Baseline coronal MR image in monkey G. **B)** Spread of contrast agent after the infusion for the same MR coronal slice as in A. **C)** A coronal tissue section from approximately the same site as in A and B; peroxidase staining reflects expression of the EYFP-reporter. **D)** Good alignment is observed between the area of EYFP expression measured with

surface epifluorescence (dark green areas) and with histological staining (light green lines). These include the region of vector spread estimated from MR images (white line); white dots indicate injection sites, and the entire black region represents the area exposed by the craniotomy. The two left most injection sites are located in M1, and the two right most sites are located in S1. E) Low magnification image of the coronal section stained with anti-GFP antibody showing the medio-lateral aspect of YFP expression in the somatosensory cortex of monkey J (areas 1, 2, 3). The black arrowhead indicates the location of the cannula track; the adjacent tissue (black frame) is shown in F, at greater magnification to show laminar distribution of the YFP-positive cells. F) Densely populated regions of YFP-positive cells are located predominantly in layers II-III and V-VI, and also show typical pyramidal morphology (cells in white frames are further enlarged in panels G-I). White arrowheads on bottom panels G-I point to typical pyramidal cells in layers II-III (G); layer V (H); and layer VI (I). Scale bars: A, 2 mm; B, 200 μ m; C-E, 100 μ m. This figure has been modified from Yazdan-Shahmorad et al. 2016⁹ and Yazdan-Shahmorad et al. 2018¹⁵.

Supplementary Figure 1. The cannula injection grid prior to threading. The length and diameter of the injection grid are 15.00 mm and 12.00 mm, respectively. The injection grid pattern consists of 0.8 mm diameter holes forming three concentric circles (diameter: 1.6, 3.2 and 4.8 mm) around the center of the grid.

DISCUSSION:

Here, we outline a feasible and efficient technique for achieving large-scale optogenetic expression in NHP primary somatosensory and motor cortex by MR-guided CED. The use of MR-guided CED presents significant advantages over traditional methods of viral infusion in the NHP brain. One such advantage is the ability to attain expression over large areas with fewer required infusions. For instance, with conventional methods, multiple injections of 1-2 μ L of the vector yield expression in a 2-3 mm diameter region^{1,2,5,17}. While previous attempts at achieving large-scale vector spread have yielded expression volumes of approximately 10 mm³ with multiple injections¹⁸, here we present a method to achieve expression over 200 mm³ with only a few infusions. A single infusion of 50 μ L by CED can achieve vector spread up to 10 mm from the injection site for cortical CED⁹ and full coverage of frontal cortical areas with thalamic CED¹⁴.

Furthermore, it has been shown that using CED can achieve more homogeneous distribution of the injected agent^{12,19-22}, leading to uniform expression levels around the site of viral vector infusion, compared to traditional microinjections. In our experiments employing CED, we observed uniformly high expression levels with approximately 70-80% of neurons expressing the opsin^{8,9} (Figure 4). In contrast, conventional diffusion-based injections exhibit regions of high expression concentrated near injection sites enveloped by weakening expression levels^{4,5,17}. The reduced need for multiple injections renders CED infusion a time-efficient method of obtaining large-scale, uniform optogenetic expression. Due to the smaller number of injections and faster infusion rates, we were able to plan and infuse the viral vector under the guidance of MRI. The use of online MR images enables precise targeting of infusions and the monitoring of the spread of the viral vector during infusion. However, if the initial depth of the inserted cannula is incorrect, this may yield expression in undesired locations as a result of the initial infused \sim 10 μ L needed to visually detect the infusion via MR. Alternatively, commercial neurosurgical navigation

systems utilizing fiducial markers can also be employed in place of MR guidance. Additionally, the costs of online imaging may be circumvented by marking the desired depth of insertion on the cannula prior to insertion and visual confirmation of complete infusion, although the accuracy of the cannula placement would be reduced. However, as seen by the unsuccessful injection in M1 of monkey G (**Figure 5D**), MR guidance can be critical for ensuring successful infusion.

Previous studies have demonstrated the safety of CED infusions of both non-viral and viral particles with no observed tissue damage outside the cannula tract and no behavioral deficits^{9,11-15,20}. Here and in our previous publications^{9,14} we report similar results following CED infusion of optogenetic viral vectors. In addition to no observed behavioral deficits following infusion, NeuN and Nissl^{9,14,15} staining revealed neuronal cell death and gliosis was limited only to the cannula tract, as has been reported with diffusion-based methods⁵. To minimize the damage from the cannula track, we can potentially reduce the diameter of the cannula. However, further investigation is needed to assess the effect of cannula size reduction on achieving large areas of expression. Moreover, because high infusion rates can also lead to tissue damage¹³, it is recommended to maintain an infusion rate at or below 5 $\mu\text{L}/\text{min}$. For more detailed information about the CED technique please see our previous publication⁹.

The use of MR-guided CED for attaining broad regions of targeted optogenetic expression in the primate cortex can lead to further investigations of large-scale circuit dynamics, neural plasticity, and network connectivity.

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DISCLOSURES:

PNS has financial interest in Neuralink Corp., a company that is developing clinical therapies using brain stimulation.

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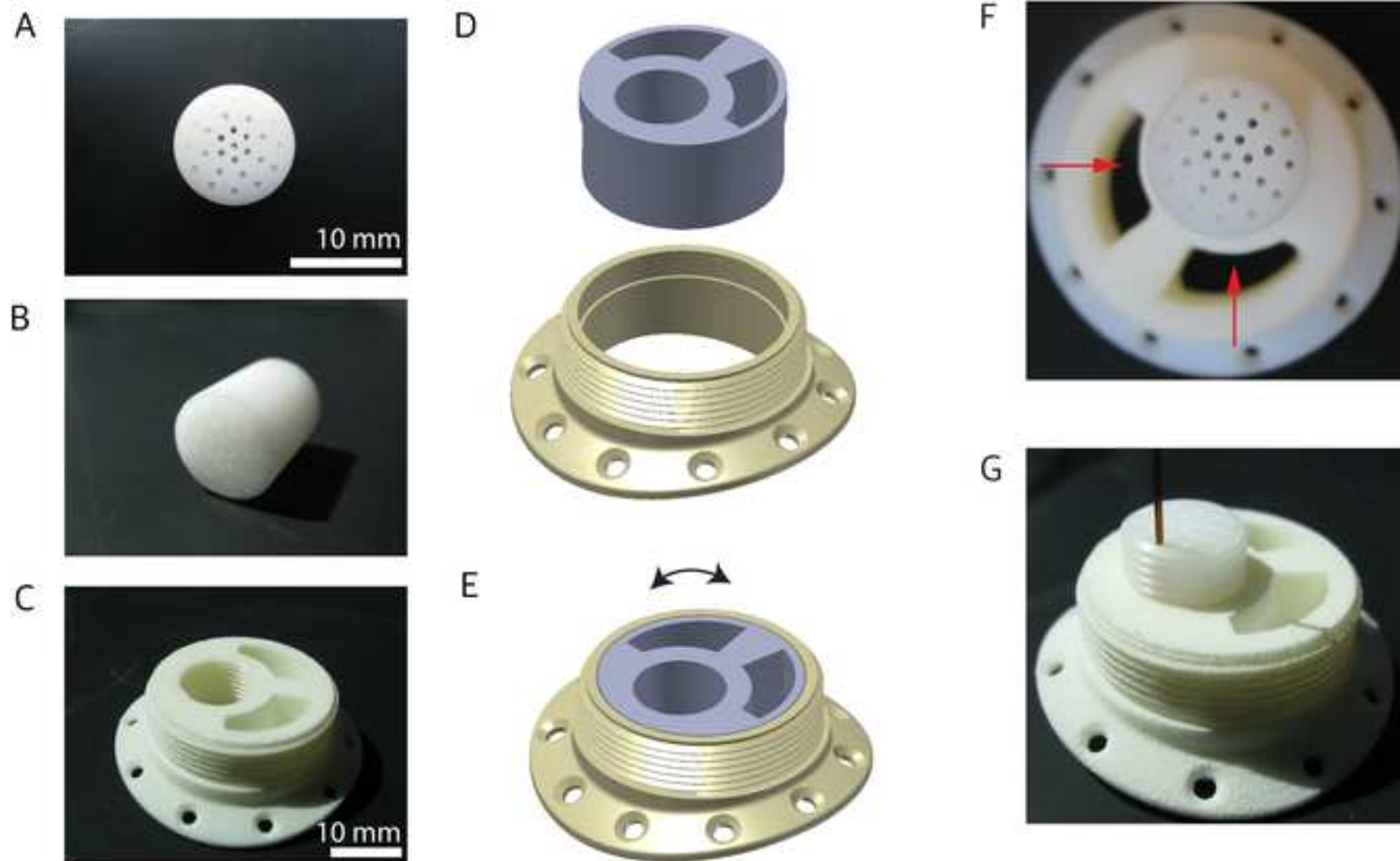
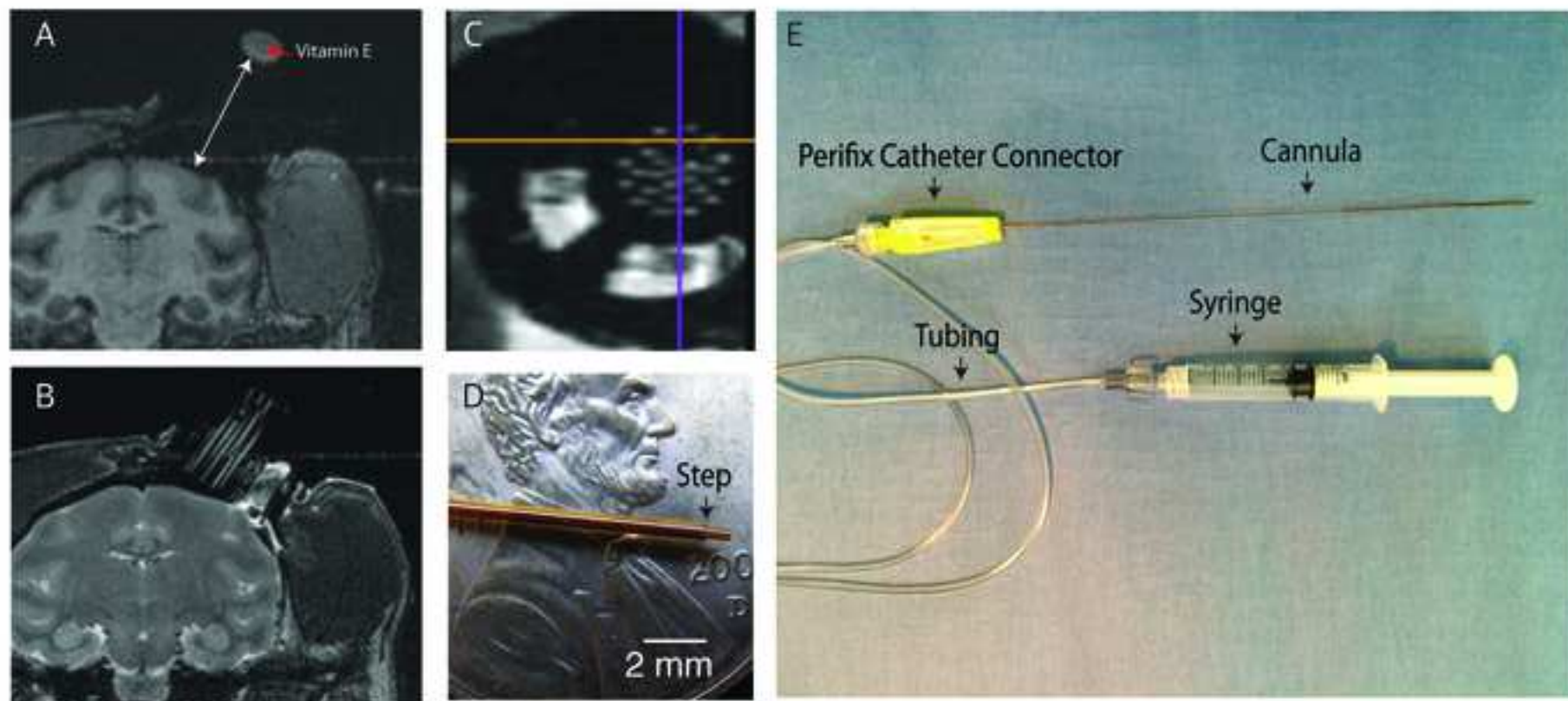
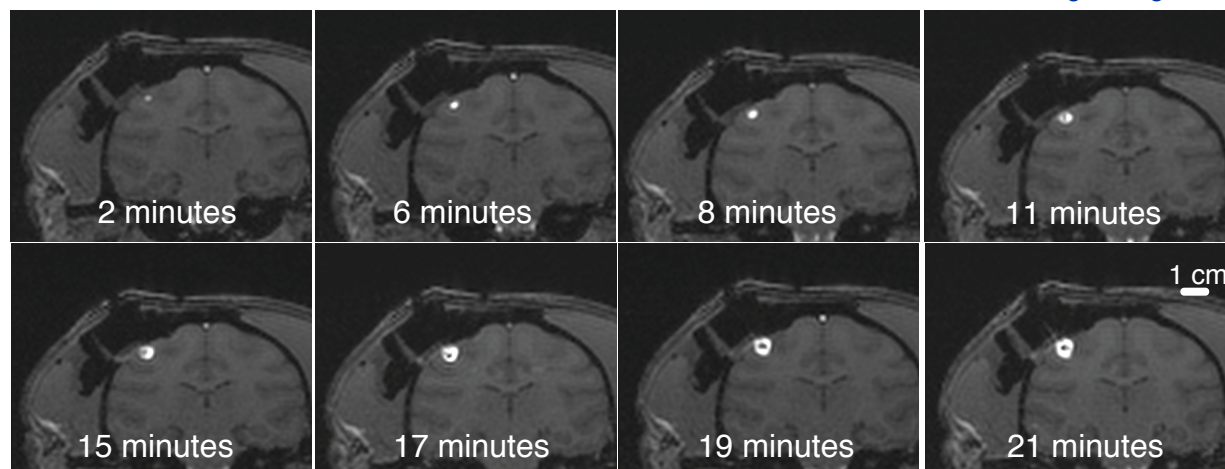


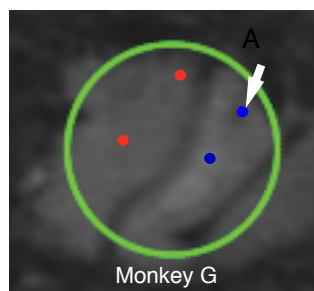
Figure 2



A



B



C

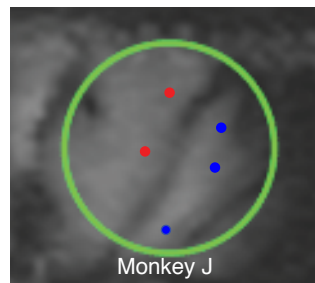
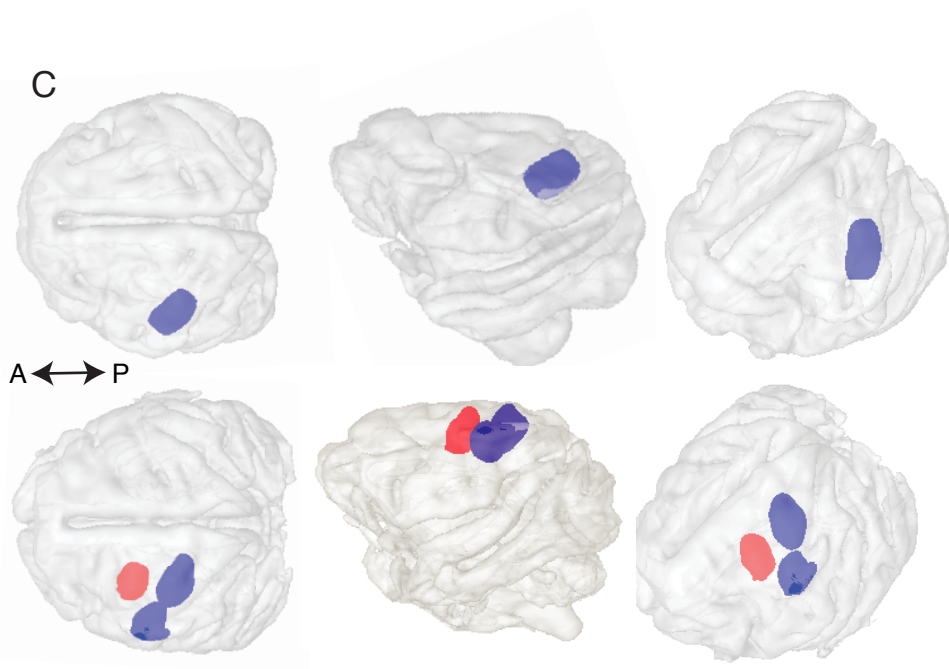
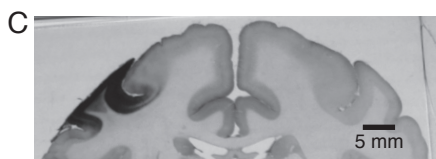


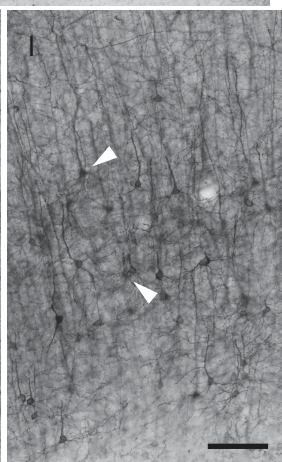
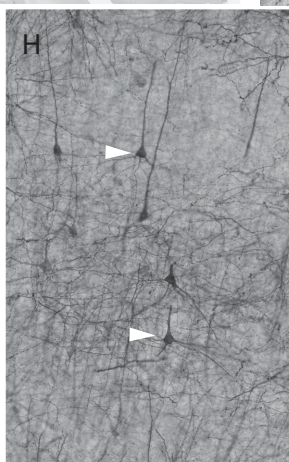
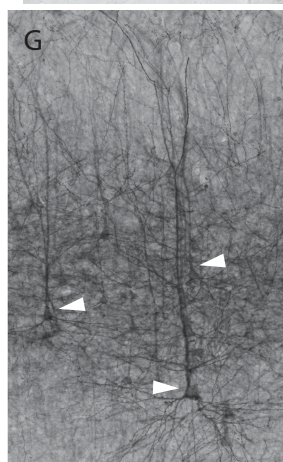
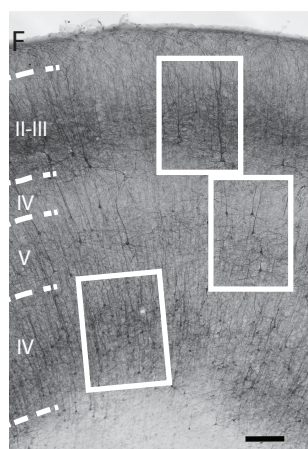
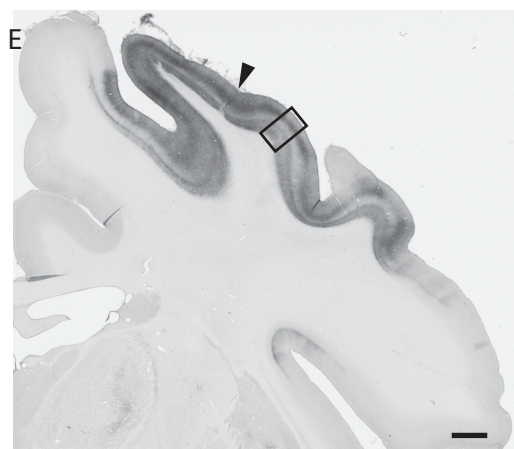
Figure 4

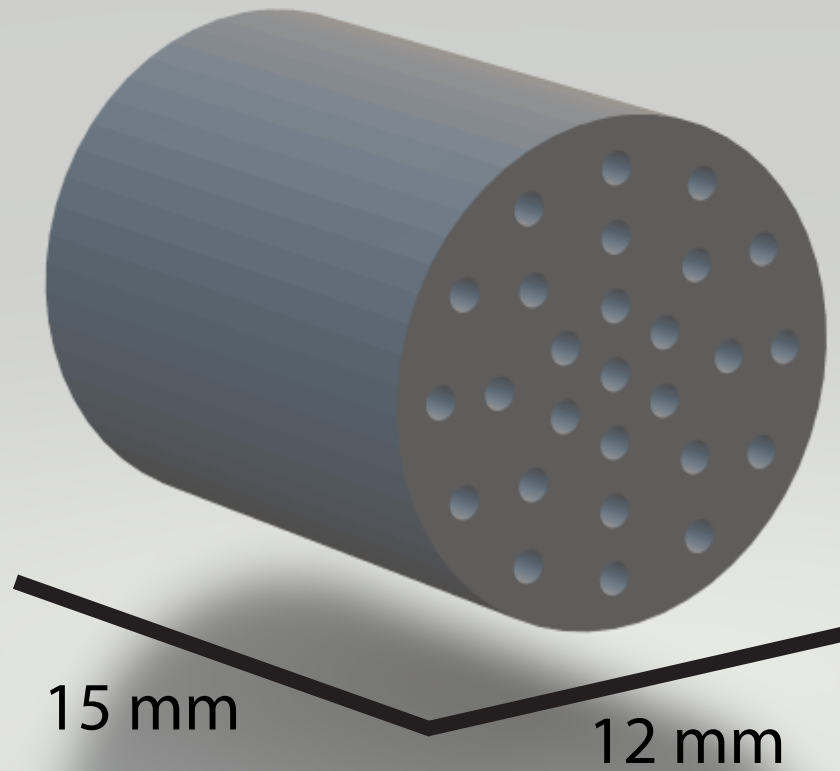
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Name of Material/ Equipment	Company	Catalog Number	Comments/Description
0.2 mL High Pressure IV Tubing	Smiths Medical Inc., Dublin, OH, USA	533640	
0.32 mm ID, 0.43 mm OD Silica Tubing	Polymicro Technologies	1068150027	
0.45 mm ID, 0.76 mm OD Silica Tubing	Polymicro Technologies	1068150625	
AAV2.5-CamKII-C1V1-EYFP	Penn Vector Core, University of Pennsylvania		
ABS plastic	Stratasys, MN, USA	AB <i>Sp</i> lus -P430	
Antimicrobial incise drape	3M	6650EZ	Ioban Drape
Dental Acrylic	Henry Schein, Inc.	1013117	Acrylic Bonding Agent
Elevators	VWR International, LLC.	10196-564	Langenbeck Elevator, Wide Tip
Fine suture	McKesson Medical-Surgical Inc.	1034505	
Gadoteridol	Prohance, Bracco Diagnostics, Princeton, NJ	0270-1111-04	
Laser for light stimulation	Omicron-Laserage, Germany	PhoxX 488-60	
MR compatible 3cc syringe	Harvard apparatus, Holliston, MA, USA	59-8377	
MR Imaging Software	Pixmeo	OsiriX MD 10.0	
MR-Compatible Pump	Harvard apparatus, Holliston, MA, USA	Harvard PHD 2000	
MR-compatible stereotaxic frame	KOPF	1430M MRI	
Perifix Clamp Style Catheter Connector	B-Braun, Bethlehem, PA, USA	N/A	
Plastic Screws	Plastics 1	0-80 x 1/8N	Nylon screws
Titanium screws	Crist Instrument Co., Inc.	6-YCX-0312	Self-tapping bone screws
Trephine	GerMedUSA Inc,	SKU:GV70-42	
uPrinter SE 3D printer	Stratasys, MN, USA	N/A	
Vitamin E Capsule	Pure Encapsulations, LLC.	DE1	
Wet sterile absorbable gelatin	Pfizer Inc.	AZL0009034201	Gelfoam

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Author(s):

Karam Khateeb, Philip N. Sabes, Azadeh Yazdan-Shamirad

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Institution:	University of Washington		
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We thank the editorial board for their help in improving our manuscript. Here we respond to their comments regarding the previously submitted manuscript.

1. Step 1.1: How is the depth of anesthesia determined?

The depth of anesthesia is determined based on multiple factors, including the respiratory rate, heart rate, and other vital signs. This has been specified in the revised manuscript.

2. Step 1.2: Mention any specification such as magnet strength.

We have specified the magnetic field strength.

3. Step 1.3: Mention settings and specifications for the scan.

We have now included specific settings for MR scans.

4. Step 1.5: What is the area you chose?

We have clarified that we targeted M1 and S1 cortical regions.

5. Step 1.5: Cite a reference for where this is available.

We have included a reference for the macaque brain atlas.

6. Step 2.3: Mention surgical tool used.

We have specified that a scalpel is used in this step.

7. Step 2.4: Mention surgical tool used.

We have specified that elevators are used in this step and have listed them in the table of materials.

8. Step 2.6: Add [fine suture] to the table of materials.

We have included fine sutures to the table of materials.

9. Step 3.1: This [Figure S1] has not been provided in the current revision.

We have uploaded Supplementary Figure 1.

10. Step 3.1: [saline] 0.1% NaCl?

We have clarified that the saline used is 0.9% NaCl.

11. Step 3.1: This [gelfoam] is a tradename, please use a generic name, e.g. sterile absorbable gelatin compressed sponge and add it to the table of materials.

We have removed “gelfoam” from the manuscript, replaced it with a generic name, and included it in the table of materials.

12. Step 3.2: This [loban] is a tradename, please use a generic name or simply delete the name loban and add it to the table of materials.

We have replaced “loban” with a generic name as in the table of materials.

13. Step 3.4: Mention any setting and specifications.

We have specified the scan settings used.

14. Step 3.5: Mention any settings and specifications.

We have now included the scan settings.

15. Step 3.5: What do you look for exactly?

We have clarified that the coronal and sagittal planes used are based on the desired site of infusion.

16. Step 3.10: This [perifix] is a tradename, please use a generic name or simply delete the name and add it to the table of materials.

We have removed “perifix” from the text.

17. Step 3.15: Mention imaging parameters.

We have included the imaging parameters.

18. Step 3.16: What do you check for?

We have clarified that we use the vector spread from the MR images to determine whether the cannula is inserted correctly.

19. Step 4.1: [Figure S1] Not present in your revision. I suggest calling this Supplementary Figure 1 throughout.

We have replaced “Figure S1” with “Supplementary Figure 1”.

20. Step 5.4: Remove/replace the tradename [perifix].

This has been removed.

21. *Validation of Viral Expression*: Define. Unclear what M1 and S1 are.

M1 and S1 were defined in both the abstract and the introduction.

22. Figure 1c: Add a space after 10 in “10mm”.

Thank you for pointing this out. We have added a space.

23. Figure 2: Add a title.

We have added a title.

24. Figure 2E: Remove the tradename “perifix”.

This has been removed.

25. Figure 3: Missing from revision. Please upload. Add a title.

We have uploaded the figure and added a title.

26. Supplementary Figure 1: Submit.

We have uploaded the figure.

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