**SPAN STANDARD OPERATING PROCEDURE**

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| **#1** | **INFORMATION** |

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| **Procedure Title** | | **Rat Middle Cerebral Artery Occlusion** |
| **Originators** | | **SPAN Coordinating Center** |
| **Creation/Revision Date** | | **2/15/21** |
| |  |  | | --- | --- | | **SOP: 40**  **Version No: 1.0**  **Effective Date: 07/05/2021** | **Supersedes**  **Document:**  **Effective Date:** | | | |
| **#2** | **POLICY** | |
| SPAN seeks to reduce sources of experimental variation. Performance and selection bias are reduced if experimenters remain unaware of treatment group assignment. A single, common model for creating focal cerebral ischemia will be used at all sites. | | |
| **#3** | **SCOPE** | |
| This procedure applies to all study sites. | | |
| **#4** | **ROLES AND RESPONSIBILITIES** | |
| Coordinating Center: draft all SOPs  Study Site Principal Investigator: read and distribute all SOPs to relevant study team members | | |
| **#5** | **APPLICABLE REGULATIONS AND GUIDELINES** | |
| ARRIVE guidelines 2019  NINDS RIGOR guidelines | | |
| **#6** | **REFERENCES TO OTHER APPLICABLE SOPS** | |
| SOP3 RapID Ear Tagging  SOP4 Enrollment  SOP5 Intention to Treat  SOP37 Randomization | | |
| **#7** | **ATTACHMENTS AND REFERENCES** | |
| Bosetti F., Koenig J. I et al. 2017. Translational stroke research: Vision and opportunities. Stroke. 48: 2632–2637.  Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. PLoS Biol. 2010;8(6):e1000412. Epub 2010/07/09. doi: 10.1371/journal.pbio.1000412. PMCID: PMC2893951. | | |
| **#8** | **TERMS AND ABBREVIATIONS** | |
| SOP: standard operating procedure  NINDS: National Institutes for Neurological Disorders and Stroke  SPAN: Stroke Preclinical Assessment Network  ARRIVE: Animal Research: Reporting of in Vivo Experiments  ITT: Intention to Treat  MCAo: Middle Cerebral Artery Occlusion  ICA: Internal carotid artery  ECA: External Carotid Artery  CCA: Common Carotid Artery | | |
| **#9** | **TRAINING REQUIREMENTS** | |
| **General Training:**  Site animal handling training  RapID training video  SPAN REDCap database training  MCAo surgical training video and Certification   |  |  | | --- | --- | | **Location Where Records Maintained:** | Site | | | |
| **#10** | **SPECIFIC PROCEDURES** | |
| |  |  | | --- | --- | | **Surgical Preparation Procedure** | **Notes** | | 1. Ensure the following:   The proposed subject has been enrolled into REDCap  ITT form has been submitted  Randomization prescription has been received  Randomization prescription is printed/available to surgeon. | Follow **SPAN SOP 4 Enrollment, SPAN SOP 5 Intention to Treat, SPAN SOP 37 Randomization** | | 1. Scan the ear tag to confirm the correct rat has been selected from cage | Follow **SPAN SOP 3 RapID Ear Tagging** | | 1. Ensure all supplies are sterile and maintain aseptic technique throughout all procedures. |  | | 1. Open Day of Surgery form in REDCap database |  | | 1. Weigh rat and record result in REDCap |  | | 1. Induce anesthesia with 4% isoflurane in an appropriate induction chamber with approved scavenging method. |  | | 1. Record the anesthesia start time on the Surgery Day Form |  | | 1. Once the rat is fully asleep, remove from induction chamber and move to surgical prep area. | **Note:** The surgical prep area is ideally NOT the surgery table. | | 1. Apply ophthalmic ointment in both eyes to prevent corneal drying during anesthesia. |  | | 1. Remove hair with clippers from the ventral neck area and the top of the head. |  | | 1. Scrub with betadine or chlorohexidine followed by alcohol in alternating wipes. |  | | 1. Transfer rat to the pre-warmed surgical station. | Note: Maintaining body heat at 37°C. | | 1. Reduce Isoflurane to 2-2.5% in 70:30 N2O:O2. |  | | 1. Inject bupivacaine subcutaneously along the anticipated ventral neck incision and top of the head incision lines. | Note: DO NOT exceed 5mg/kg total dose bupivacaine. | | 1. Pinch a toe to confirm adequate anesthesia depth. If a response is elicited, isoflurane should be increased by 0.5% and toe pinch repeated after 2 minutes have elapsed. | Note: Confirm adequate anesthesia depth before proceeding. | | **MCAo Procedure** | **Notes** | | 1. Scrub the ventral neck with betadine or chlorohexidine followed by alcohol in alternating wipes. |  | | 1. Place Glad Press ’n Seal on the neck and cut a surgical window. | /Users/karismanagarkatti/Desktop/Screen Shot 2020-03-31 at 2.17.03 PM.pngNote: Available in any grocery store. | |  | A picture containing indoor, bird, penguin, rodent  Description automatically generated  Rodent on station pre-drape  A picture containing text, indoor, cluttered  Description automatically generated  Rodent on station post-drape. In live scenario Press ’n Seal will be up against the skin to minimize obscuring the model. | | 1. Make a midline incision in the neck. |  | | 1. Gently and bluntly dissect the right neck muscles to expose the CCA. | Note: Do not transect muscles | | 1. Place a temporary ligature or fine vessel clamp around the CCA. Avoid manipulating the vagus nerve. | Note: Silk sutures work best for ligating vessels | | 1. Place a distal permanent ligature around the ECA. Include the superior thyroid and occipital artery if possible. |  | | 1. If above step is not possible, ligate or cauterize those arteries separately. |  | | 1. Place a loop of suture around the ICA; do not tighten | Note: This will provide hemostasis as the filament is advanced. | | 1. Place a loop of suture around the proximal ECA as close to the bifurcation as possible; do not tighten. |  | | 1. Make a small arteriotomy in the ECA just distal to the temporary loop of suture but proximal to the permanent ligature. |  | | 1. Insert the Doccal filament or equivalent (Doccol ; See notes for size selection guidance) and guide the filament around (or down the CCA first) and then up the ICA to occlude the middle cerebral artery. Advance until there is resistance (usually around 17 mm from the bifurcation). Note the time of occlusion in the surgery log.     Note: Sutures should be marked at 17mm to facilitate insertion depth | Occluding the pterygopalatine artery with cotton tipped applicator may be necessary to prevent inadvertent insertion and direct the filament up the internal carotid.   |  |  | | --- | --- | | Weight | Size (highlighted in product number) | | Up to 250 grams | 503123 | | 250-280 grams | 503323 | | 280 grams and over | 503523 | | | 1. Tighten the loop of suture around the proximal ECA to secure the filament. |  | | 1. Remove the loop of suture around the ICA. |  | | 1. Remove the temporary ligature or vessel clamp from around the CCA. | Watch for any bleeding before closing the skin. Cauterize or re-ligate as necessary. | | 1. Close skin on the ventral neck. | If animals will remain asleep during the occlusion period, monitor breathing rate and adjust isoflurane level up or down accordingly. | | **For Rats Awake During the Occlusion Period** | **Notes** | | 1. Remove Press ‘n Seal. |  | | 1. Remove from anesthesia and allow to recover in a pre-warmed recovery cage. |  | | 1. Transcribe all details, times, notes, etc. into the REDCap Surgery Day form. | See Experimental protocol for Stage specific details regarding occlusion period and intervention administration time. | | 1. Under **Form Status**, mark the record “**Complete**” when all fields are complete. |  | | 1. Select “**Save & Exit Form**” |  | | | |

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| **#11** | **REVIEWED AND APPROVED BY** |
| *Patrick Lyden, Principal Investigator*  *\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_*  *(Printed Name/Title) (Signature)*  *Jessica Lamb, SPAN Manager*  *\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_*  *(Printed Name/Title) (Signature)*  *\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_*  *(Printed Name/Title) (Signature)*  *\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_*  *(Printed Name/Title) (Signature)*  *\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_*  *(Printed Name/Title) (Signature)*  *\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_*  *(Printed Name/Title) (Signature)*  *\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_*  *(Printed Name/Title) (Signature)*  *\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_*  *(Printed Name/Title) (Signature)* | |