#1	INFORMATION

Procedure Title	MRI Acquisition Stage 2 Pilot
Originators	SPAN Coordinating Center
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MRI Acquisition protocol for Stage 2 Pilot:

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

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'**, **using naming convention below,** and email MRI group and/or the Coordinating Center (spancc@usc.edu) when Stage 2 pilot files have been uploaded.

MRI Stage 2 Pilot Naming Convention for IDA Upload
Sites to include timepoint and model (AGE/OB/SHR) 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

Details from SPAN Stage 1 MRI Acquisition

4 Types of Scans:

1. Anatomical T2-weighted imaging using FSE/TSE/RARE fmethod (fast spin echo)
   - **Purpose:** normalization/segmentation and comparison with later time point at 30 days
   - Use a long TR (e.g., 6000) and an effective echo time of 60 ms or longer
2. A series of spin-echo images in order to create T2 maps
   - **Purpose:** lesion segmentation
   - Use a minimum echo time of 15 ms or shorter to provide a low-contrast volume for analysis
   - Use a maximum echo time of 70 ms or longer (lesion T2 will be about 70 ms)
   - Suggested echo times for multi-echo imaging using a volume transmitter: 0 to 100 ms in steps of 10 ms
   - Suggested echo times for single-echo imaging using a surface coil transmitter: 15, 45, 75 ms
3. A series of diffusion-weighted images in order to create ADC maps
   - **Purpose:** lesion segmentation and CSF discrimination
   - Suggested b-values: 0, 500, 1000 along the z direction

All scans except for #1 above should use "conventional" imaging with one readout per excitation in order to avoid distortions. Furthermore, all image data above should use the same matrix, resolution, and geometry:

Field of view	19.2 mm in-plane x 15 mm in slice direction
Matrix	128 x 128 x 30 slices
Resolution	150 x 150 um with 500 um slice thickness
BW	50k

Individual sites have some latitude to use methods appropriate to their hardware and sequences. In particular, some sites will want to use multi-echo imaging for the generation of spin-echo T2 maps, whereas other sites may not have an available sequence or may need to use single-echo imaging due to the use of a surface coil for RF transmission and reception. **Importantly, now that each site has defined their protocol, it should remain fixed for the duration of the SPAN study.**

Explicit example protocol on a Bruker scanner (numbering scheme matches basic protocol above)

Hardware: 9.4T magnet, Bruker volume transmit coil, Bruker 4-channel phased array surface receiver coil
Software: PV5.1

The numbering scheme below matches the "Basic Imaging Protocol" above. The time per animal, including all setup, should be less than 1 hour.
- 0. Setup (these setup scans are not needed in the upload)
    - Sagittal localizer (sequence = FLASH) to position animal accurately along bore
    - Tri-plane localizer with large FOV = 30 mm (sequence =RARE) for adjustments
    - Tri-plane localizer with small FOV = 15 mm (sequence =RARE) for geometry planning
- 1. Anatomical T2-weighted image volume   (3.5 min)
    - Sequence = RARE (rapid acquisition with relaxation enhancement)
    - fTR/TE=6000/15, 8 segments, 4 averages, effective TE=60
- 2. Multi-echo (spin-echo) scan to enable T2 map   (6.5 min)
    - Sequence = MSME (multi-slice multi-echo); TR = 4500
    - 10 spin echo times from 10 to 100 ms
      Or
    - Sequence = MSME using 1 echo per scan
    - 3 spin-echo TE values = 15, 45, 75 ms
- 3. Diffusion-weighted scan to enable ADC map
      (9.5 min)
    - Sequence = DtiStandard (*), TR/TE=1500/25
    - 3 b-values: 0, 500, 1000

(*) Note that sequence DtiStandard enforces a minimum inter-slice delay time to prevent high gradient duty cycles during long runs (at least this is true on PV5.1). This delay can lead to excessively long TR values, so it may be necessary to edit the sequence to shorten this delay, which is not necessary for short diffusion scans.

Potential pitfalls and best practices

Pitfall	Some scans are not co-aligned with others within a single dataset.
Best practice	After defining the geometry on the first scan (e.g., RARE anatomical), always copy geometry from the first scan to other scans.
Pitfall	Image volumes within a given "mapping" dataset (e.g., multi-echo data) do not have a consistent scale factor.
Best practice	1) When possible, collect all data within a mapping series using a single scan that collects multiple time points (e.g., multiple TE values or b values) to ensure self-consistent scaling

	2) If hardware warrants the use of multiple scans (e.g., a multiple spin-echo sequence like MSME should not be used with a transmit surface coil), then take special care to ensure that each scan has the correct relative signal (**).
Pitfall	The stroke lesion appears on the wrong side of the brain, complicating analysis.
Best practice	Ensure that all animals are registered correctly during the initiation of the scan. Using "feet first" when the animal is "head first) will cause a parity change in the image coordinate system.
Pitfall	A surface coil provides insufficient spatial coverage or signal to noise ratio (SNR) across the whole brain, leading to a failed segmentation.
Best practice	While surface coils provide excellent SNR in general, ensure that 1) the surface coil in use is large enough to provide full brain coverage under optimal conditions, and 2) there is reproducible method to accurately position the coil on the animal head. If initial images indicate poor volume coverage due to a shifted coil or animal head, remove the animal and reposition the coil before continuing the scan.

(**) On Bruker scanners, set the parameter "Reco_map_mode = ABSOLUTE_MAPPING"

Upload to LONI Repository

MRI Data will be uploaded to LONI in DICOM format. These files will contain much of the information that is needed to analyze the data, but unfortunately some information will be missing. For instance, Bruker DICOM files do not incorporate b-values. Moreover, it would simplify identification of each image series if it was labeled in some manner. To help facilitate, please include the following text strings into your "protocol name", which is a standard DICOM field that will be carried along with the data.

1. RARE anatomical image volume: protocol name includes "RARE_anatomy"
2. T2-weighted image scan(s): protocol name includes "T2_map"
3. Diffusion-weighted image scan(s): protocol name includes "ADC_map"

Additionally, specific information describing items 2-3 above should be copied into a text file or data structure template to accompany each dataset upload. See an example below for the text file. Note: This data structure template should be emailed to the CC during the pilot study.

T2 information

Sequence = multi-echo multi-slice or single-echo multi-slice
TE = specify 10 values for multi-echo or 3 values for single-echo (in units of ms)

ADC information
Sequence = DtiStandard or whatever was used
b-values = specify 3 values used (in units of s/mm2)

<mark>Protocol must remain fixed for the duration of the SPAN study.</mark>

Example Data Structure template

Each experiment will have a folder SS3691_Ya_d2_1_1)

SS3691**: ear tag id**
Ya: Yale
d2: day 2 scan

      a. RARE anatomical image volume: **RARE_anatomy**
      b. T2 map:

If using Single echo multi slice

      c. T2_map_15ms
      d. T2_map_45ms
      e. T2_map_65ms

If using multi echo multi slice

T2_map (10 to 100 ms)

      f. Diffusion weighted image scan: **ADC map**
      Sequence= DtiStandard
      b values: 0, 500, 1000