SPAN STANDARD EXPERIMENT

#1	LAB INFORMATION

Report Title	**SPAN: Stage 2 Run-in**
Report Author	The Coordinating Center
Dates of Experiment	March 2021-June 2021
Responsible Person(s)	Patrick Lyden Cenk Ayata Lauren Sansing Enrique Leira Anil Chauhan Raymond Koehler David Hess Jaroslaw Aronowski Louise McCullough
Location of Experiment	At Sites

#2	Purpose

Stage 2 Run-In: To verify the feasibility of parameters in the new models under development for Stage 2 (aged mice, obese mice, and SHR rats) including: behavior testing, surgical approach, MRI acquisition, mortality and post-operative handling. Tentative modifications to the MRI protocol have been made intending to optimize scan results in these animals.

#3	REFERENCES TO OTHER SOPS

SPAN SOP 6 Mouse Middle Cerebral Occlusion
SPAN SOP 17 Reperfusion
SPAN SOP 40 Rat Middle Cerebral Occlusion
SPAN SOP 45 Tissue Banking
SPAN SOP 52 Obesity Induced Hyperglycemic Mouse
SPAN SOP 53 Aging Model
SPAN SOP 54 Spontaneously Hypertensive Rat (SHR)
SPAN Stage 2 MRI Acquisition Memo

#4	METHODS

Description	Notes/Observations
1) Sites will assess feasibility of Stage 2 parameters for Surgery, Behavior, and MRI in as many subjects as possible in the two Stage 2/3 models they were assigned: aged mice, obese mice, or SHR. 2) CC has organized weekly huddles on Fridays that will continue into the onset of Stage 2. Sites will share experiences using parameters outlined this experimental protocol.	**SPAN SOP 52** Obesity Induced Hyperglycemic Mouse **SPAN SOP 53** Aging Model **SPAN SOP 54** Spontaneously Hypertensive rat (SHR)
Surgical Approach	**Notes/Observations**
Perform right side MCAo according to appropriate protocol. Sites may experiment with keep the animals asleep or awake during the occlusion period. Occlusion Duration by Model □ **Aged Model:** 45 min. □ **Obesity Induced Hyperglycemic Model:** 45 min. or 60 min. □ **Spontaneously Hypertensive Rats (SHR):** 60 min. Or 90 min.	**SPAN SOP 6** Mouse Middle Cerebral Occlusion **SPAN SOP 40** Rat Middle Cerebral Occlusion **SPAN SOP 17** Reperfusion

Behavior Testing	Notes/Observations
Perform Corner Test ☐ Bolted apparatus moving towards animal ☐ Guiding unbolted boards towards animal ☐ Testing unbolted boards in rat cage ☐ Ways to stimulate turns with Corner Test apparatus	**Note:** If behavior videos are recorded, these can be uploaded into IDA using the visit code: ***Stage 2 Pilot.*** Sites will email spancc@usc.edu when videos are uploaded into this IDA visit code.
Perform Grid Test ☐ SHR Grid trials with existing Stage 1 Grid Parameters ☐ Proposed apparatus for SHR: 1" 3x3 ft Grid apparatus ☐ Ways to stimulate movement on grid	
MRI Run-in	
Sites will assess feasibility of Day 2 MRI (and Day 29 if the sites wish) scanning using the **SPAN Stage 2 MRI Acquisition memo** in the two Stage 2/3 models they were assigned: aged mice, obese mice, or SHR. As a reminder of what the Stage 2 MRI protocol entails: 1) Scan n=3 normal brains and n=3 stroked animals for each animal model for each site. You may scan the same animal before and 48 hours after stroke. 2) Obtain RARE + T2 map + ADC map 3) Field of view: ☐ **Aged mice:** original 19.2 mm in-plane x 15 mm in slice direction. ☐ **Obese mice:** original 19.2 mm in-plane x 15 mm in slice direction **AND** 10% larger (21.12 mm). ☐ **Spontaneously hypertensive rats (SHR):** 25.6 mm in-plane, 0.8 mm slice thickness	**SPAN Stage 2 MRI Acquisition Memo**

4) Matrix density 128 x 128 x 30 slices in all scans.
5) Use fat suppression for all scans.
6) Upload these scans into IDA as Visit code ***Stage 2 Pilot*** and email MRI group and/or the Coordinating Center (spancc@usc.edu) when Stage 2 pilot files have been uploaded.

MRI Naming Convention:

Sites to include timepoint and model after Animal Record in each scan uploaded into IDA.

- **Aged Model :(AGE)**
  XX####D2AGE
  XX####D30AGE

- **Obesity Induced Hyperglycemic Model :(OB)**
  XX####D2OB
  XX####D30OB

- **Spontaneously Hypertensive Rats: (SHR)**
  XX####D2SHR
  XX####D30SHR

#5	LESSONS LEARNED/ NEXT STEPS