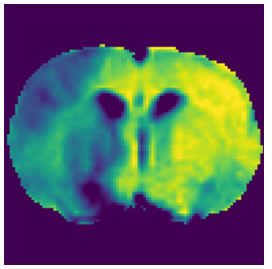
Mar 18, 2026

2-deoxy-D-glucose chemical exchange sensitive spin-lock (CESL) MRI of the rodent brain



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We use this protocol and it's working.

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Abstract

Magnetic resonance imaging (MRI) of glucose metabolism shows significant potential for identifying disease biomarkers and monitoring therapeutic responses in neurological conditions. Here, we present a protocol utilizing chemical exchange-sensitive spin-lock (CESL) MRI with the glucose analogue 2-deoxy-D-glucose (2DG) in the rat brain. We employed this method to characterize metabolic changes in ischemic tissue within a rat model of stroke. However, the technique is not limited to stroke and may be adapted to other disease models with minimal modifications. Previous research has demonstrated that CESL MRI is sensitive to various glucose analogs, including regular D-glucose, which is suitable for human application. Consequently, our protocol provides a foundation for a wide range of future applications in both basic and translational research, with potential utility in animal models and, eventually, human studies.

Image Attribution

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Materials

Bruker 7 Tesla BioSpec (70/20 USR) with Avance III HD Electronics and Paravision 6.0.1 Software
Transmit-only Volume Coil for Mice and Rats - 86mm (Bruker, Ettlingen, Germany)
Phased Array Surface Coil for Rat Head (Rapid Biomed, Rimpar, Germany)

Small Animal Monitoring System (SA Instruments, Stony Brook, NY, USA)

Femoralis catheter: Portex Fine Bore Polythene 30 Tubing, 0.58 mm inner diameter, 0.96 mm outer diameter, 90 cm long

Filament for MCAO: 390 µm diameter, 4039910PK10Re, Doccol, Sharon, 5 MA, USA

2-Desoxy-D-Glucose (Carl Roth, Art.-Nr. CN96.3; Germany)

Matlab Toolbox ANTx2 https://github.com/ChariteExpMri/antx2

Protocol materials

2-Desoxy-D-Glucose **Carl Roth** **Catalog #**CN96.3

Gibco DPBS without Calcium and Magnesium **Fisher Scientific** **Catalog #**14190136 or equivalent

Troubleshooting

Animal preparation

1 Animals undergo two surgical procedures before the MRI examination:
a. Femoralis catheter implantation to inject 2DG during the MRI measurements.
b. 90 min middle cerebral artery occlusion (MCAO) to induce focal cerebral ischemia.

Note

All animal procedures mentioned herein are specific to stroke modeling in the rat using 90 min MCAO and can be replaced with other disease models.

2 Anesthetize animals using isoflurane in a 70%/30% N2O/O2 mixture. Anesthesia is induced with 5% isoflurane and maintained at 1.75-2.5% isoflurane during the femoralis catheter placement and the MCAO and 1.75% during MRI to achieve a breathing rate of ~60-100/min.

3 For MRI, place the animal on a heated water blanket to ensure constant body temperature of 37±0.5 °C.

4 Use ear and toothbar fixation to minimize motion artifacts.

5 Monitor the respiration rate using a small animal monitoring system (SA Instruments, Stony Brook, NY, USA).

Preparation of 2-deoxy-D-glucose (2DG) solution

6 Goal: injected dose of 1 g/kg body weight.

6.1 Reagent: 2-Desoxy-D-Glucose **Carl Roth Catalog #**CN96.3

6.2 Preparation of the injection solution: 25% 2DG w/v, i.e. 0.25 g in 1 mL of solution.
~3.3 mL NaCl and 1 g 2DG were needed to achieve 4 mL of the final solution.

6.3 Injection volume: v = dose * weight / concentration = 1 g/kg * weight / 0.25g / mL = 4 mL/kg.
Example: 1.6 mL for a rat of 400 g body weight.

6.4 Injection period: around 1-2 min.

MRI hardware

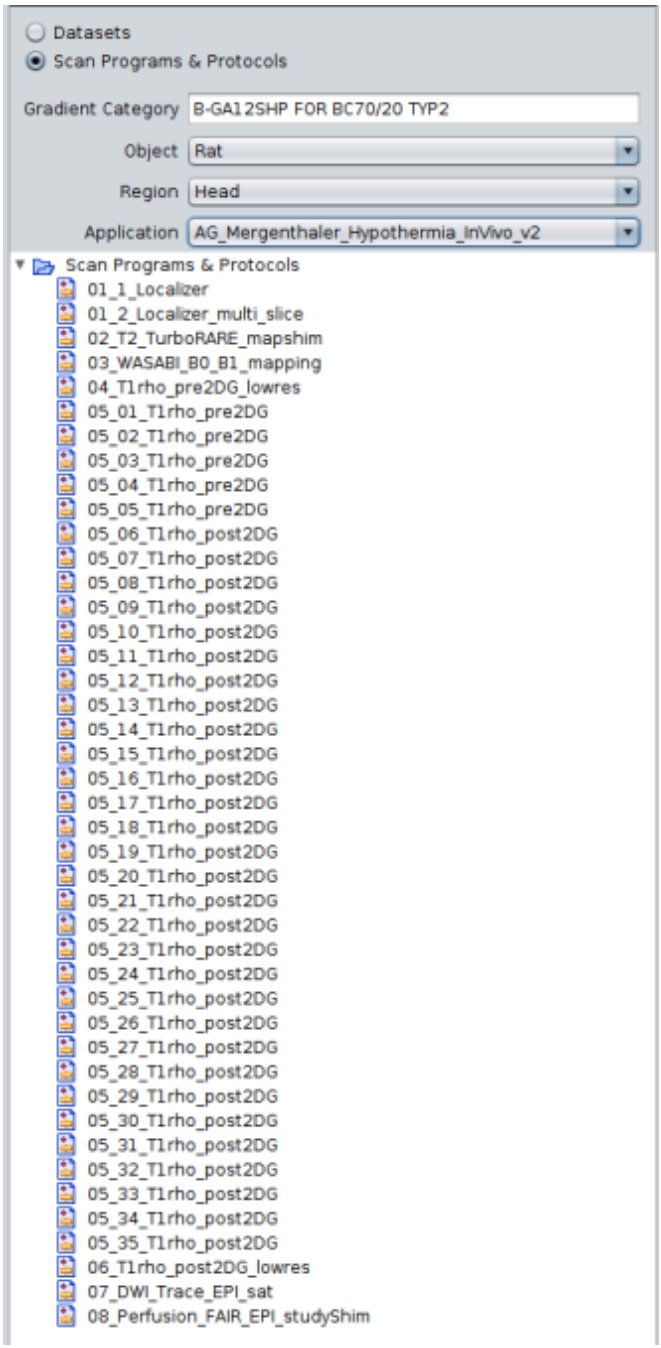
7

Equipment	
BioSpec	NAME
70/20 USR	TYPE
Bruker	BRAND
https://www.bruker.com/	LINK

Bruker 7 Tesla system (BioSpec 70/20 USR, Bruker, Ettlingen, Germany) + 86 mm transmit volume resonator (Bruker) + receive rat brain surface coil (Bruker). Paravision 6.0.1 software.

MRI pulse sequences

8 The MRI protocol includes the following scan protocols (Boehm-Sturm, et al., 2025).



Glossary:

- RARE: rapid acquisition with relaxation enhancement (turbo spin echo sequence)
- WASABI: Simultaneous mapping of water shift and B1 (Schuenke P, Windschuh J, et al., 2017)
- T1rho: CESL sequence to map R1rho=1/T1rho
- DWI: diffusion weighted imaging
- FAIR: flow sensitive alternating inversion recovery (sequence for perfusion imaging)
- EPI: echo planar imaging (DWI and FAIR use spin echo EPI readout)

In vivo MRI protocol

9 **01_1_Localizer**

Note

When the animal is placed into the scanner first tune and match (wobble) the volume resonator.

Run scan with Automatic Setup / Adjustments.

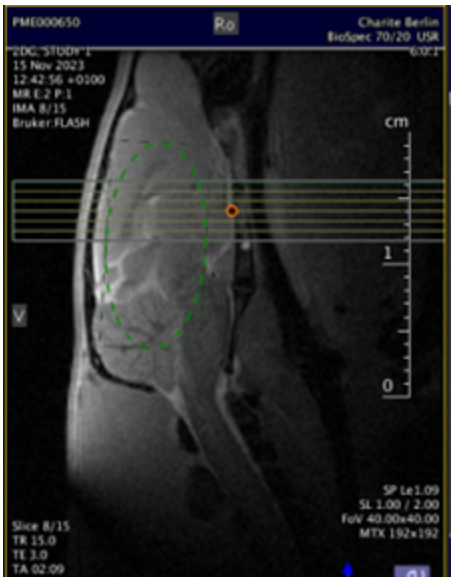
10 **01_2_Localizer_multi_slice**

Run scan.

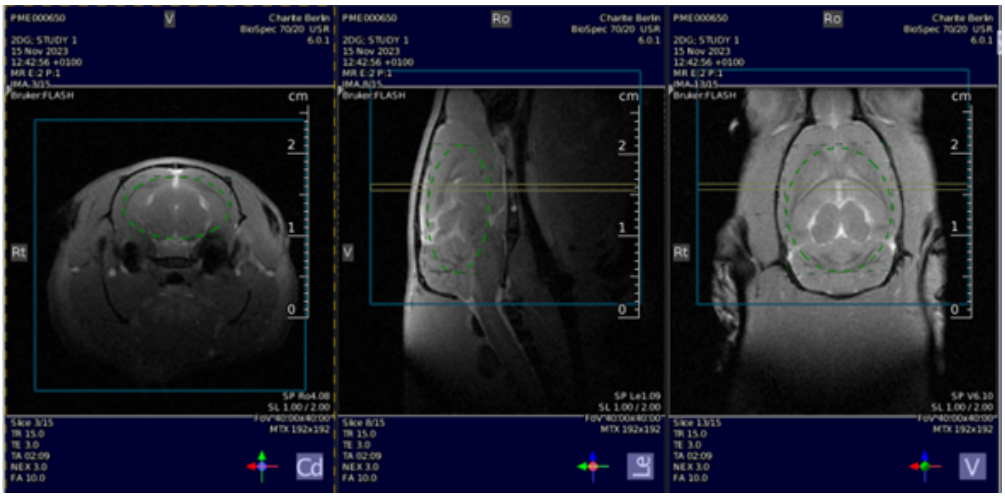
11 **02_T2_TurboRARE_mapshim**

11.1 **Set Geometry**

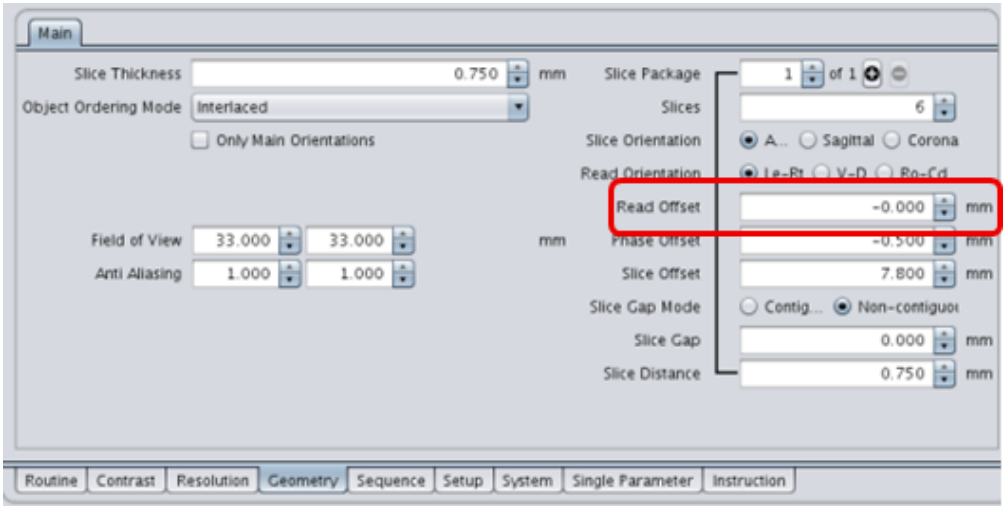
a. Reduce the number of slices to 6, adjust the slice position so that the anterior commissure is covered by the uppermost slice.
b. Switch back to 38 slices and adjust center to trachea.
c. Adjust the ellipsoid shim volume to the center of the brain.
d. Make sure that the **read offset is 0.00.**



a. slice positioning



b. /c. final geometry and shim volume position



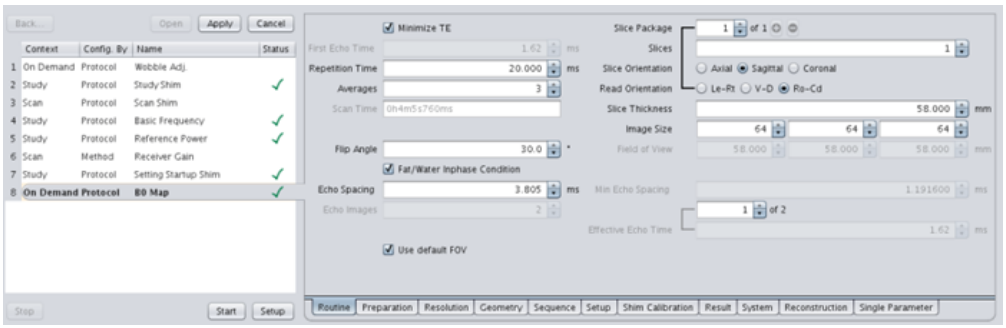
d. check read offset

11.2 **B0 map**

Create a B0 map with:

a. 3 averages, default FOV and

b. Map S/N 20.



a. B0 map settings FOV and averages

b. B0 map setting Reconstruction

Run B0 mapping (Start).

11.3 Run scan 02_T2_TurboRARE_mapshim.

12 **03_WASABI_B0_B1_mapping**

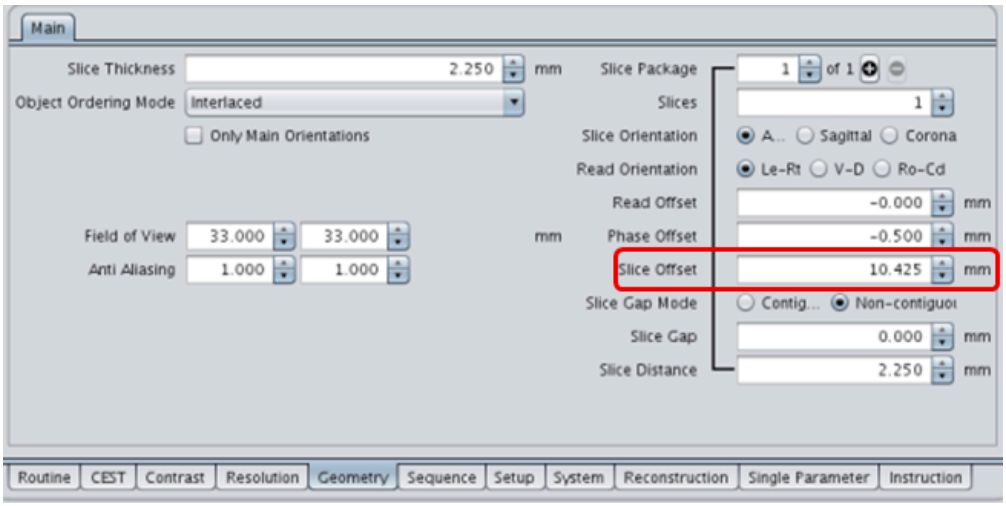
Import the slice orientation of 02_T2_TurboRARE_mapshim and add 2.625 mm to the slice offset!

Note

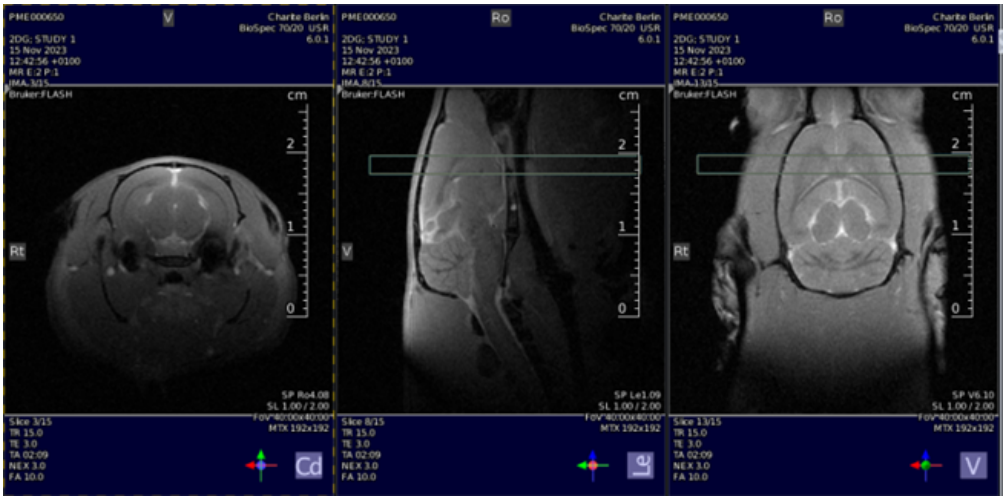
B0 and B1 mapping is recommended to check B0/B1 homogeneity in the given experimental setup and to correct for inhomogeneities in the image post processing step.
In our experimental setting, the B0 and B1 maps were only used once to check sufficient homogeneity and not used for corrections.
WASABI is particularly efficient in mapping B0 and B1 but other sequences can be used.

Note

The slice offset is specific to the MCAO model to ensure the center of the striatum is covered, which is the brain region most severely affected by the stroke. In other disease models different offsets can be used to perform 2DG-CESL MRI in the brain regions of interest.



a. set the slice offset



b. final geometry to cover the center of the striatum

Run scan.

13 **Import the slice orientation of 03_WASABI_B0_B1_mapping to all following T1rho protocols**

(04_T1rho_pre to 06_T1rho_post)

14 **04_T1rho_pre2DG_lowres**
05_1_T1rho_pre2DG to 05_5_Tirho_pre2DG
Run scans.

15 **⇒ Slow injection of 2DG solution through the femoralis catheter over 2 min!**
Inject the calculated volume plus the dead volume of the catheter.

16 **05_6_T1rho_post2DG to 05_35_T1rho_post2DG**
Run scans.

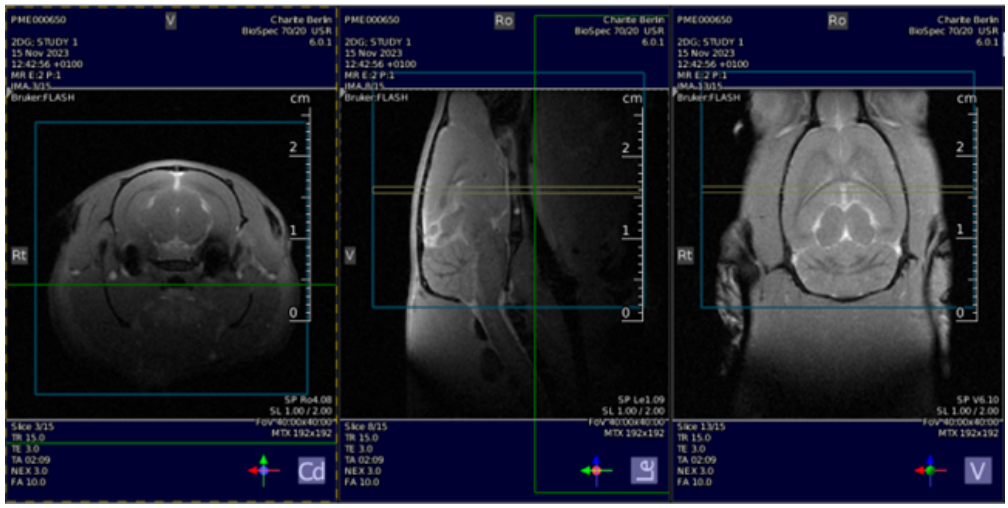
17 **06_T1rho_post2DG_lowres**
Run scan.

18 **07_DWI_Trace_EPI_sat**

Note

Diffusion (DWI) and perfusion (PWI) MRI are of specific interest in the stroke model. These sequences can also be of interest in other disease contexts but are not mandatory for CESL imaging.

Import the slice orientation of 02_T2_TurboRARE_mapshim and adjust the FOV Saturation slice.



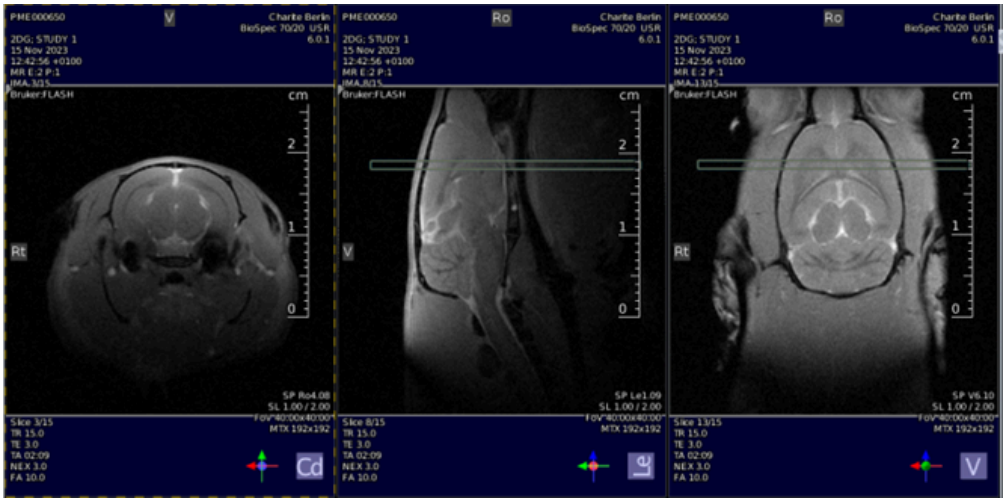
final geometry with FOV saturation slice

Run scan.

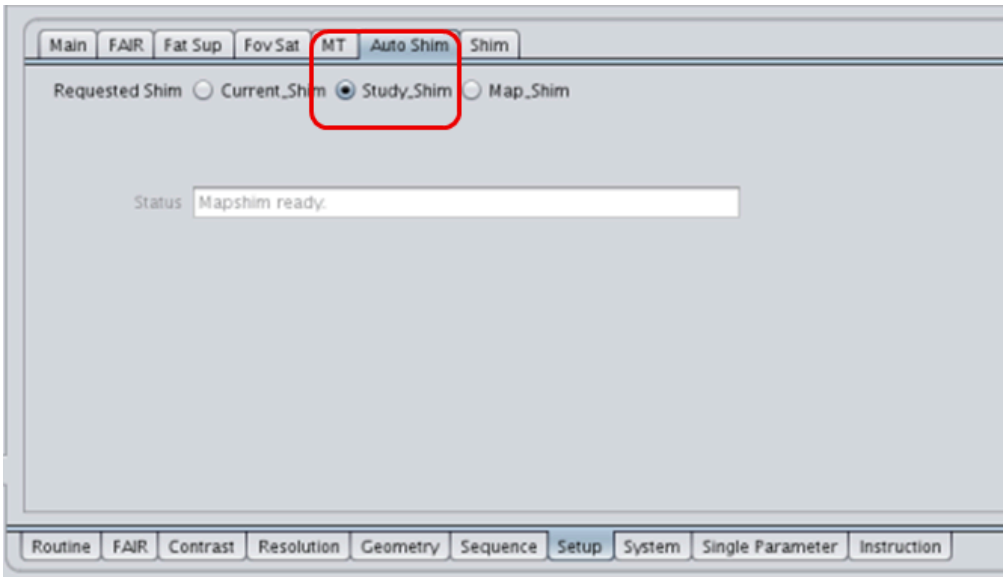
19 **08_Perfusion_FAIR_EPI_studyshim**

a. Import the slice orientation of 03_WASABI scan.

b. Use the **study shim** option. Use of a global shim ensures a better global inversion pulse of the FAIR sequence.



a. final geometry



b. check study shim option

Run scan.

Image data processing

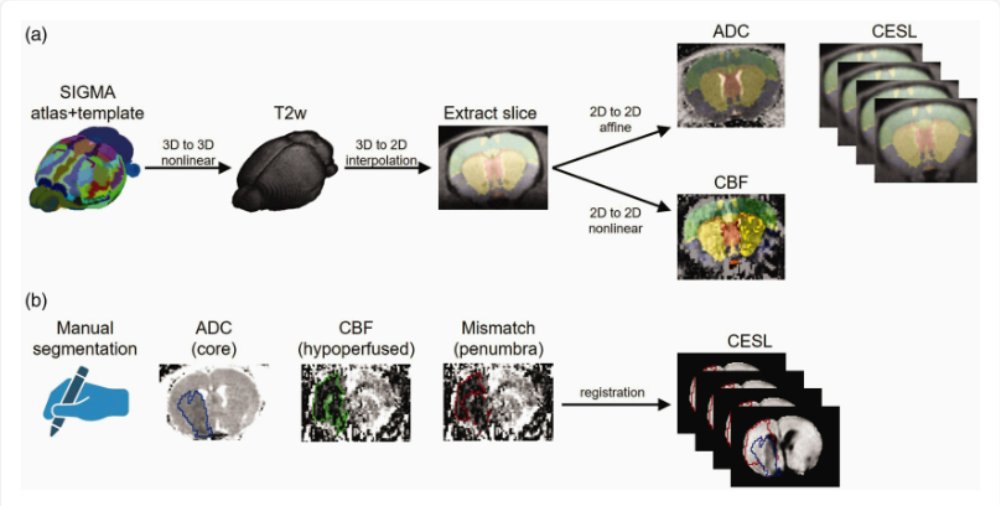
20 **Generate $R_{1\rho}$ maps**

Calculate $R_{1\rho}(t_i)$ at time t_i voxelwise via monexponential fitting of the signal over the spin lock times TSL:

$$S\left(TSL, t_i\right) = e^{-R_{1\rho}(t_i)\cdot \mathrm{TSL}};\ i = 1 \ldots 35$$

21 **Region of Interest (ROI) Analysis**

ROI analysis can be performed in two ways:



ROI analyses. CESL images can be quantified in anatomical regions defined in the SIGMA rat brain atlas (a) or using manual delineation (b) of the lesion on ADC, CBF and late $\Delta R_{1\rho}$ maps (mean of the last 5 maps). Penumbra is defined via perfusion/diffusion mismatch.
*Figure reproduced from Boehm-Sturm et al., 2025 (doi: **10.1177/0271678X251355049**) under a CC-BY-NC license.*

21.1 **Automatic analysis of brain regions of interest**

Segment brain regions automatically on the different MRI contrasts (T2, ADC, CBF, CESL) using a series of 3D to 3D and 3D to 2D image registrations using the SIGMA

rat brain template (Barriere, et al., 2019). The registration can be performed for example in ANTx2 software (Koch, et al., 2019).

Software

ANTx2	NAME
all all	OS
ChariteExpMRI	DEVELOPER
https://github.com/ChariteExpMri/antx2	REPOSITORY
https://github.com/ChariteExpMri/antx2	SOURCE LINK

Apply the registration to the SIGMA atlas, which matches the template. For registration, use the b = 0 image for diffusion MR images, the first inversion time image for perfusion MR images and the first TSL image for CESL images. For perfusion MRI, images will be distorted due to shimming artefacts of the EPI sequence and a nonlinear registration is needed. For ADC and CESL images, affine (12 degrees of freedom) registrations are sufficient.

21.2 Manual analysis of stroke regions of interest

Note

This step is only executed for the stroke model. It could be of interest for other animal models to segment special regions of interest manually but is not mandatory.

Segment the hypointense lesion on ADC, CBF and $\Delta R_{1\rho}$ maps (mean image of the last 5 images) using an initial automated histogram-based segmentation followed by manual corrections in ANALYZE software (v5.0, AnalyzeDirect, Overland Park, KS, USA). Using the image registration transforms of the first approach, transform the lesion masks from ADC and CBF to the atlas and then register them to the $\Delta R_{1\rho}$ maps. In atlas space, generate ROIs mirrored at the midline and transform them to the $\Delta R_{1\rho}$ maps. Define lesion core via hypointense ADC, hypoperfused tissue via hypointense CBF and the hypometabolic lesion via hypointense areas on the mean $\Delta R_{1\rho}$ maps (mean of the last 5 measurements). The penumbra is defined via perfusion diffusion mismatch, i.e.

$$mask_{penumbra} = mask_{hypoperfused} \setminus \left(mask_{hypoperfused} \bigcap mask_{core} \right)$$

22 **Statistics**

Extract mean values from regions of interest. Perform group statistical analyses on those values with tests matching the experimental design of your study.

In vitro quality assurance

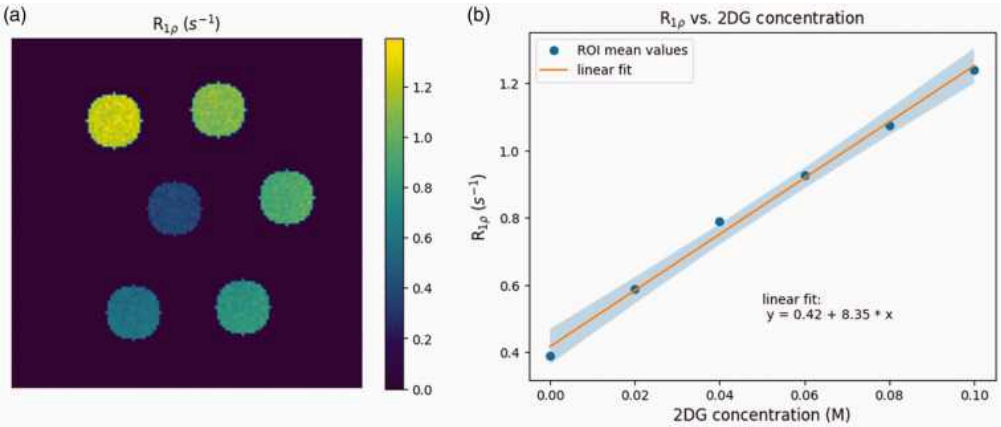
23 Prepare phantom with 5 mm NMR tubes containing different concentrations of 2DG in buffer. Typical range of concentrations: (0, 0.02 M, 0.04 M, 0.06 M, 0.08 M, 0.10 M) 2DG in DPBS:

Gibco DPBS without Calcium and Magnesium **Fisher Scientific Catalog #**14190136 or equivalent

24 Perform the in vivo CESL MRI protocol excluding the DWI and PWI scans. A single T1rho scan is sufficient. Use a single slice perpendicular to the NMR tubes (cf. T1rho protocol in section "In vivo MRI").

25 Generate $R_{1\rho}$ map (cf. section "Image data processing").

26 Plot $R_{1\rho}$ over 2DG concentration and perform a linear fit. The result should be similar to this



CESL MRI of tubes containing different concentrations of 2DG in DPBS. $R_{1\rho}$ map (a) and quantification including linear fit (b).
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Protocol references

Boehm-Sturm P, Schuenke P, Foddis M, Mueller S, Koch SP, Beard DJ, Holloway P, Mottahedin A, Schröder L, Buchan AM, Mergenthaler P. 2-deoxy-D-glucose chemical exchange-sensitive spin-lock MRI of cerebral glucose metabolism after transient focal stroke in the rat. *J Cereb Blood Flow Metab* 2025; 271678X251355049. DOI: **10.1177/0271678X251355049**.

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