Supplementary Information

Supplementary Theory

Adaption of manual lesion volume correction

Supplementary Fig. S1 shows a schematic view of a coronal section through the mouse brain after focal ischemia on the left hemisphere and explains our notation for MLC. We follow the framework of Loubinoux et al. and Gerriets et al.¹² and generalize it in the following way: instead of working with the whole hemispheric volumes, we consider an arbitrary region on the contralateral side with volume $V_{cu}$ that can also be completely delineated on the MRI scan on the ipsilateral side and has an ipsilateral volume $V_{iu}$, the $u$ stands for edema uncorrected volume. A good example would be the whole brain (used in this study) or only forebrain, which is common in most published studies. Other examples could be the striatum or the hippocampus if one were interested in the extent of damage to specific brain structures only. The ipsilateral and contralateral volumes are assumed symmetric without swelling, i.e. $V_{le} = V_{ce} = \frac{1}{2} V_{te}$, where $V_{te}$ is the edema corrected total volume of the brain region. The edema uncorrected lesion volume within the brain region as delineated on a T2w image is $LV_u$. Assuming a homogenous expansion of the lesion due to edema (compression factor $F_1 < 1$) and a uniform compression of healthy tissue outside the lesion by a factor $F_2 > 1$, the lesion volume excluding edema would be

$$LV_e = F_1 \cdot LV_u \quad (1)$$

For the contralateral brain region the edema corrected brain region volume:

$$V_{ce} = F_2 \cdot V_{cu} = \frac{1}{2} V_{te} \quad (2)$$

The ipsilateral brain region can be divided into swollen damaged tissue and compressed undamaged tissue, which leads to

$$V_{le} = F_1 \cdot LV_u + F_2 \cdot (V_{iu} - LV_u) = \frac{1}{2} V_{te} \quad (3)$$

The uncorrected volumes can be delineated on an MR image which leaves 4 unknown $LV_e, F_1, F_2, V_{te}$. To solve the underdetermined linear system, equations (1)-(3) can be divided by the total brain region volume, transforming edema corrected lesion volume and the other unknown parameters to units of percent of hemispheric brain region volume, i.e. $LV_e \rightarrow LV_e / (\frac{1}{2} V_{te}), F_1 \rightarrow F_1 / (\frac{1}{2} V_{te}), F_2 \rightarrow F_2 / (\frac{1}{2} V_{te}), \frac{1}{2} V_{te} \rightarrow 100\%$.

Solving for $LV_e$ leads to

$$\%LV_e = \frac{V_{eu} - V_{iu} + LV_u}{V_{cu}} \times 100 \quad (4)$$

i.e. we have shown that equation (2) from Gerriets et al. for the relative hemispheric edema corrected lesion volume can be generalized to an arbitrary subregion of the brain that is present on both hemispheres. If the region contains the whole lesion, no additional assumptions are needed compared to the original framework and we have managed a way to analyze data without whole brain coverage by the MR scan. Moreover, when interested only in damage to a specific subregion, MR images do not need to cover the whole lesion.
but only the specific region of interest. In this case, however, an additional, quite crude approximation is needed that tissue inside the lesion is homogenously expanded.

It is important to note that generally

\[ V_{lu} + V_{cu} \neq V_{le} + V_{ce} \]  

(5)

i.e. the total volume of the brain region can also be altered due to edema since there are no stiff boundaries. This means that the other equations derived by Gerriets et al. for the space-occupying effect or absolute edema corrected lesion volume are generally not applicable. Only in the case of the whole ipsilateral hemispheric volume \( HV_i = V_{lu} \) and whole contralateral volume \( HV_c = V_{cu} \) the skull acts as an inelastic barrier and equation (5) holds, leading to the expression for the absolute edema corrected lesion volume (in \( \text{mm}^3 \)) derived by Gerriets et al.

\[ LV_e = HV_c + HV_i - (HV_c + HV_i - LV_u) \cdot \frac{HV_c + HV_i}{2HV_c} \]  

(6)

Note that this requires whole brain coverage by the MRI scan. Only then \( LV_u \) and \( LV_e \) can both be expressed in the same absolute/relative units and the absolute/relative space occupying effect can be calculated via

\[ SE = LV_u - LV_e \]  

(7)

or expressed in percent of the lesion volume as delineated on T2w images

\[ \%SE = \frac{LV_u - LV_e}{LV_u} \times 100 \]  

(8)

**Supplementary Figures**

**Supplementary Fig. S1.** Schematic of a coronal section of the mouse brain explaining the nomenclature used for the manual edema correction of lesion volume. Infarcted tissue is assumed to be homogenously expanded (gray) whereas healthy tissue is assumed to be compressed (white). We will show how to calculate the edema corrected percent damage to an arbitrary brain region. Here, the striatum is taken as an example (solid lines). The ipsilateral region is expanded whereas the contralateral region is compressed compared to a brain in absence of edema (dashed lines). The advantage of this generalized framework is that full brain coverage by MRI is not required and the lesion can extend beyond the brain region of interest.
Supplementary Fig. S2. Analysis Pipeline. First, T2w image volumes of each mouse were coregistered automatically to the Allen template with an initial rigid body transformation with 6 degrees of freedom (dof) followed by an affine transformation (12 dof) using ELASTIX. The affine coregistration step only extracted the transformation parameters, which were later concatenated with the parameters from non-linear transformation (warping) to allow a single combined transformation from mouse space into Allen space (and vice versa). This avoids artifacts from concatenated interpolations. The T2w image was then segmented into tissue compartments of gray matter (GM), white matter (WM), and cerebrospinal fluid (CBF) using the ‘unified approach’ for segmentation and normalization provided by SPM8. In this step, we used the basic parameters settings and tissue probability maps (TPMs) from SPMMouse. TPMs were previously brought in registration with the Allen template using SPMMouse's warping. Although warping into target space is also conducted by SPM's unified approach, we used ELASTIX due to better performance in registering T2w images i) from mice with large lesion territory and ii) from mice that had PT-related surgery wounds. Detailed ELASTIX parameters can be found in supplementary table 1. We used two synthetic images generated by linear combination of i) the three TPMs from SPMMouseAllen as fixed image and ii) the three tissue compartment maps derived from the T2w-segmentation step as moving image by setting the image intensity I of voxel (x,y,z) to

\[ I(x,y,z) = a \cdot \text{GM}(x,y,z) + b \cdot \text{WM}(x,y,z) + c \cdot \text{CSF}(x,y,z), \quad a=10000, \quad b=20000, \quad c=40000 \]

Here GM, WM, and CSF represent the respective calculated tissue probabilities.

To cope with high-intensity values in the T2w lesion territory and resulting defective values in the tissue compartments, values inside the lesion of the moving image were downsampled by an empirically determined factor 1/3. At the end of the warping step the concatenation of linear and nonlinear transformation parameters allowed to transform the T2w image and other images (in registration with the T2w) such as the lesion mask into Allen space. To save memory, registered images were downsampled to 70 µm isotropic resolution.
Supplementary Fig. S3. PT lesion territory and local tissue swelling and compression. Incidence maps depict the lesion territory across PT mice after transformation into Allen space (panel 1). Numbers above slices denote the distance to the Bregma (mm). Panel 2 depicts the voxel-wise mean edema-induced volume changes across PT mice. While voxels in green represent local volume preservation, voxels in red represent a local expansion. Voxelwise t-statistics over maps of PT and sham mice confirmed non-homogeneous volume changes and the largest cluster identified with high spatial correspondence tissue swelling in the region of the PT-lesion (panel 3).

Supplementary tables

| ELASTIX parameter       | Value                                      | Description                                               |
|-------------------------|--------------------------------------------|-----------------------------------------------------------|
| Transform               | "BSplineTransform"                         | Type of transformation function                           |
| Registration            | "MultiMetricMultiResolution Registration"  | Allows combination of different cost functions             |
| Interpolator            | "BSplineInterpolator"                      | Registration method                                        |
| Optimizer               | "AdaptiveStochasticGradientDescent"        | Optimization routine                                      |
| MaximumNumberOfIterations | 1000                                      | Optimizer parameter                                       |
| MaximumStepLength       | 0.015                                      | Optimizer parameter                                       |
| Metric                           | "AdvancedMattesMutualInformation" "TransformBendingEnergyPenalty" | Cost function                                                                 |
|---------------------------------|------------------------------------------------------------------|--------------------------------------------------------------------------------|
| ImageSampler                    | "RandomCoordinate"                                               | Subset selection of voxels used for optimization                                |
| NumberOfSpatial Samples         | 2048                                                             | ImageSampler parameter                                                         |
| NewSamplesEvery Iteration       | "true"                                                           | ImageSampler parameter                                                         |
| NumberOfResolutions             | 4                                                                | Intermediate resampling steps (image pyramid levels)                            |
| GridSpacingSchedule             | 8 4 2 1                                                          | Image pyramid parameter                                                         |
| FinalGridSpacingIn Voxels       | 5 5 5                                                            | Image pyramid parameter                                                         |
| FinalBSpline InterpolationOrder | 3                                                                | Interpolation method                                                           |

**Supplementary Table 1.** ELASTIX parameters used for nonlinear image warping. Full parameter files available online at [http://elastix.bigr.nl/wiki/index.php/Parameter_file_database](http://elastix.bigr.nl/wiki/index.php/Parameter_file_database).
| Species      | Stroke model               | MRI time point | MRI sequence | FOV, covers whole brain? | Appropriate equation was used? | Reference               |
|--------------|----------------------------|----------------|--------------|----------------------------|-------------------------------|-------------------------|
| rat          | MCAO                       | 24h            | RARE         | no                         | yes                           | Baskerville et al. 4    |
| mouse        | MCAO                       | 24h            | RARE         | NA                         | no                            | Berthet et al. 5        |
| mouse        | MCAO                       | 24h            | RARE         | yes                        | yes                           | Frieler et al. 6        |
| mouse        | MCAO                       | 24h            | RARE         | no                         | yes                           | Hochmeister et al. 7    |
| mouse        | MCAO                       | 24h            | RARE         | yes                        | no,                           | Igarashi et al. 8       |
| rat          | MCAO                       | 24h            | MSME         | no                         | yes                           | Juenemann et al. 9      |
| rat          | MCAO                       | multiple       | RARE         | no                         | yes                           | Kang et al. 10          |
| rat          | MCAO                       | multiple       | RARE         | no                         | yes                           | Kang et al. 11          |
| mouse        | MCAO                       | 48h            | RARE         | NA                         | yes                           | Khanna et al. 12        |
| mouse        | MCAO                       | 48h            | RARE         | NA                         | yes                           | Khanna et al. 13        |
| rat          | MCAO                       | 24h            | RARE         | yes                        | yes                           | Leoni et al. 14         |
| rat          | ICH                        | multiple       | FLASH        | no                         | no                            | Marinkovic et al. 15    |
| rat          | PT                         | NA             | RARE         | no                         | yes                           | Möller et al. 16        |
| rat          | MCAO                       | 24h            | RARE         | no                         | yes                           | Reid et al. 17          |
| dog          | MCAO                       | 24h            | RARE         | yes                        | yes                           | Rink et al. 18          |
| rat          | MCAO                       | 48h            | RARE         | NA                         | yes                           | Rink et al. 19          |
| mouse        | MCAO                       | multiple       | RARE         | no                         | yes                           | Stubbe et al. 20        |
| rat          | MCAO                       | 24h            | RARE         | no                         | yes                           | Wayman et al. 21        |
| rat          | MCAO                       | 24h            | RARE         | no                         | yes                           | Weise et al. 22         |
| mouse        | thrombo-embolic MCAO       | 24h            | RARE         | no                         | no                            | Zhang et al. 23         |

**Supplementary Table 2. Systematic review of studies using MLC.** A Pubmed search of studies that cite the original papers of Gerriets et al. and Loubinoux et al. 1,2 was performed, 20 original contributions were found. All used coronal MRI slices. A FOV<sub>z</sub> < 12 mm (mouse) or 22 mm (rat) was considered non-whole brain coverage. If the used equation for absolute/relative edema corrected lesion volume did not match the corresponding equation from Gerriets et al. this lead to a "no" in the corresponding column. Abbreviations: intracerebral hemorrhage (ICH) rapid acquisition with relaxation enhancement (RARE), multi slice multi spin echo (MSME), rostral-caudal field of view (FOV<sub>z</sub>), not available (NA)
### Supplementary Table 3. Signal to noise ratios (SNR) and ratios of gray matter to white matter volume for different groups

| Group               | SNR in gray matter | Gray matter/white matter ratio |
|---------------------|--------------------|--------------------------------|
| MCAO                | 66.648 ± 14.673    | 2.326 ± 0.172                  |
| PT                  | 61.532 ± 3.764     | 2.806 ± 0.318                  |
| sham                | 77.591 ± 4.140     | 2.303 ± 0.449                  |
| all                 | 69.516 ± 11.911    | 2.414 ± 0.373                  |
| Allen brain atlas   | NA                 | 2.566 ± 0.000                  |

Supplementary Table 3. Signal to noise ratios (SNR) and ratios of gray matter to white matter volume for different groups. For SNR, signal was measured in voxels with a gray matter probability 1% in the corresponding tissue probability map resulting from the SPMMouse segmentation. Noise was measured as the SD of signal across voxels in a 20x20 voxel square in all four corners and all slices of the image volume. Gray and white matter volumes were assessed by adding probability values over all voxels in the corresponding tissue probability map from the SPMMouse segmentation. Note that SNR values of sham animals were higher since these were scanned with only 20 slices closer to the sensitivity center of the MRI coil, whereas all other animals were scanned with 32 slices. Values represent mean±SD.

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