



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
<p>1) Sites will assess feasibility of Stage 2 parameters for Surgery, Behavior, and MRI in as many subjects as possible in the <u>two</u> Stage 2/3 models they were assigned: aged mice, obese mice, or SHR.</p> <p>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.</p>	<p>SPAN SOP 52 Obesity Induced Hyperglycemic Mouse</p> <p>SPAN SOP 53 Aging Model</p> <p>SPAN SOP 54 Spontaneously Hypertensive rat (SHR)</p>
Surgical Approach	Notes/Observations
<p>Perform right side MCAo according to appropriate protocol. Sites may experiment with keep the animals asleep or awake during the occlusion period.</p> <p><u>Occlusion Duration by Model</u></p> <ul style="list-style-type: none"><input type="checkbox"/> <u>Aged Model:</u> 45 min.<input type="checkbox"/> <u>Obesity Induced Hyperglycemic Model:</u> 45 min. or 60 min.<input type="checkbox"/> <u>Spontaneously Hypertensive Rats (SHR):</u> 60 min. Or 90 min.	<p>SPAN SOP 6 Mouse Middle Cerebral Occlusion</p> <p>SPAN SOP 40 Rat Middle Cerebral Occlusion</p> <p>SPAN SOP 17 Reperfusion</p>



Behavior Testing	Notes/Observations
<p>Perform Corner Test</p> <ul style="list-style-type: none"> <input type="checkbox"/> Bolted apparatus moving towards animal <input type="checkbox"/> Guiding unbolted boards towards animal <input type="checkbox"/> Testing unbolted boards in rat cage <input type="checkbox"/> Ways to stimulate turns with Corner Test apparatus 	<p>Note: If behavior videos are recorded, these can be uploaded into IDA using the visit code: Stage 2 Pilot.</p> <p>Sites will email spancc@usc.edu when videos are uploaded into this IDA visit code.</p>
<p>Perform Grid Test</p> <ul style="list-style-type: none"> <input type="checkbox"/> SHR Grid trials with existing Stage 1 Grid Parameters <input type="checkbox"/> Proposed apparatus for SHR: 1" 3x3 ft Grid apparatus <input type="checkbox"/> Ways to stimulate movement on grid 	
MRI Run-in	
<p>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 <u>two</u> Stage 2/3 models they were assigned: aged mice, obese mice, or SHR.</p> <p>As a reminder of what the Stage 2 MRI protocol entails:</p> <ol style="list-style-type: none"> 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: <ul style="list-style-type: none"> <input type="checkbox"/> Aged mice: original 19.2 mm in-plane x 15 mm in slice direction. <input type="checkbox"/> Obese mice: original 19.2 mm in-plane x 15 mm in slice direction AND 10% larger (21.12 mm). <input type="checkbox"/> Spontaneously hypertensive rats (SHR): 25.6 mm in-plane, 0.8 mm slice thickness 	<p>SPAN Stage 2 MRI Acquisition Memo</p>



- 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.

#5

LESSONS LEARNED/ NEXT STEPS