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TITLE:

Targeted Neuronal Injury for the Non-Invasive Disconnection of Brain Circuitry

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Focused ultrasound, non-invasive, neurosurgery, neuronal lesion, MRI, brain.

SUMMARY:

The goal of the protocol is to provide a method for producing non-invasive neuronal lesions in the brain. The method utilizes Magnetic Resonance-guided Focused Ultrasound (MRgFUS) to open the Blood Brain Barrier in a transient and focal manner, in order to deliver a circulating neurotoxin to the brain parenchyma.

ABSTRACT:

Surgical intervention can be quite effective for treating certain types of medically intractable neurological diseases. This approach is particularly useful for disorders in which identifiable neuronal circuitry plays a key role, such as epilepsy and movement disorders. Currently available

surgical modalities, while effective, generally involve an invasive surgical procedure, which can result in surgical injury to non-target tissues. Consequently, it would be of value to expand the range of surgical approaches to include a technique that is both non-invasive and neurotoxic.

Here, a method is presented for producing focal, neuronal lesions in the brain in a non-invasive manner. This approach utilizes low-intensity focused ultrasound together with intravenous microbubbles to transiently and focally open the Blood Brain Barrier (BBB). The period of transient BBB opening is then exploited to focally deliver a systemically administered neurotoxin to a targeted brain area. The neurotoxin quinolinic acid (QA) is normally BBB-impermeable, and is well-tolerated when administered intraperitoneally or intravenously. However, when QA gains direct access to brain tissue, it is toxic to the neurons. This method has been used in rats and mice to target specific brain regions. Immediately after MRgFUS, successful opening of the BBB is confirmed using contrast enhanced T1-weighted imaging. After the procedure, T2 imaging shows injury restricted to the targeted area of the brain and the loss of neurons in the targeted area can be confirmed post-mortem utilizing histological techniques. Notably, animals injected with saline rather than QA do demonstrate opening of the BBB, but dot not exhibit injury or neuronal loss. This method, termed Precise Intracerebral Non-invasive Guided surgery (PING) could provide a non-invasive approach for treating neurological disorders associated with disturbances in neural circuitry.

INTRODUCTION:

The purpose of this method is to provide a means for producing non-invasive neuronal lesions in a targeted region of the brain. The rationale for developing such an approach is to disconnect neuronal circuitry contributing to neurological disorders. For instance, surgery can be quite effective in treating certain medically intractable neurological disorders, such as drug resistant epilepsy (DRE)[1]. However, each of the available surgical modalities possess limitations in terms of producing undesirable collateral damage to the brain. Traditional resective surgery can be highly invasive with the risk of bleeding, infection, blood clots, stroke, seizures, swelling of the brain, and nerve damage[2]. Alternatives to resective surgery that are minimally invasive or non-invasive include laser interstitial thermal therapy and radiosurgery, which have also proved to be effective in suppressing seizures in DRE. More recently, thermal lesions produced by high-intensity focused ultrasound (HIFU) have shown promise in reducing seizures. HIFU is non-invasive; however, its treatment window is currently limited to more central areas of the brain because of the risk of thermal injury to non-target tissue located in the vicinity of the skull. Despite such limitations, the benefits of surgery often outweigh the potential risks. For instance, although surgery for DRE can produce collateral brain damage, its beneficial effects in suppressing seizures and improving quality of life typically prevail over the surgical risks.

The method described herein, Precise Intracerebral Non-invasive Guided surgery (PING), was developed for the purpose of disconnecting neural circuitry, while limiting collateral brain damage. The method utilizes low intensity focused ultrasound combined with intravenous injection of microbubbles to open the BBB, in order to deliver a neurotoxin. This approach does not produce thermal lesions to the brain[3–7], and the period of BBB opening can be exploited to deliver BBB-impermeable compounds to the brain parenchyma. The opening of the BBB is

transient, and can be produced in a targeted manner using magnetic resonance imaging guidance. In our studies, the period of BBB opening has been utilized to deliver a circulating neurotoxin to a targeted area of the brain parenchyma in rats and mice[8,9]. Quinolinic acid is a neurotoxin that is well tolerated when administered intravenously[10], intraarterially[10], or intraperitoneally[8,9,11]. The lack of QA toxicity is due to its poor BBB permeability, which has been reported to be negligible[10]. In contrast, direct injection of QA into the brain parenchyma produces neuronal lesions that spare neighboring axons[12,13]. Thus, when circulating QA gains access to the brain parenchyma in the targeted area of BBB opening, neuronal death is produced[8,9]. The present method thus produces focal neuronal loss in a precisely targeted and non-invasive manner.

PROTOCOL:

All methods described here have been approved by the University of Virginia Animal Care and Use Committee.

1. Preparation of reagents

1.1 On the day of surgery, prepare 6.0 mL of injectable quinolinic acid (QA). Dissolve 450 mg of QA in 4.0 mL of 1.0 N NaOH. Add 0.6 mL of 10x PBS, pH to 7.4, and bring to a final volume of 6.0 mL with dH_2O. Filter through 0.22 µm syringe filter. The solution is stable for 2 weeks at 4 °C.

1.2 Prepare an aqueous dispersion of microbubbles in normal saline by probe sonication from decafluorobutane gas and stabilize with DSPC/PEG stearate monolayer shell[12].

1.3 Size microbubbles by flotation at normal gravity. Determine microbubble concentration and size by electrozone sensing using Multisizer III counter. Microbubble concentration and size distribution should be 6 x 10^8/mL and ~2 µm (mean particle diameter), respectively. The largest bubbles are removed by flotation exclusion/separation.

1.4 Alternatively, purchase commercially available microbubbles.

2. Preparation of animals

2.1 Acclimatize the animal (rat or mouse) for 3 days after delivery. The experiments described here used Sprague-Dawley rats (5–6 weeks of age) or telencephalic internal structural heterotopia (tish) rats (local colony).

2.2 House the animals under a 12 hour light: 12 hour dark cycle.

2.3 Record the animals' weights. This information is important throughout the procedure.

2.4 Obtain T2-weighted MR images the day before the FUS procedure in order to establish preoperative baseline images. Use the following parameters for T2 imaging: repetition time/echo

time [TR/TE] =3,000/138 milliseconds, 3 averages, field of view=29 x 45 mm^2, matrix size = 125 x 192, slice thickness = 0.23 mm.

2.5 Anesthetize the animal with isoflurane (4% induction, 2% maintenance). Confirm adequate depth of anesthesia using a toe pinch. Apply an ophthalmic ointment to the eyes.

2.6 Shave the animal's scalp, and remove the remaining hair with depilatory cream.

2.7 Use a tail vein catheter for the infusion of microbubbles, contrast agent, and QA. The catheters consist of a length of PE10 tubing fitted with a 30 G x ½ inch needle. Leave a 1 mL syringe with heparinized saline in line, to be removed and reattached when the line is used for infusions.

3. MRI and PING procedures

3.1 Perform the MRI on a 7 T MR unit with a gradient strength of 600 mT/m/ms (**Figure 1** and **Figure 2**). Perform MRI acquisitions using a surface coil incorporated in the FUS system.

NOTE: The FUS system used for the experiments comprises three parts: (i) the sonication system is a MR-compatible pre-focused, 8-element annular array, 1.5 MHz transducer (spherical radius = 20 mm ± 2 mm, active diameter = 25 mm (f-number = 0.8), with 80% electric-acoustic efficiency, which is connected to a phased array generator and RF power amplifier; (ii) a MR-compatible motorized positioning stage to move the transducer in the anterior-posterior direction and medio-lateral direction; (iii) a Thermoguide workstation to control the delivery of sonication, including electronic focusing through phase modulation to adjust the focal depth (**Figure 2**).

3.2 Place the anesthetized animal on the coil sled assembly (**Figure 1**) of the MR-compatible FUS system in the prone position. Immobilize the animal using the incisor bar and ear bars incorporated in the cradle of the sled.

3.3 Apply acoustic gel to the water-wetted scalp; ensuring that no bubbles exist, place the membrane barrier of the water circulator portion of the transducer assembly above the animal's skull, and lower the transducer assembly as far as possible in a parallel planar orientation relative to the skull plate. Place the diaphragm of the transducer firmly against the animal's shaven scalp directly over the skull plate.

3.4 Attach a pneumatic sensor to the body with surgical tape, to monitor respiration. Position the pneumatic sensor on the left lower rib cage.

3.5 Move the FUS arm assembly with coil, sled, and animal into the 7T MRI unit (**Figure 1**).

3.6 Run a T2-scout sequence to determine the general physical position of the transducer assembly relative to the animal's head, and make mechanical adjustments as necessary (**Figure 2**). The parameters for T2 imaging are: repetition time/echo time [TR/TE] = 3,000/138

milliseconds, 3 averages, field of view = 29 x 45 mm^2, matrix size = 125 x 192, slice thickness = 0.23 mm. Thermometry is typically not used in this protocol.

3.7 Obtain T2 images to refine the transducer positioning. Precisely, define the transducer location and specify the focal point(s) of sonication using the targeting function of the software. The parameters for T2 imaging are: repetition time/echo time [TR/TE] = 3,000/138 milliseconds, 3 averages, field of view = 29 x 45 mm^2, matrix size = 125 x 192, slice thickness = 0.23 mm.

3.8 Just prior to sonication, inject 300 µL/kg of microbubbles[14] via the tail vein.

3.9 Use a 1.5 MHz transducer to produce sonications (30 ms wave packet, 3% duty cycle, 1 Hz burst repetition frequency, 240 s duration/sonication.

3.10 Immediately after sonication, inject gadodiamide contrast agent via the tail vein, and then perform T1-weighted plus contrast scans to confirm opening of the BBB and the accuracy of targeting. The parameters for T1-weighted imaging: TR/TE = 900/12 milliseconds, 2 averages, field of view = 24 x 30 mm^2, matrix size = 208 x 256, slice thickness = 0.7 mm. Typically, a single T1 scan is performed.

3.11 Remove the FUS arm and sled from the MRI, and place the animal on a heating pad set to 40 °C, while maintaining 2% isoflurane anesthesia.

3.12 Starting 30 min after sonication, use a syringe pump to infuse QA (75 mg/mL stock solution) via the tail vein for 1 h at a rate of 16.8 µL/min to achieve a final dosage of 225 mg/kg (q.s. to 1.0 mL saline).

3.13 When the infusion is complete, discontinue anesthesia, keeping the animal on a heating pad until alert. Place the animal in a cage and make routine checks for its activity every 15 min, for several hours after the procedure.

3.14 Return the animal to the vivarium and check every 6 h for the first day for distress or irregular activity.

3.15 One day post-sonication, perform T2-weighted MR imaging to assess any damages in the area of sonication. The parameters for T2 imaging: repetition time/echo time [TR/TE] = 3,000/138 milliseconds, 3 averages, field of view = 29 x 45 mm^2, matrix size = 125 x 192, slice thickness = 0.23 mm. Images are evaluated for areas of hyperintensity to identify possible tissue damage/edema.

4. Post-mortem analysis of neuronal loss

4.1 Allow a post-sonication, survival period of 4–5 days for assessing neuronal loss with Fluoro-Jade histochemistry.

4.2 Deeply anesthetize the animal with isoflurane, and euthanize via intracardial perfusion with 0.1 M phosphate buffer (pH 7.4) followed by 4% paraformaldehyde in phosphate buffer.

4.3 Remove the brain from the skull and post-fix for 2 days in 4% paraformaldehyde.

4.4 Immerse the brain in 30% sucrose for cryoprotection, and cut sections at a thickness of 20–30 μm with a cryostat.

4.5 Mount cryostat sections onto gelatinized slides and air-dry overnight.

4.6 Rehydrate slides in distilled water, and then dehydrate in ascending graded ethanols. After dehydration, rehydrate slides in descending graded ethanols.

4.7 Transfer slides to a solution of 0.06% potassium permanganate for 15 min on an orbital shaker.

4.8 Rinse slides for 1 min in distilled water and transfer to a 0.001% solution of Fluoro-Jade B in 0.1% acetic acid. Incubate under gentle agitation for 30 min at room temperature. Rinse slides three times for 1 min in distilled water.

4.9 Dry the slides on a slide warmer, equilibrate in xylenes for 3 min, and coverslip using DPX mounting media.

REPRESENTATIVE RESULTS:

This section describes the effect of PING on neurons located in a neocortical dysplasia. Tissue dysplasias are a common feature in the brains of patients with drug resistant epilepsy, and surgical removal of seizure-genic dysplasias can provide excellent control of seizures[15]. Defining the effect of PING on dysplastic brain tissue is therefore an important priority. A rat model of genetic cortical dysplasia, the tish rat, was selected for studying this issue because the tish brain exhibits dysplastic tissue (subcortical band heterotopia) located below a normally positioned neocortex (**Figure 3**)[16]. Both the dysplasia and the overlying neocortex contain neurons that are functional and exhibit characteristic neocortical connectivity[17,18].

PING targeting the heterotopia of the tish rat brain was effective in producing focal neuronal loss of the dysplastic neurons (**Figure 3**). T2-weighted images taken one day post-PING exhibit areas of hyperintensity, consistent with tissue damage in the targeted areas of sonication. Post-mortem staining using Fluoro-Jade after a 5-day post-PING survival period demonstrated degenerating neurons in the areas of T2-hyperintensity, corroborating the ability of PING to produce neuronal loss in the targeted dysplastic neocortical tissue.

Figure 1. Magnetic resonance (MR)-compatible sled and 7T MR magnet. The key features of the sled used to position the animal in the MR magnet and to deliver sonication are depicted (**A**). The sled assembly is shown inserted into the 7T MR magnet (**B**).

Figure 2. **Control room for Magnetic Resonance (MR) imaging and MR-guided Focused Ultrasound (FUS).** The control room comprises two primary stations. The station where the investigator is sitting is the planning area for targeting FUS (**A**). The second station is the control area for the MRI system (**B**). The copper-reinforced window behind the MRI station looks into the room housing the 7T magnet. The monitor at the FUS station displays the software controlling the guidance and parameters of sonication (**C**). This example shows a sonication targeting the striatum superimposed on a T2-weighted MR image (**D**).

Figure 3. PING produces neuronal loss in the dysplastic neocortex of the tish rat brain. T2-weighted MR images (**A,B**) were obtained one day after PING. The neocortex [N], heterotopia [H], and the lateral ventricles [LV] are labeled for the purpose of orientation (**A**). Areas of hyperintensity [white and yellow arrows], indicative of tissue damage, can be seen in the targeted areas of the heterotopia on both sides of the brain (**B**). Post-mortem staining with Fluoro-Jade(**C–F**). Bright green cells representing degenerating neurons are observed in the regions corresponding to the areas of hyperintensity on both the left (**C,E**; white arrows) and right (**D,F**; yellow arrows) sides of the brain. Scale bars: C,D = 1 mm; E,F = 0.5 mm.

DISCUSSION:

The PING method is designed to produce non-invasive, targeted neuronal lesions. The method derives from a strong and growing foundation of research in the field of focused ultrasound[3–7]. The ability to provide focal access to specific areas of the brain parenchyma via transient opening of the BBB has created an avenue for delivering a wide variety of agents that would normally not gain access to the brain. This opportunity is largely being advanced for the central delivery of therapeutic agents that possess poor BBB permeability. The PING method leverages the same opportunity in a different manner by delivering a neurotoxin, with the ultimate goal of destroying circuits that contribute to neurological dysfunction. Aberrant neural circuitry represents an effective target for surgical intervention to attenuate the impact of certain neurological disorders[2,15]. The long-term goal for the PING method is to augment the currently available approaches for disconnecting circuitry contributing to neural dysfunction.

The design of a comprehensive study using the PING method would incorporate multiple control groups. These groups would include: (a) an untreated group [No FUS/No QA]; (b) a group controlling the direct effects of FUS [FUS/No QA]; (c) a group assessing the direct effects of systemic neurotoxin in the absence of FUS [No FUS/QA]. Outcomes in these groups would be compared with the primary PING group [FUS/QA].

The PING method requires basic skills for the handling of small animals. These include the induction and maintenance of anesthesia, placement of a tail vein line, intravenous administration of multiple agents in the setting of an MRI unit, intravenous infusion of a drug, and animal care during the operative and postoperative periods. It also requires the ability to perform intracardial perfusion, tissue sectioning, histochemical staining, and microscopic analyses. Institutional training and approval from an Animal Care and Use Committee, or equivalent agency, is necessary for several of the steps in the protocol.

The initial protocol for the PING procedure utilized repetitive intraperitoneal injection of QA over multiple days[8,9]. This approach is still viable and effective. However, the current protocol modified the route and rate of QA administration. In particular, QA was administered intravenously during a 1 h post-sonication period of infusion. Again, either administration protocol is effective. The 4–5 day survival period utilized in the current protocol was adopted to optimize the use of the Fluoro-Jade method for detecting degenerating neurons. If longer survival periods are required, then alternative tissue staining methods would be indicated. One such approach would be to use a neuron-specific antibody (e.g., anti-NeuN), to demonstrate immunohistochemically where surviving neurons remained after a lengthier post-sonication survival period. A variety of outcomes, in addition to the current structural analyses, would also be useful. For instance, post-PING assessments of electrophysiological and behavioral outcomes would significantly advance the characterization of the impact of the PING method.

One potential complication of FUS procedures is that temperatures could be elevated in the vicinity of the skull, particularly when targeting cortical areas located near the skull. This complication currently restricts the range of sites (treatment envelope) amenable to thermal lesioning using high intensity FUS (HIFU). In the context of PING, thermal increases near the skull may also represent a limitation of the technique. However, the low intensity of sonication used with PING reduces the risk of thermal injury, and should expand the treatment envelope as compared to HIFU.

The PING method possesses several features that are both infrastructure-intensive and training-intensive. MRI-compatible FUS equipment and an MRI facility equipped for animal research are required. This represents a considerable front-end investment, in order to provide the necessary research infrastructure. However, it is encouraging to note that the number of sites equipped with FUS technology is rapidly expanding for both research and clinical endeavors. Thus, as the number of available sites for performing such work increases, the opportunities for on-site and/or collaborative investigation will grow. In terms of training, any investigator seeking to undertake FUS studies would benefit from training by a research group experienced in FUS experimentation. For instance, the MRgFUS targeting software, while relatively straightforward, is more easily acquired when advised by an experienced investigator.

Multiple, alternative surgical modalities exist for the removal of disturbed neuronal circuitry. These include resective surgery, stereotactic laser ablation, radiosurgery, high-intensity focused ultrasound, and radiofrequency treatment. Each of these approaches is effective and can be of considerable therapeutic value for certain medically intractable disorders. However, these surgical modalities possess one or more specific limitations: (a) invasive procedure, (b) pannecrotic impact on target tissue, (c) damage to adjacent, non-target tissue (e.g., axons of passage), and (d) delay in achieving effective outcomes. Significant advantages of the PING method are that it: (a) is non-invasive, (b) is not pannecrotic, (c) limits effects on adjacent non-target tissue, and (d) should provide rapid outcomes.

There are several potential future directions for the PING method, including both preclinical and clinical applications. With respect to animal experimentation, the method provides a means for

producing focal neuronal lesions in preclinical models of neurological disease. For instance, we have recently used this approach to test the effect of PING in a rodent model of limbic epilepsy[19]. Treatment with PING reduced the frequency of chronic, spontaneous seizures in a pilocarpine model of limbic epilepsy. This study provided the first, preclinical proof of concept for the utility of PING in treating a neurological disorder.

Quinolinic acid was selected for the PING procedure for two primary reasons. First, existing literature[11] indicates that neuronal cell body lesions can be produced while sparing other non-target tissue, such as axons of passage. Second, although QA is a neurotoxic when delivered directly to the brain, it is well tolerated when administered systemically because of its limited permeability through the BBB. The other toxins that are well tolerated peripherally, such as monosodium glutamate (MSG), could be considered as alternatives for QA. A key advantage of MSG would be that there exists a substantial literature regarding the use of this compound by humans. An important goal for future research will be to define the specificity of injury produced by QA or other systemically administered neurotoxins in terms of potential cell-type specificity. Another potential preclinical application for this general approach would be to administer a peripherally tolerated compound that is toxic to other cell types in the brain parenchyma, such as glial cells. For instance, peripheral injection of a toxin that affects oligodendroglia could allow focal demyelination in an area of white matter. This would allow targeted demyelination of part of a targeted pathway. Moreover, the approach might conceivably allow assessments of serial demyelination of the same site, which is a hallmark of relapsing-remitting multiple sclerosis.

With respect to the possible future applications of PING in humans, neurological disorders in which disturbed neural circuitries are a target could be amenable to treatment with PING. Based on our initial preclinical findings[19], Drug Resistant Epilepsy (DRE) is a promising example of a disorder that might benefit from PING. Surgical treatment of DRE can be highly beneficial, but remains one of the most underutilized treatments that is actually effective for a major neurological disorder. An advantage of PING in this setting is that the volume of the target area can be conformal and enlarged by utilizing serial sonications at multiple sites. This would allow more precise targeting of irregularly shaped and irregularly sized targets in the brain parenchyma. The translational process for PING would necessarily involve testing for the safety of systemic administration of QA in non-human primates, and ultimately in humans. Kynurenine metabolites are typically cleared from the blood through the kidneys and eliminated via urination[20,21]; however, the fate of injected QA has not been assessed in this model. Nonetheless, it is important to note in this context that high levels of systemically administered QA are well tolerated in rodents. Importantly, the use of PING would not preclude Standard of Care treatment. Were single or multiple PING treatments to prove ineffective in a given patient, then other procedures, such as resective or ablative surgery, would remain viable options. It is also important to recognize that the clinical environment into which PING would emerge is ideally poised for translation and implementation[22–26]. Structural and functional imaging modalities are rapidly becoming more refined, which will better allow for the identification of appropriate target(s) for precise interventions. Also, there is no need for the design, construction, or approval of a new medical device for PING. MR-guided, high-intensity FUS is already an established and approved modality for multiple indications, including neurological applications. And, as

mentioned earlier, recent years have witnessed a proliferation of sites at which FUS is already in clinical use. Together, these advantages suggest a promising course of development for PING for multiple applications.

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DISCLOSURES:

The authors have nothing to disclose.

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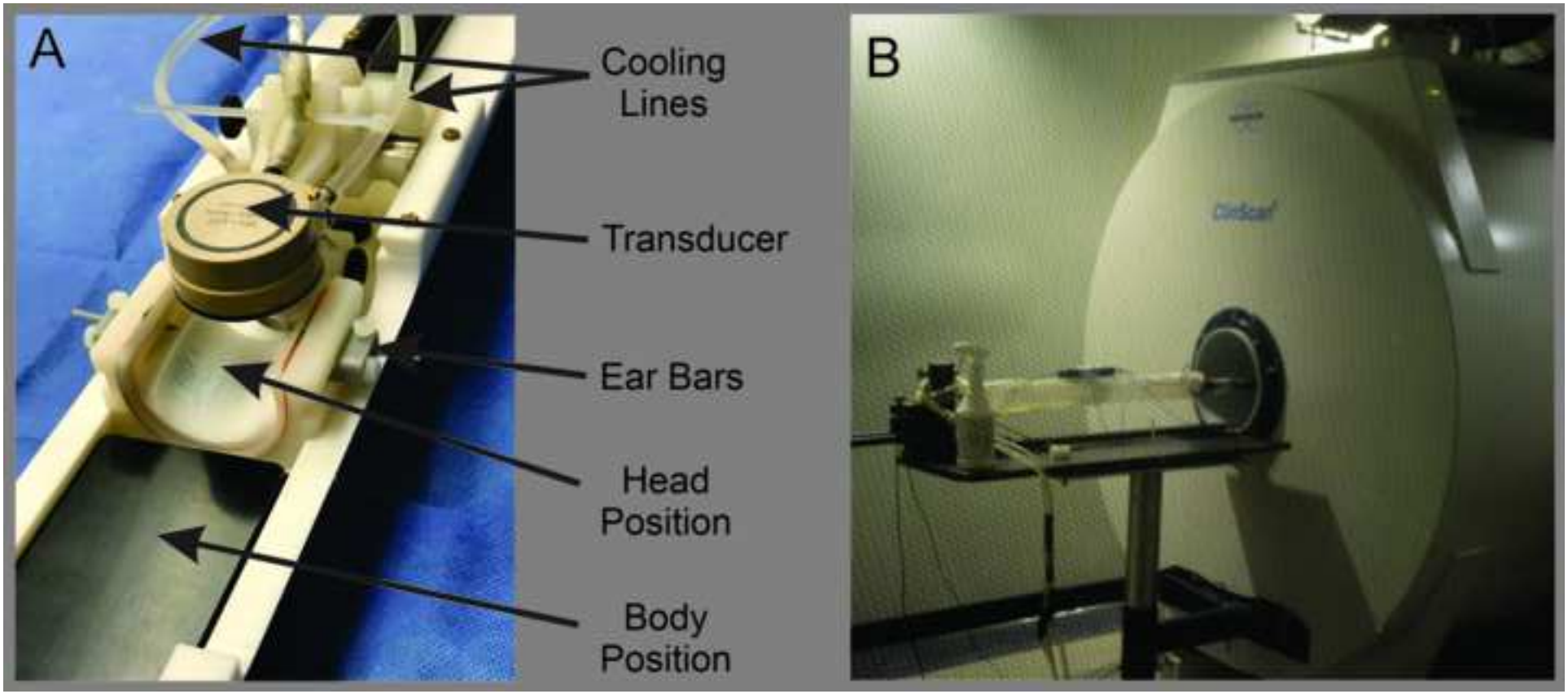

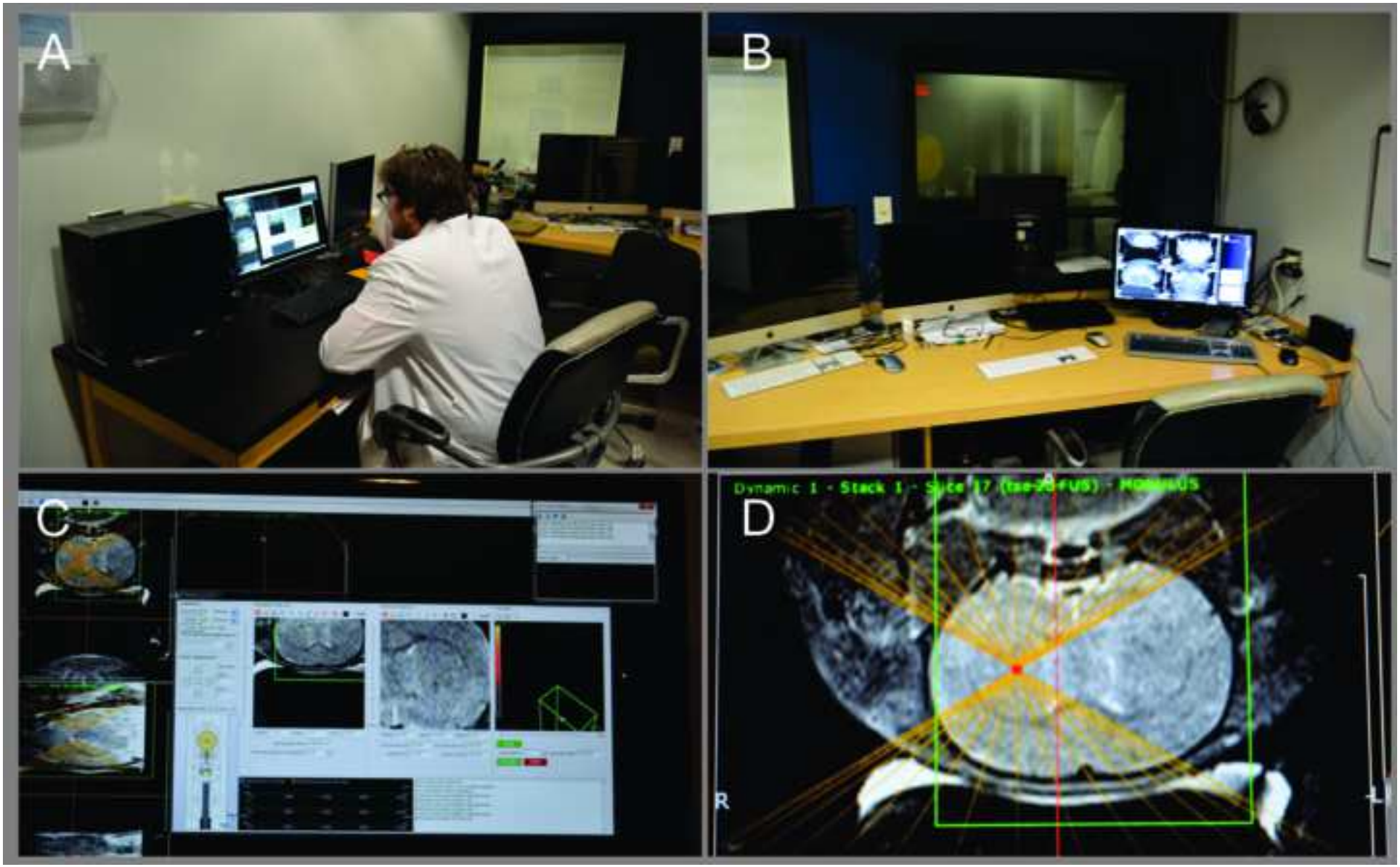

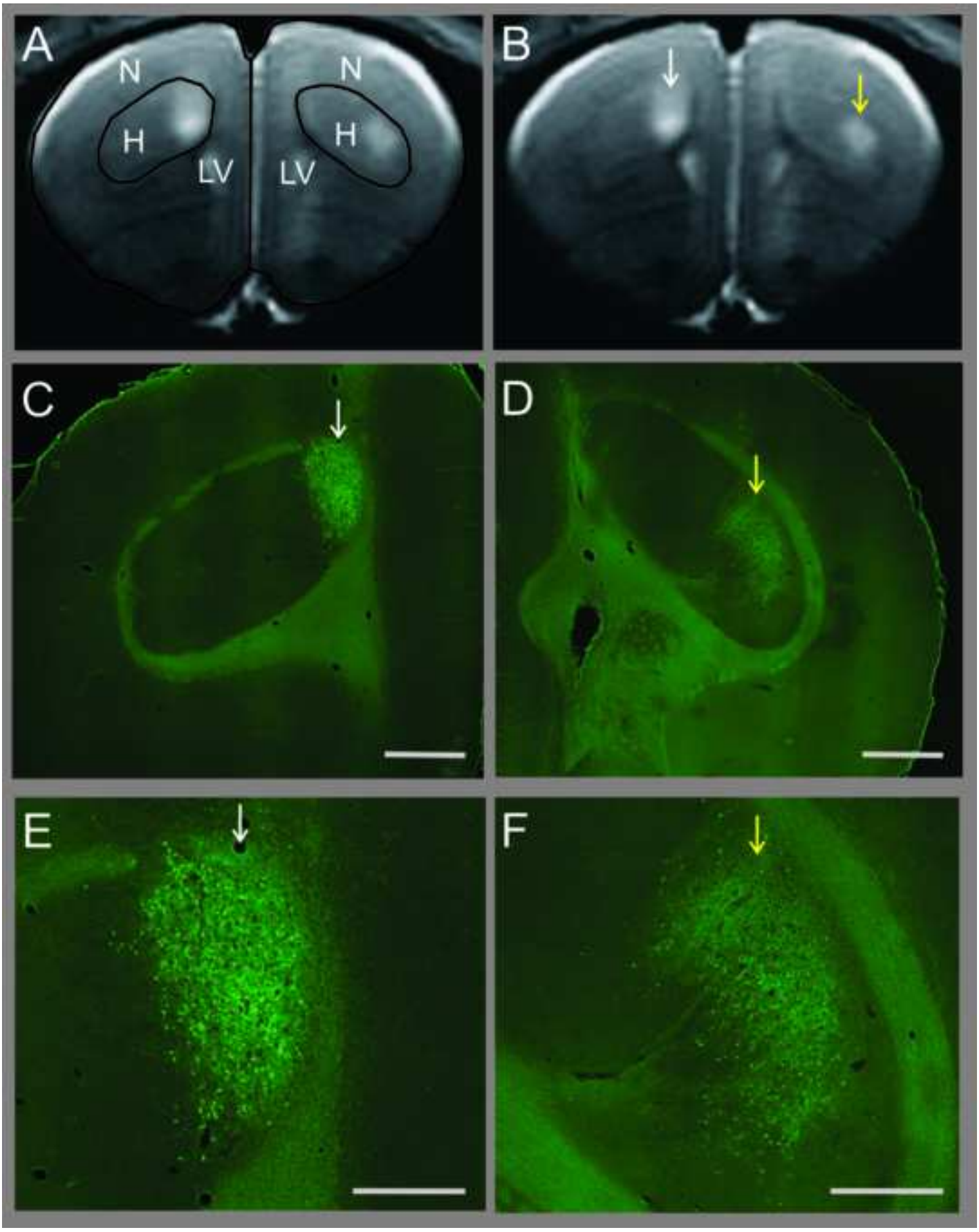

Table of Materials

Name of Material/ Equipment	Company	Catalog Number	Comments/Description
7T-ClinScan MRI System	Bruker Biospin, Ettinglen, Germany		MR Image Acquisition
Acoustic Gel	Litho CLEAR	11-601	High Viscosity Accoustic Transmission Gel
DPX Mounting Medium	Electron Microscopy Sciences	13512	Resin Based Cover Glass Mountant
Fluoro-Jade B	EDM Millipore	AG310	High Affinity Stain For Degenerating Neurons
Fluovac anesthetic adsorber	Harvard Apparatus	34-0388	Organic Anaesthesia Scavenger
FUS System	Image Guided Therapy, Pessac, France	LabFUS	MR Compatible Small Animal Focused Ultrasound System
Gadodiamide	GE Healthcare AS, Oslo, Norway	Omniscan	MR Contrast Agent
Heparin	SAGENT	NDC2502140010	Anti-Coagulant
Hypodermic needle 30G x 1/2	Becton-Dickinson	26027	Tail Vein Catheterization
Insulin syringe 28G1/2 (1ml)	EXEL	26027	Administration of Injectables to Tail Vein Catheter
Isofluorane atomizer	SurgiVet	VCT302	Anaesthesia Administration
Isoflurane	Henry Schein	NDC1169567762	Anaesthesia
KMnO4	Sigma	223468	Reagent Used in Fluoro-Jade B Staining
Microbubbles	Produced internally: A. Klibanov	305106	Blood Brain Barrier Disrupting Agent
Microbubbles (commercial source)	Lantheus Medical Imaging, North Billerica, MA	Definity microbubbles	Blood Brain Barrier Disrupting Agent
Monitoring & Gating System	Small Animal Instruments	Model 1030	Respiration Monitoring
Multisizer 3 Coulter counter	Beckman-Coulter, Hialeah, FL	Multisizer 3	Used to Determine Average Size of Microbubbles
Optixcare EYE LUBE	CLC MEDICA, Ontario, Canada	11611	Corneal Protectant-Eye Lube
PE10 tubing	Becton-Dickinson	427401	Tail Vein Catheter Component
Quinolinic Acid	Santa Cruz Biotechnology, Dallas, TX	CAS 89-00-9	Neurotoxin
Sprague-Dawley Rats	Taconic Biosciences	SD-M	Rat Model
Syringe Pump	Carnegie Medicin	CMA 100	Controlled Delivery of Quinolinic Acid
Thermoguide Software	Image Guided Therapy, Pessac, France	Thermoguide	Drives Lab FUS System
Tish Rats	In-house colony		Rat Model
Veet depilatory cream	Reckitt Benckiser		Removal of Scalp Hair

Nam Nguyen

Manager of Review, JoVE

RE: Rebuttal Letter for JoVE61271

August 20, 2020

Dear Nam,

Thank you again for your help during the revision of this submission. (Targeted neuronal injury for the non-invasive disconnection of brain circuitry," (JoVE61271).

The changes to the video and manuscript are detailed below. The Editorial comments are in black font, and the revisions made are described in blue font.

We believe that the reviews and subsequent revisions have improved the video and manuscript substantially.

Sincerely,

Kevin

Kevin S. Lee, Ph.D.
Harrison Foundation Professor, Department of Neuroscience
Professor, Department of Neurosurgery
School of Medicine
University of Virginia

The following contains the editorial comments in black font, with our revisions in blue font.

1. Please increase the homogeneity between the video and the written manuscript. Ideally, all figures in the video would appear in the written manuscript and vice versa. The video and the written manuscript should be reflections of each other.

2. Furthermore, please revise the narration to be more homogenous with the written manuscript. Ideally, the narration is a word for word reading of the written protocol.

Response to Comments # 1 and 2: The manuscript has been revised to improve the homogeneity between the video and manuscript. In particular, two new figures have been added (Figures 1 and 2) that correspond with clips, images, and voice overs presented in the video. Also, parts of the video have been removed as suggested (i.e. anesthesia segment) or edited (e.g. fine placement in sled segment).

3. 08:51 "ClinScan magnet" is mentioned several times. Please use a more generic term throughout.

The ClinScan magnet is now referred to as the 7T magnet in the voice over.

4. On-Screen Text & Graphics

• In the main title card, in the affiliations listing, consider breaking up the individual institutions into separate lines. There is an extra space between "Image" and "Guided Therapy"

The affiliations listing have been changed to present the individual institutions on separate lines.

• For main and chapter title cards, please center (vertically and horizontally) the text and background stripe the text appears on. For the main title card, please fill the frame with the dark gray (leave no black bars on the edges.)

The background has been filled in with the dark grey color for the title card. And, the text has been centered vertically and horizontally.

• Please speed up or eliminate the "type-on" effect, or convert it to a simple fade-on. It is taking too long for the information to come on-screen. This is particularly evident during the title card's transition 00:00-00:13.

The type-on effect has been removed where it was taking too long, and replaced with either a more rapid fade-in or direct appearance.

• Please ensure that graphics and images are scaled to fill as much of the video frame as possible without losing key information.

Graphics and videos have been re-scaled.

5. JoVE Animal Use Standards

• 02:03-02:43 Please eliminate the anesthesia segment here. Describing an anesthesia regimine is okay, but depicting one is not okay for our videos. You can pick it up from where the animal is placed on a heating pad. Depiction of maintence anesthesia during the protocol is okay.

The induction anesthesia segment has been removed, and the video now picks up from the point that the animal is placed on the heating pad for maintenance anesthesia, etc. This also streamlines the video by reducing its duration by ~ 40 seconds.

6. Edit Pacing & Narration

• 05:06-05:55 Showing the complete fine placement of the mouse in the sled is unnecessary. You can eliminate most of this segment, as it is a fairly common procedure for protocols like this one.

The segment showing the fine placement of the animal in the sled, has been edited and reduced in duration.

• 06:26-07:55 This segment is one long shot, consider chopping it up and using dissolves (or fades) to move time along a little more quickly. There are many "dead" or "in-between" spots that can be lifted out of the video that will shorten the video and make it more concise and better-paced.

The segments demonstrating the placement of the animal in the sled has been edited to divide it into segments separated by fade-outs and fade-ins. This streamlined the presentation of the sled placement from a duration of 3:42 to 2:31.

• Narration audio note: Be sure to go through and trim the starts and ends of the narration so extra noise (room noise, breaths, furniture moving around) from the recordings is not played. Prime example is heard @08:52-08:55 with the chair noise.

Audio has been reviewed. Where extraneous sounds were heard, the voice over tracks have either been edited or replaced.

As per, the request in the previous review, scale bars have been added to Figure 3 (formerly Figure 1) in the manuscript.