



## SPAN STANDARD EXPERIMENT

#1	LAB INFORMATION		
Report Title		SPAN: MRI Pipeline Validation	
Report Author		Patrick Lyden Cenk Ayata	
Dates of Experiment		March 2021-June 2021	
Responsible Person(s)		Patrick Lyden Cenk Ayata Ryan Cabeen	
Location of Experiment		At Sites	
#2	Purpose		

The Stage 1 MRI pipeline yields several morphometric measures, including total cerebral volume, lesion volume, ventricular volume, and midline shift. The values obtain from each time point scan will be compared to:

**Chronic Lesion comparison:** Day 29 scan will be compared to morphometric values obtained from Cresyl Violet stained sections from Stage 1 banked brain tissue.

**Acute Lesion comparison:** TTC imaged sections obtained immediately after Day 2 MRI scan from 4 young mice (60 min MCAo) at each site. Each site performs 4 animals =total sample size is 24.





#3

## **REFERENCES TO OTHER SOPS**

**SPAN SOP 6 Mouse Middle Cerebral Occlusion** 

**SPAN SOP 17 Reperfusion** 

SPAN SOP 35 MRI Acquisition Stage 1

**SPAN SOP 45 Tissue Banking** 

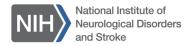
**SPAN SOP 59 TTC Staining** 

#4

## **METHODS**

Cili	onic Lesion (Stage 1 Day 30 Scans vs Cresyl violet)	Notes/Observations
1.	CC will select brains from tissue bank to cover a range of lesion sizes. Selection will be made via LONI image analyst (Ryan Cabeen)	Retrospective analysis of Stage 1 subjects already
2.	Sites will perform Histology for total n=20 mouse brains, approximately n=3-4 per site	obtained.  SPAN SOP 45 Tissue
3.	25 micron thick, 2 sections taken every 500 microns, exhaustive sectioning from frontal pole to midbrain	Banking
	Cresyl violet staining	
	Mount/cover slip/image Using Image J or other software, delineate area	
0.	of hemispheres (ipsi and contra), ventricles and visible lesion.	
	Acute Lesion (Day 2 scans vs TTC)	Notes/Observations
1.	Each site to perform 60 min. right side MCAo on n=4 young mice.	SPAN SOP 6 Mouse Middle Cerebral Occlusion SPAN SOP 17 Reperfusion
	<ul><li>n=4 young mice.</li><li>Perform 48-hour MRI followed immediately (or within 12 hrs. of scan) with sacrifice and TTC</li></ul>	
2.	<ul><li>n=4 young mice.</li><li>Perform 48-hour MRI followed immediately (or within 12 hrs. of scan) with sacrifice and TTC staining.</li></ul>	Cerebral Occlusion SPAN SOP 17 Reperfusion SPAN SOP 35 MRI
2.	n=4 young mice.  Perform 48-hour MRI followed immediately (or within 12 hrs. of scan) with sacrifice and TTC staining.  Follow SOP 59 for TTC Staining procedure.	Cerebral Occlusion SPAN SOP 17 Reperfusion SPAN SOP 35 MRI Acquisition Stage 1 SPAN SOP 59 TTC Staining
2.	n=4 young mice. Perform 48-hour MRI followed immediately (or within 12 hrs. of scan) with sacrifice and TTC staining. Follow SOP 59 for TTC Staining procedure. Upload the D2 MRI Scan in IDA with the visit	Cerebral Occlusion SPAN SOP 17 Reperfusion SPAN SOP 35 MRI Acquisition Stage 1 SPAN SOP 59 TTC Staining Note: if sites have an existing
2.	n=4 young mice.  Perform 48-hour MRI followed immediately (or within 12 hrs. of scan) with sacrifice and TTC staining.  Follow SOP 59 for TTC Staining procedure.	Cerebral Occlusion SPAN SOP 17 Reperfusion SPAN SOP 35 MRI Acquisition Stage 1 SPAN SOP 59 TTC Staining
2.	n=4 young mice.  Perform 48-hour MRI followed immediately (or within 12 hrs. of scan) with sacrifice and TTC staining.  Follow SOP 59 for TTC Staining procedure.  Upload the D2 MRI Scan in IDA with the visit code: "Stage 2 Pilot". In the animal ID, indicate	Cerebral Occlusion SPAN SOP 17 Reperfusion SPAN SOP 35 MRI Acquisition Stage 1 SPAN SOP 59 TTC Staining Note: if sites have an existing TTC protocol in current use,





2.	Data Transfer  Sites will transcribe data onto a CSV Send CSV upon completion to the Coordinating Center ( spancc@usc.edu) Include images of slides with the CSV	
	Power Analysis	
<ol> <li>Brain and lesion volumes will be compared MRI vs histology using Pearson's or Spearman's correlation coefficient.</li> <li>Power analysis with power 80%, alpha 0.05, with n = 20 gives sufficient sample size to detect whether the correlation coefficient is greater than 0.97</li> </ol>		
#4	RESULTS	
#5	LESSONS LEARNED/ NEXT STEPS	