Video Article

Circumscribed Capsular Infarct Modeling Using a Photothrombotic Technique

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Abstract

Recent increase in the prevalence rate of white matter stroke demands specific research in the field. However, the lack of a pertinent animal model for white matter stroke has hampered research investigations. Here, we describe a novel method for creating a circumscribed capsular infarct that minimizes damage to neighboring gray matter structures. We used pre-surgery neural tracing with adeno-associated virus-green fluorescent protein (AAV-GFP) to identify somatotopic organization of the forelimb area within the internal capsule. The adjustment of light intensity based on different optical properties of gray and white matter contributes to selective destruction of white matter with relative preservation of gray matter. Accurate positioning of optical-neural interface enables destruction of entire forelimb area in the internal capsule, which leads to a marked and persistent motor deficit. Thus, this technique produces highly replicable capsular infarct lesions with a persistent motor deficit. The model will be helpful not only to study white matter stroke (WMS) at the behavioral, circuit, and cellular levels, but also to assess its usefulness for development of new therapeutic and rehabilitative interventions.

Video Link

The video component of this article can be found at https://www.jove.com/video/53281/

Introduction

Until recently, the "gray matter stroke (GMS) models" have been exclusively used to understand the pathophysiology of stroke and to guide the development of new treatments. However, there has been an increasing prevalence of stroke that affects subcortical white matter in elderly individuals, which constitutes 15 - 25% of all strokes[1,2]. Numerous studies have been conducted regarding stroke using GMS models, whereas there are few studies that have used white matter stroke (WMS) models. White matter in rodents is substantially less than the white matter in humans or primates. Consequently, it is more difficult to selectively access and destroy the target regions in the white matter[3]. Additionally, no efficient tools have been developed to date to selectively destroy the planned extent of targeted white matter. Therefore, there has been lack of appropriate models for study of white matter strokes.

Animal stroke models are often used to monitor the progress of motor recovery for development of new rehabilitative and therapeutic methods. It is ideal to utilize an animal model that exhibits a long-term neurological deficit concordant with the anatomical alterations demonstrated in human stroke[4,5]. In this regard, rapid recovery of the motor deficit and wide involvement of the brain following infarct lesioning may not be realistic in the pursuit of stroke research. Previous capsular infarct models have been made by the occlusion of the internal carotid or anterior choroidal arteries and diffusion of endothelin-1 (ET-1) into the internal capsule[6-9]. Nonetheless, artery occlusion requires careful dissection of arteries, but it produces a wide area of infarct lesion, including the internal capsule, without persistent behavioral deficits. Moreover, ET-1 was not diffused to completely destroy the posterior limb of the internal capsule, and hence less marked or persisting behavioral deficit.

A photothrombotic infarction model has been widely used to generate various types of infarct lesions in the cortex and subcortical structures[10]. The technique include intravenous administration followed by focal illumination, which leads to platelet aggregation in the small vessels and generation of infarct lesions[10]. Photothrombotic technique has been extensively used to create GMS lesions, whereas it has rarely been used to generate WMS lesions[5,11]. For this technique, a combination of Rose Bengal dye and light irradiation has been demonstrated to be useful in destruction of the target structure, causing corresponding functional deficits. The key element of the photothrombotic technique is light irradiation, because it determines the size of infarct lesions. Light irradiation results in different effects on gray matter and white matter, because the scattering of light is more than 4 times higher in white matter compared with gray matter[12]; Accordingly, if the light intensity has a sufficiently low irradiance (<1,140 mW/mm^2), one can limit the extension to which photothrombotic lesion affect the extent to the white matter (*i.e.*, internal capsule). For example, light of higher energy can induce infarcts in both gray and white matter, yet lower energy light may induce photothrombosis only in white matter. Furthermore, the penetration of light energy was very limited. Approximately 99% of light energy was lost beyond 1 mm from the source of light[13]. Therefore, it is expected that accurately targeted, lower energy light induces photothrombosis only in the white matter with a minimal encroachment of the neighboring gray matter.

Here, we describe a novel method to create infarct lesions in the forelimb area of the internal capsule in rodents. We describe the method of identification of the forelimb area in the internal capsule, the technology of light irradiation, including the adjustment and delivery of light, and the generation of an infarct lesion. We also describe behavioral testing used to evaluate the completeness of the capsular modeling.

Protocol

All procedures were conducted according to the institutional guidelines of Gwangju Institute of Science and Technology (GIST), and all procedures were approved by the Institutional Animal Care and Use Committee at GIST.

1. Pre-lesioning Steps

1. **Identification of the Forelimb Area in the Internal Capsule using AAV-GFP**
    1. House and handle Sprague Dawley rats (~400 g, 11 - 13 weeks) in compliance with institutional and national guidelines.
    2. Sterilize all surgical tools and electrodes using an appropriate sterilizer (Steam or Plasma sterilizer). Use steam sterilizer at 121 °C as setting of 30 min for sterilization and 30 min for dry.
    3. Anesthetize the animal with a mixture of ketamine hydrochloride (100 mg/kg) and xylazine (7 mg/kg) via an intramuscular injection. Check the depth of anesthesia by paw pinching. Maintain the body temperature at 37.5 ± 0.5 °C via a heating pad under the body of the animal.
    4. Place the animal in a stereotactic frame using an ear bar and mouth holder.
    5. Clean and disinfect the surgical site with 70% alcohol and povidone iodine solution. Infiltrate 2% lidocaine hydrochloride under the scalp in the intended skull incision area to reduce the intraoperative pain.
    6. Apply vet ophthalmic ointment to prevent drying of the eyes. Place a sterile drape over the animal to the operative sites. Maintain all procedures in sterile conditions.
    7. Perform a midline skull incision of 2 cm using a scalpel and retract the skin bilaterally with wire retractors. Dry the skull with cotton swabs and 30% hydrogen peroxide.
    8. Make a hole using a hand piece drill over the forelimb area of motor cortex (AP: +2.5 from bregma, ML: ±2.5 from the midline) and clear the tract with micro-currette for virus-injection.
    9. Thaw the AAV-GFP (2 x 10^{12} virus molecules/ml) on ice and load 1 µl of the virus in a 10 µl syringe. Place the syringe on the stereotactic frame.
    10. Move the needle to the pre-made hole and lower the needle 1 mm deep into the dura.
    11. Inject the virus slowly (0.1 µl/min) using a high precision micropump and leave the needle in place for an additional 10 min to allow the virus to diffuse out.
    12. After cleaning the operative site with saline irrigation, secure the wound with 3-0 nylon suture; release the rat from the stereotactic frame and transfer it to a recovery chamber. Administer ketoprofen (2 mg/kg) via an intramuscular injection for postoperative pain control.
    13. Maintain the body temperature (37 °C) with heating pad and administer second generation cephems-class antibiotics (0.1%, 1 ml) via an intramuscular injection and 2% lidocaine hydrochloride via subcutaneous injection as necessary. Do not leave an animal unattended until it has regained sufficient consciousness to maintain sternal recumbency. Single house the animal until full recovery.
    14. Following 2 - 3 week recovery, deeply anesthetize the rat with an overdose of ketamine hydrochloride (300 mg/kg) via an intramuscular injection in the hood. Confirm the death of animal by lack of toe pinching response, pulse, and breathing. Place the rat supine in the hood.
    15. Open the abdominal cavity via a 'Y'-shaped incision to open the chest cavity. Tightly clamp the descending aorta with a hemostat and rupture the right atrium of the rat heart for blood drainage. Initiate perfusion into the left ventricle of the heart with cold 1% paraformaldehyde for 5 min (10 ml/min) followed by 4% paraformaldehyde for 30 min (10 ml/min).
    16. Remove the rat head from carcass using a pair of scissors. Make a midline incision from the neck to the nose and remove the neck muscles using scissor or rongeur so that skull is exposed. Gently dissect the skull bones and duras out from the brain.
    17. Extract the brain and place the rat brain in a 50 ml conical tube filled with 4% paraformaldehyde overnight. The next day, wash the brain with 1x PBS 3 times and place it in a 30% sucrose solution.
    18. After the brain completely sinks to the bottom of the 30% sucrose solution, place the brain in the cryomold with OCT compound at -20 °C in cryotome. Slice the brain in coronal plane at a thickness of 40 µm and an interval of 200 µm.
    19. Perform GFP immunohistochemistry staining using the slide method[14]. Apply primary antibody (1:200 of Anti-Green Fluorescent Protein, Rabbit IgG fraction) to brain slices overnight at 4 °C. On 2nd day, wash with 1% Phosphate Buffered Saline with Tween-20 (PBST) solution 3 times and apply the secondary antibody (1:500 of Goat Anti-Rabbit IgG (H + L)) for 1 hr. Rinse the slide with 1% PBST 3 times. Place the cover glass on the brain slice.
    20. Using a fluorescent microscope (excitation wavelength 470 nm, emission wavelength 525 nm, magnification 5X), observe the AAV-GFP transduced axons in the internal capsule. Compare the locations of transduced axons with the Rat Brain Atlas[15] to determine the stereotactic coordinates of the transduced axons

2. **Pre-lesioning Adjustment of the Light Intensity Appropriate for Capsular Infarct Modeling**
    1. **Construction of the Optical Neural Interface**
        1. Cut an appropriate length (4 cm) of a 27 gauge spinal needle with a stylet inside using a cutting drill.
        NOTE: Cutting may compress and crush the spinal needle tip; remove the stylet and polish the spinal needle tip to remove the crushed portion of the spinal needle and maintain the inner caliber of the spinal needle.
        2. Strip an appropriate length (10 cm) of the jacket of the optical fiber (125 µm with a 62.5 µm core) of one side patch cord.
        3. Insert the unjacked optical fiber into the metal tube (external diameter: 3.8 mm, internal diameter: 3.3 mm and length: 17 mm), which is then clamped around the fiber. The metal tube is helpful to fill the space between the optical fiber and the hub of the spinal needle. Clamp the lower 1/2 of the metal tube with a presser twice.

4. Apply the heat-curable epoxy on the optical fiber and insert the optical fiber into the spinal needle. Apply an additional epoxy to the empty space in the hub. Cure the epoxy for 20 min at 100 °C for stable fixation.
5. Cleave the optical fiber that protrudes out of the spinal needle and polish the optical fiber at the tip of the spinal needle using diamond lapping (polishing) sheets.
6. Connect the FC/PC connector part of patch cord to the coupler of green laser system and measure the light intensity from the tip of the optical fiber using digital optical power and energy meter.

2. Photothrombotic Infarct Lesioning in the Internal Capsule

1. Sterilize all surgical tools and electrodes using an appropriate sterilizer (Steam or Plasma sterilizer). Use steam sterilizer at 121 °C as setting of 30 min for sterilization and 30 min for dry.
2. Anesthetize the animal (~400 g, 11 - 13 weeks) with a mixture of ketamine hydrochloride (100 mg/kg) and xylazine (7 mg/kg) via an intramuscular injection. Check the depth of anesthesia by paw pinching. Maintain the body temperature at 37.5 ± 0.5 °C via a heating pad under the body of the animal.
3. Place the animal in a stereotactic frame using an ear bar and mouth holder.
4. Clean and disinfect the surgical site with 70% alcohol and povidone iodine solution. Infiltrate 2% lidocaine hydrochloride under the scalp in the intended skull incision area to reduce the intraoperative pain. Apply vet ophthalmic ointment to prevent drying of the eyes.
5. Apply a sterile drape over the animal and expose the operative sites. Maintain all procedures in sterile conditions.
6. Perform a midline skull incision of 2 cm and retract the skin bilaterally with wire retractors. Dry the skull with cotton swaps and hydrogen peroxide.
7. Adjust the height of the nose clamp until the bregma and lambda are aligned at the same level. CRITICAL STEP: This alignment is critical to correctly approach a deeper structure, such as in performing an infarct lesion in the internal capsule in the main experiment.
8. Make a hole (diameter: 2 mm; AP: -2.04 from bregma; ML: ±3.0 from the midline) using a drill to induce photothrombosis.
9. Polish and clean the optical fiber tip of the optical interface. Fix the ONI to the stereotaxic frame without bending. Check the tip of the ONI and wipe it out clearly before and after the insertion of the optical interface.
10. Measure the laser intensity from the tip of the optical fiber prior to the insertion of the optical interface to the target site of the rat brain. Adjust the laser intensity to 3.5 mW, as confirmed by pre-surgery steps, at the tip of the optical fiber.
11. Insert the ONI into the target area of the internal capsule (-7.8 mm confirmed from pre-surgery step) through the drill hole.
12. Maintain the body temperature at 37.5 ± 0.5 °C during photothrombosis. A lower body temperature may not produce the expected extent of infarction. Inject Rose Bengal (2 ml/kg) through the tail vein.
13. Turn on the 532 nm green laser for 90 sec 1 min after the Rose Bengal injection. After irradiation, gently remove the ONI from the brain. After cleaning the operative site, secure the wound secure the wound with 3-0 nylon suture; release the rat from the stereotactic frame and transfer it to a recovery chamber.
14. For sham-operated group (SOG), perform an identical lesion-making procedure, except for injection of saline (0.2 ml/100 g) instead of Rose-Bengal.
15. Maintain the body temperature (37 °C) with heating pad after surgery and administer antibiotics (second generation cephalosporin, 0.1%, 1 ml) via an intramuscular injection. Do not leave the animal unattended until it has regained sufficient consciousness to maintain sternal recumbency. Do not return post-surgical animals to the cage occupied by other animals until fully recovered.
NOTE: Preliminary experiment was performed in the same procedures to find the optimal light intensity from 1 mW to 5 mW and the procedure may be required to acquire the satisfactory extent of lesion in different condition.

3. Evaluation of Capsular Infarct Lesioning

1. **Behavioral Testing and Animal Grouping**
   1. Perform single pellet reaching tasks as described by Whishaw *et al.*[14] to evaluate the motor deficit of the forelimb every day for 1 week after the stroke modeling. Perform a single pellet reaching task (SPRT) in food-restricted animals (90% of control body weight) using clear Plexiglas (30 x 15 x 35 cm height) with a 1 cm wide slit and a food shelf in the front of the middle of the front wall.
   2. Place a pellet on the food shelf obliquely contralateral to the preferred forelimb. Administer 20 pellets per session for 3 weeks.
   NOTE: A successful number of SPRTs is defined as a reach in which the animal grasps a food pellet and puts it into the mouth without dropping it.
   3. Calculate the score as a percentage of successful reaches, which is defined by the following formula:
   $$\frac{\text{Number of successful reaches X 100}}{20}$$
   NOTE: We divide animals into 3 groups: the sham-operated group (SOG), moderate recovery group (MRG), and poor recovery group (PRG). If a post-stroke SPRT score >50%, we classify the rats as the MRG, which indicates the presence of a substantial lesion, but not complete destruction of the target. If the post-stroke SPRT score is <50% compared with the pre-stroke SPRT score, we classify the group as PRG, which indicates complete lesioning in the target.

2. **Neurohistological Confirmation of Infarct Lesioning**
   1. Perform cardiac perfusion with 4% paraformaldehyde as previously described. After the brain completely sinks in the 30% sucrose solution, perform coronal sectioning at a thickness of 10 µm and an interval of 200 µm using a microtome or cryotome[4].
   2. Stain with H&E, Nissl, Luxol fast blue-PAS, Neurofilament protein-L or Glial fibrillary acid protein staining and observe the histological findings to determine the optimal light intensity that can cover the entire breadth of the internal capsule in the target area to observe staining[4,17].
   3. Using ImageJ software, measure the volume of the photothrombotic infarct area of the internal capsule on the brain slides.

1. To measure the volume of infarct area, launch the 'ImageJ' software. To open the files to be stacked, select 'Image to Stacks' ('Image' → 'Stacks' → 'Image to Stacks'). Edit file name and select 'Set the Scale' ('Analyze' → 'Set the Scale') to edit scale.
2. In 'Plugins', select 'Measure Stacks' to calculate volume or area of images. Insert the distance interval of 2 images into 'Slice Spacing'. Make a drawing of the ROI (Region Of Interest) of all images and click 'Measure'.
   NOTE: The software 'ImageJ' automatically calculates the area and volume of each image and total volume of them.

Representative Results

The method presented here is intended to create a circumscribed capsular infarct with a persistent motor deficit. Therefore, it is critical to correctly determine the target within the internal capsule in the pre-surgery step. The somatotopic mapping of pyramidal fibers in the internal capsule has not been settled to date. To correctly identify the target within the internal capsule, the forelimb area must be delineated. An injection of AAV-GFP into the forelimb area of the motor cortex can trace the axons of the pyramidal fibers in the internal capsule **(Figure 1).** Other neural tracers, such as biotinylated dextran amine (BDA), may be used for the same purpose. The stereotactic coordinates of the target within the internal capsule can be elucidated by tracing the axonal projections that originate from the forelimb area of the motor cortex to the internal capsule.

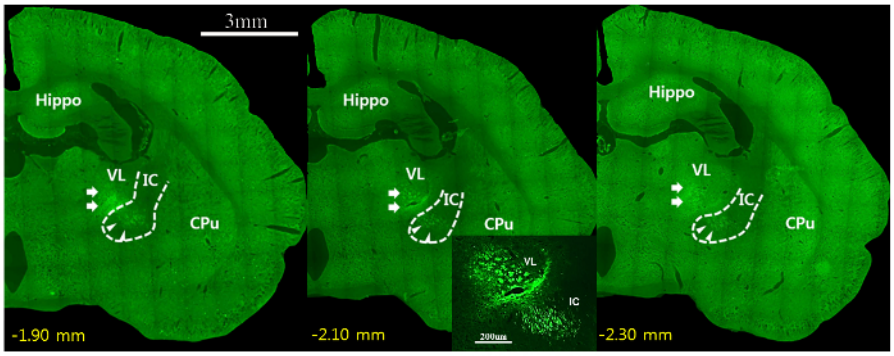

Figure 1. Identification of the Forelimb Area in the Internal Capsule 2 Weeks after the Injection of AAV-GFP. GFP-transduced axonal fibers that originated from the forelimb area of the motor cortex are shown in the ventrolateral nucleus of the thalamus (arrows) and the caudal portion of the internal capsule (arrowhead). The dotted line indicates the contour of the internal capsule, and the numbers refer to the distances from bregma. Hippo, hippocampus; Cpu, caudate putamen; VL, ventrolateral nucleus; IC, internal capsule. Please click here to view a larger version of this figure.

The optimal light intensity may be different depending on the strain and body weight of the animal and the types and diameters of optical fibers. Therefore, the optimal light intensity should be determined separately prior to the main infarct lesioning experiment. Using the photothrombotic procedure, the light intensity can be gradually increased until the extent of the lesion covers the entire breadth of the internal capsule without destroying the neighboring gray matter structures **(Figure 2).** The optimal light intensity can be verified by comparing the histological extent of the infarct lesion and locations.

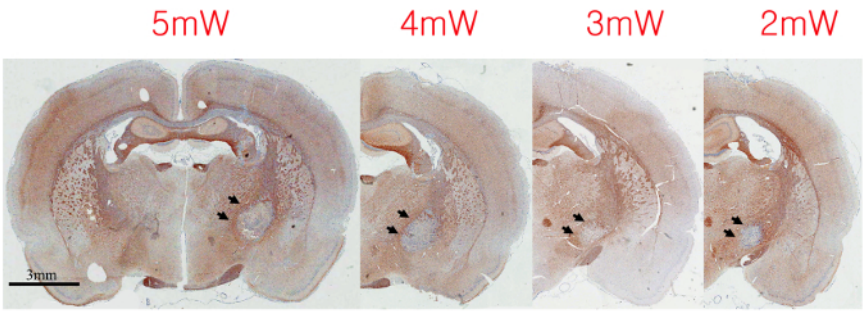

Figure 2. Extent of the Infarct Lesions Across Varying Intensity of Laser Light from 2 mW to 5 mW 2 Weeks after Photothrombotic Lesioning. The optimal light intensity is considered to be between 3 mW and 4 mW in this experimental setting. Arrows indicate the infarct lesion. Please click here to view a larger version of this figure.

We prefer to use the ONI in which the optical fiber is contained in a thin metal tube (spinal needle). The optical fiber may produce minimal light scattering from the side of the fiber, which is likely to generate additional neural damage along the optical fiber tract. Encasement of the optical fiber is also advantageous to prevent the bending of the optical fiber in deeper targets, as well as to attach the ONI to the stereotactic frame **(Figure 3).**

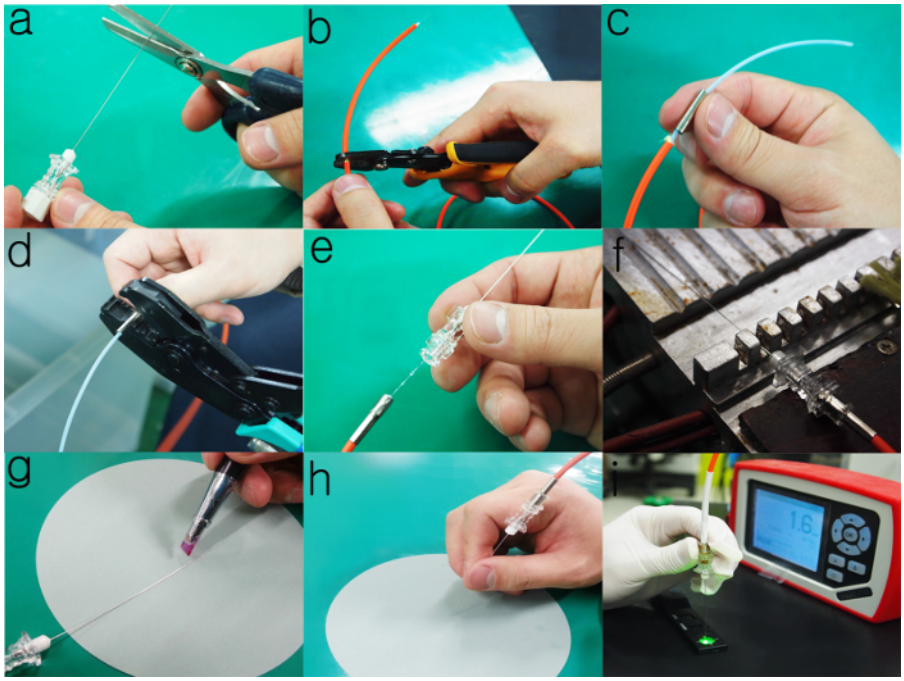

Figure 3. Construction of the Optical-neural Interface (ONI). (a) Cutting of the spinal needle. (b) Stripping of the optical fiber. (c & d) The anchoring metal tube is inserted over the stripped optical fiber and cramped to secure the optical fiber to the hub of the spinal needle. (e) The epoxy-added optical fiber is inserted into the spinal needle. (f) The epoxy is hardened at 100 °C for 20 min. (g) The optical fiber is cleaved at the tip of the spinal needle. (h) The optical fiber is polished. (i) The light intensity is measured from the tip of the optical fiber. Please click here to view a larger version of this figure.

The photothrombotic procedure will produce reproducible lesions and locations with ~70% success in motor impairment. Typical capsular infarct lesion encompasses the ventrodorsal dimension of capsular fibers **(Figure 4A).** Furthermore, the infarct lesion extends along the anteroposterior axis of the internal capsule because of increased light scattering inside the capsular fiber **(Figure 4B).** The optical tract located below the internal capsule consists of white matter fibers; thus, it is often damaged by irradiation of an increased light intensity. Serial sections and staining are required to confirm the whole volume and extent of infarction. The infarct volume was 0.63 ± 0.37 mm^3. To evaluate the destruction of the capsular fiber, neurofilament and Luxol fast blue-PAS stains are helpful.

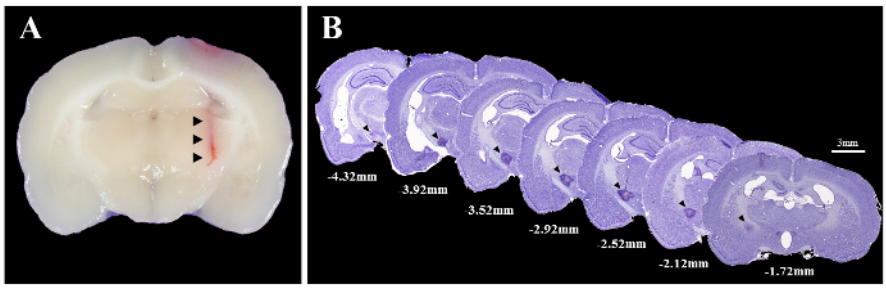

Figure 4. Microscopic Appearance of Capsular Infarct 3 Weeks after Photothrombosis. Microscopic appearance of capsular infarct 3 weeks after photothrombosis. A) Brain slice of coronal section in the rat brain. Arrowhead indicate the tract of needle containing optical fiber in the thalamus and up to internal capsule. B) Serial Nissl staining of the coronal brain slices showing the whole extent of infarct lesion in the internal capsule. Arrowheads indicate the infarct lesion. Please click here to view a larger version of this figure.

The success of the modeling can be evaluated by behavioral testing using a single pellet reaching task. The behavioral performance after 1 week following the infarct lesioning is a good guide to confirm accurate lesioning, which accompanies the persistent and marked impairment of SPRT despite the daily single pellet reaching training **(Figure 5).** Once the motor deficit is shown in the PRG, the neurological deficit persisted during 3 month period of observation. Sham-operated group did not show the significant decrease of SPRT performance after operation.

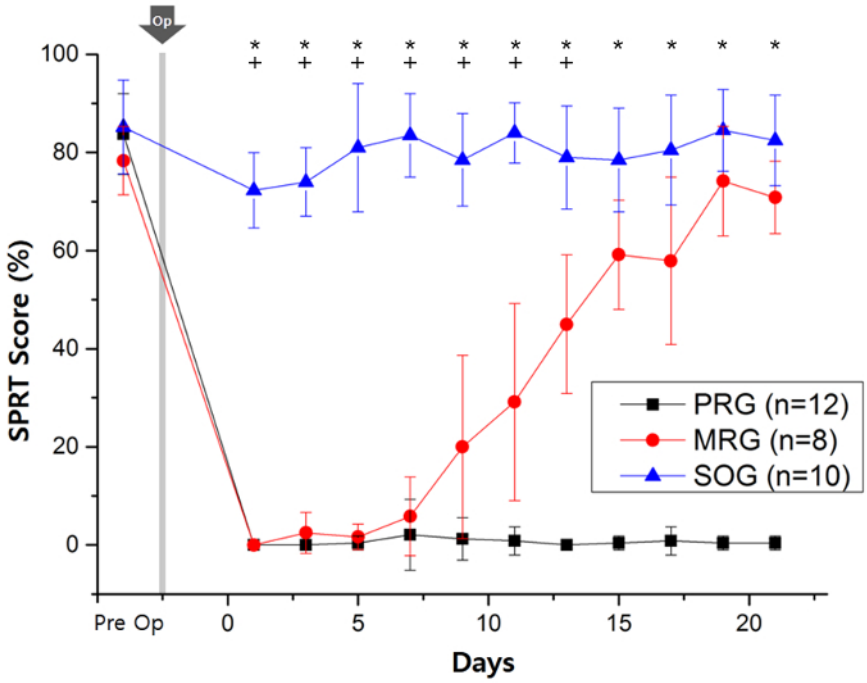

Figure 5. Changes in Single Pellet Reaching Scores after Capsular Infart[4,20]. The experimental groups (PRG and MRG) exhibited significantly decreased scores immediately after the infarct lesioning compared with the sham-operated group (SOG). The MRG exhibits a gradual recovery of SPRT performances, whereas the PRG exhibits persistent motor impairment over time. Op, photothrombotic infarct lesioning; PRG, poor recovery group; MRG, moderate recovery group. Statistical significance was determined using repeated measure analysis of variances. +SOG versus MRG; *SOG versus PRG. Data are means ± sem. Please click here to view a larger version of this figure.

Discussion

The capsular infarct model presented here demonstrates a targeted lesion with marked and persistent motor impairment in forelimb function. Previous models of subcortical capsular stroke have demonstrated an insufficient degree of motor impairment and a rapid recovery process[6,8,9]. In this sense, this model resembles the clinical capsular infarct cases which exhibit long-term functional impairment.

The most critical steps in the development of a circumscribed capsular infarct model are: 1) to correctly identify the somatotopic representation of the body part intended to disable the function within the internal capsule; 2) to identify the optimal intensity of the green laser, which can destroy the entire breadth of the internal capsule with minimal encroachment of neighboring gray matter structures; and 3) to accurately place the optical fiber in the target structure. Although the presented techniques can induce circumscribed capsular infarct model with a high replication rate (>70%), small differences in the targeting and degree of completeness of destruction covering the entire breadth of the internal capsule may account for different motor deficits.

The corticospinal tract is situated in the anterior half of the posterior limb in the internal capsule in humans despite the controversy of somatotopic organization[15]. By contrast, there has been no equivalent classification or detailed elucidation of the somatotopic organization of the internal capsule in rodents. The lack of knowledge regarding the somatotopic organization often leads to incorrect targets of infarct lesioning within the internal capsule with different motor outcomes among capsular infarct models. However, we identified the GFP-transduced axons in the caudal portion of the internal capsule, which likely represent the path of the forelimb motor fibers. Furthermore, lesioning of this area demonstrated a marked and persistent deficit of the forelimb reaching skill. Therefore, we recommend the caudal portion of the internal capsule for stereotactic lesioning to improve the validity of the capsular infarct model.

Pre-adjustment of light intensity is mandatory to produce a uniform extent of infarct lesion in stroke models because the animal strain, body weight, light source and types of ONI may generate different sizes of infarction. Therefore, preliminary experiments using different light intensities in experimental animals with the same strain and body weight should be conducted until the satisfactory infarct lesion is achieved with minimal light intensity.

Strong light intensity that can destroy the entire breadth of the capsular fiber (anterior-posterior and dorsoventral extent) that corresponds to the forelimb area with minimal damage to the neighboring structures is considered to be the optimal light intensity. The forelimb area of the internal capsule is bounded by the thalamus superiorly and the optic tract inferiorly. Therefore, the depth of ONI insertion should be accurate to destroy the entire extent of the IC in the dorsoventral direction, with simultaneous preservation of the superior and inferior neighboring structures. Inaccurate placement of the ONI results in an incomplete destruction of the IC, which leads to rapid recovery of the motor deficit as a result of the synaptic plasticity of the remaining pyramidal fibers in the internal capsule. In serial histological examinations, the most confounding factor in the induction of a persistent motor deficit was the incorrect positioning of the ONI, which leads to the failure to destroy the entire breadth of the PLIC[4,16]. Therefore, careful attention should be paid to reach the correct target. Recently, Blasi *et al.* reported that lasting pure-motor deficit can

be produced by making an infarct lesion in posterior internal capsule using endothelin-1(ET-1)[17]. However, ET-1 may destroy the neighboring gray matter structure by the diffusion of ET-1.

Behavioral testing is an immediately available benchmark in the laboratory to assess the formation of infarct lesion in the internal capsule. However, evaluation of motor performance one week after infarct lesioning is recommended to divide the animals into moderate and poor recovery groups. Moderate recovery was defined as an increase in the performance score by >50% compared with the score prior to lesioning, whereas poor recovery was defined as recovery of <50%. Among the forelimb motor behavioral tests, the single pellet reaching task is one of the most sensitive tests for both quantitative and qualitative measurements of stroke-induced motor performances[14]. The task quantitatively measures the reaching success while simultaneously providing an analysis of forelimb use, such as grasping and retrieving a food pellet. The qualitative analysis of the reaching movement is also helpful to differentiate the quality of stroke recovery by distinguishing genuine functional recovery or compensation[20]. Here, we briefly described the quantitative measurement of the SPRT; however, qualitative analysis using filming and scoring based on a frame-by-frame analysis is recommended for further detailed analysis.

The techniques presented here need not be confined to the induction of circumscribed capsular infarct modeling. The technique may be applied to the induction of an infarct lesion in other areas of white matter, such as the corpus callosum, anterior commissures and connecting fibers among neural structures. The combination of the tiny ONI and photothrombotic technique based on the optical properties of white matter is likely to destroy the targeted structures with minimal damage to the neighboring structures. For example, lacunar infarctions can be easily produced by targeting the subcortical structures related to motor, cognitive, and memory functions. When the target structure is large, multiple insertions of the ONI and different targeting and angled trajectories may be required to produce the desired extent of lesions.

There are several limitations in this technique. The technique is sufficient to demonstrate the consequence of infarct lesioning in the PLIC and subsequent recovery. However, this model does not reflect the full spectrum of human WMS because photothrombotic destruction of white matter slightly differs from human WMS. Consequently, neurobiological or MRI imaging findings may exhibit different features in the early stage of photothrombotic lesioning. Therefore, this model should be appropriately used to trade off the model's advantages and disadvantages. Technically, not all surgeries can produce the marked and permanent motor deficit in this model because it requires very accurate procedures. Specifically, trained and experienced hands are required to produce the high reproducibility in the generation of this model.

In conclusion, the combined use of a photothrombotic technique, optimization of light intensity, and correct targeting is a useful technique to produce a circumscribed capsular infarct model. This model will be helpful not only to study WMS at the behavioral, circuit, and cellular levels but also to assess the usefulness of new therapeutic and rehabilitative interventions.

Disclosures

The authors have nothing to disclose.

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References

1. Roger, V. L. *et al.* Heart disease and stroke statistics--2012 update: a report from the American Heart Association. *Circulation.* **125**, e2-e220 (2012).
2. Debette, S., & Markus, H. S. The clinical importance of white matter hyperintensities on brain magnetic resonance imaging: systematic review and meta-analysis. *Bmj.* **341**, c3666 (2010).
3. Zhang, K., & Sejnowski, T. A universal scaling law between gray matter and white matter of cerebral cortex. *PNAS.* **97**(10), 5621-5626 (2000).
4. Kim, H. S. *et al.* A rat model of photothrombotic capsular infarct with a marked motor deficit: a behavioral, histologic, and microPET study. *J Cereb Blood Flow Metab.* **34**(4), 683-689 (2014).
5. Kleim, J. A., Boychuk, J. A., & Adkins, D. L. Rat models of upper extremity impairment in stroke. *ILAR J.* **48**(4), 374-384 (2007).
6. Frost, S. B., Barbay, S., Mumert, M. L., Stowe, A. M., & Nudo, R. J. An animal model of capsular infarct: endothelin-1 injections in the rat. *Behav Brain Res.* **169**(2), 206-211 (2006).
7. He, Z. *et al.* Definition of the anterior choroidal artery territory in rats using intraluminal occluding technique. *J Neurol Sci.* **182**(1), 16-28 (2000).
8. Tanaka, Y. *et al.* Experimental model of lacunar infarction in the gyrencephalic brain of the miniature pig: neurological assessment and histological, immunohistochemical, and physiological evaluation of dynamic corticospinal tract deformation. *Stroke.* **39**(1), 205-212 (2008).
9. Shibata, M., Ohtani, R., Ihara, M., & Tomimoto, H. White matter lesions and glial activation in a novel mouse model of chronic cerebral hypoperfusion. *Stroke.* **35** (11), 2598-2603 (2004).
10. Watson, B. D., Dietrich, W. D., Busto, R., Wachtel, M. S., & Ginsberg, M. D. Induction of reproducible brain infarction by photochemically initiated thrombosis. *Ann Neurol.* **17**(5), 497-504 (1985).
11. Kuroiwa, T. *et al.* Development of a rat model of photothrombotic ischemia and infarction within the caudoputamen. *Stroke.* **40**(1), 248-253 (2009).
12. Bashkatov, A. N., Genina, E.A., Tuchin, V.V.,. in *Handbook of biomedical optics.* (ed D.A. Boas, Pitris, C., Ramanujam) 83 CRC Press, Boca Raton, Fl (2011).
13. Yizhar, O., Fenno, L. E., Davidson, T. J., Mogri, M., & Deisseroth, K. Optogenetics in neural systems. *Neuron.* **71**(1), 9-34 (2011).

14. Whishaw, I. Q., Whishaw, P., & Gorny, B. The structure of skilled forelimb reaching in the rat: a movement rating scale. *J Vis Exp.* 8(18), 816 (2008).
15. Jang, S. H. A review of corticospinal tract location at corona radiata and posterior limb of the internal capsule in human brain. *NeuroRehabilitation.* **24**(3), 279-283 (2009).
16. Kim, D. *et al.* Longitudinal changes in resting-state brain activity in a capsular infarct model. *J Cereb Blood Flow Metab.* **35**(1), 11-9 (2014).
17. Blasi, F., Whalen, M. J., & Ayata, C. Lasting pure-motor deficits after focal posterior internal capsule white-matter infarcts in rats. *J Cereb Blood Flow Metab.* **35(6)**, 977-984 (2015).
18. Metz, G. A., Antonow-Schlorke, I., & Witte, O. W. Motor improvements after focal cortical ischemia in adult rats are mediated by compensatory mechanisms. *Behavioural brain research.* **162**(1), 71-82 (2005).

