**SPAN STANDARD OPERATING PROCEDURE**

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

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| **Procedure Title** | | | | **Aging Model** |
| **Originators** | | | | **SPAN Coordinating Center** |
| **Creation/Revision Date** | | | | **3/5/21** |
| |  |  | | --- | --- | | **SOP: 53**  **Version No: 1.0**  **Effective Date: TBD** | **Supersedes**  **Document:**  **Effective Date:** | | | | | |
| **#2** | **POLICY** | | | |
| SPAN seeks to reduce variation among study sites. All sites will use the same procedures to induce comorbidities in research models. | | | | |
| **#3** | **SCOPE** | | | |
| This procedure applies to all Sites and the Coordinating Center. | | | | |
| **#4** | | **ROLES AND RESPONSIBILITIES** | | |
| Coordinating Center: draft all SOPs  Study Site Principal Investigator: read and distribute all SOPs to relevant study team members and assure compliance | | | | |
| **#5** | **APPLICABLE REGULATIONS AND GUIDELINES** | | | |
| <https://olaw.nih.gov/sites/default/files/Guide-for-the-Care-and-Use-of-Laboratory-Animals.pdf> | | | | |
| **#6** | **REFERENCES TO OTHER APPLICABLE SOPS** | | | |
| SPAN SOP 2 Animal Acquisition | | | | |
| **#7** | **ATTACHMENTS AND REFERENCES** | | | |
| None | | | | |
| **#8** | **TERMS AND ABBREVIATIONS** | | | |
| SOP: standard operating procedure  NINDS: National Institutes for Neurological Disorders and Stroke  SPAN: Stroke Preclinical Assessment Network  CC: Coordinating Center | | | | |
| **#9** | **TRAINING REQUIREMENTS** | | | |
| **General Training:**  Site animal handling training   |  |  | | --- | --- | | **Location Where Records Maintained:** | Site | | | | | |
| **#10** | | | **SPECIFIC PROCEDURES** | |
| |  |  | | --- | --- | | **Description** | **Notes** | | Animals should be 15-17 months old on Surgery day. This will mean that animal will be 16-18 months at final behavior testing/MRI. |  | | 1. After arrival at site facility, animals must acclimate for at least 4 weeks (preferably longer) prior to surgery day. |  |   The procedure below outlines two methods for acquiring aged animals. The first is in-house aging, the second is ordering through NIA.   |  |  | | --- | --- | | **In House Aging/Vendor Order** | **Notes** | | 1. Order animals from the closest vendor site. | **See SPAN SOP 2 Animal Acquisition** | | 1. Use strain/species specified in Experimental Protocol | **See Experimental Protocol** for Stage specific details for vendor site/strain/species. | | 1. Quantity of animals to be ordered will be determined based on NIA supply. Coordinating Center will communicate with site regarding anticipated numbers. | Note: NIA has promised 60 animals per month for the entire network. | | **NIA Ordering** | **Notes** | | 1. Site PI to register Grant at: [https://ros.nia.nih.gov/](https://urldefense.com/v3/__https:/ros.nia.nih.gov/__;!!LIr3w8kk_Xxm!8ZVhgpi6vl5Qp-aCGuphCEsyrLjX1OV6fxls7L7OtP33hm51CR9YvemO50sSaw$) | Note: Site PI must note in registration that grant is part of SPAN. | | 1. Site will schedule surgery dates in alignment with CC timelines. Sites must place their orders on the NIA Rodent Ordering website no more than **30 days in advance** of the requested delivery. |  | | 1. Site will coordinate with own institution to determine the procedures for importing animals from NIA. |  | | 1. CC to send animal order plan (covering the entire project) to NIA. CC to provide NIA with quarterly updates that include number of animals to be sent to each site in the upcoming 3 months. |  |  |  |  | | --- | --- | | **Post-Operative Care** | **Notes** | | 1. Administer subcutaneous injections of 0.9% sodium chloride or LRS (1 ml divided into two sites) twice a day for 7 days (total of 2ml daily). |  | | 1. Remove penile plugs with 70% ethanol- soaked cotton wipes. This helps to avoid any distress associated with bladder emptying. | Note: Penile plugs are very common in aged mice post MCAo. | | 1. Provide animals with wet chow in their cages. |  | | 1. Animal should be weighed immediately after to administering fluids. Record Body weight for 2 days in REDCap database. |  |  |  |  | | --- | --- | | **Animal Handling Guidelines** | **Notes** | | 1. Change the cage before surgery day and 3-4 days post MCAo. This will minimize stress due to cage change. |  | | 1. Limit the surgery duration <10 minutes, defined as the time from incision to closure. This will limit over-exposure to anesthesia. | Note: This implies awakening animal during occlusion and briefly de-anesthetizing for de-occlusion | | 1. Place the animal in the recovery cage on heating pads. Arrange the heating pad in the cage so that the heating pad is on one half of the cage. This will allow the animal to move to the cold side of the cage if overheated. |  | | 1. Perform NDS for the first 2 days after MCAo to minimize overhandling of the animal. | Note: They look fragile and sick for almost a week. Most of the mortality is observed within first 4-7 days post-surgery | | 1. Place shacks or little house in the cages so that the animal feels secure | Note: Sites to consult with on-site vet regarding enrichment. | | **Euthanasia Criterion** | **Notes** | | Euthanize Animal in Case of:  Body Weight Loss of > 20%  Severe deficit such that the animal is not able to eat or drink.  Complete the **End of Study form** in the REDCap database. |  | | | | | |

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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)* | |