Supplementary Materials and Methods

Edema correction by Gerriets and colleagues, 2004.

Infarct volumes measured at 24 hours were corrected with an alternative edema correction, according to the formula provided by Gerriets and colleagues:[1]

$$LV^c = HV_c + HV_i - (HV_c + HV_i - LV^u) \times \frac{HV_c + HV_i}{2 \times HV_c}$$

Where LV^c and LV^u represent the corrected and uncorrected lesion volume, respectively, and HV_c and HV_i represent the contralateral and ipsilateral hemispheres volume, respectively. For this purpose, the ipsilateral lesion areas, and the ipsilateral and contralateral hemisphere areas were manually defined on T2-weighted images using MIPAV software (Medical Image Processing And Visualization, NIH, Bethesda, MD; http://mipav.cit.nih.gov/).[2] Subsequently, HV_i, HV_c, and LV^u (expressed in mm^3) where calculated as the summation of the slice thickness (1 mm) multiplied by the ipsilateral hemispheric, contralateral hemispheric, and ipsilateral lesion areas, respectively.

Unpaired Student's *t*-test was used for two-group analysis, while correlation and linear regression analysis were computed with Pearson's *r* test. Regression analysis were not forced through 0. A value of $p<0.05$ was considered significant.

Cerebral tissue processing and Cresyl violet staining.

After animals were euthanized under deep anesthesia, brains were post-fixed by immersion in 10% formaldehyde for at least 24 hours, and coronal sections (50 µm thick) were cut on a vibratome. A number of 19 consecutive sections (250 µm interval) were stained with Cresyl violet (Bioptica, Milano, Italy). A digital camera (Nikon Coolpix P5000) adaptable to a stereomicroscope was used to obtain images of each section. Ischemic lesion, ipsilateral, and contralateral hemisphere areas where manually delineated using ImageJ image processing software (National Institute of Health, Bethesda, MD, USA). In order to correct the infarct volume for asymmetries due to cerebral edema, the lesion areas were corrected using the formula from Leach and colleagues:[3]

$$corrected\ infarct\ area = \frac{infarct\ area\ \times contrataleral\ hemisphere\ area}{ipsilateral\ hemisphere\ area}$$

Corrected infarct volume (expressed in mm^3) was subsequently calculated as the summation of the corrected lesion areas multiplied by slice thickness (1 mm).

Correlation and linear regression analysis were computed with Pearson's *r* test. Regression analysis was not forced through 0. A value of $p<0.05$ was considered significant.

Receiver-operator curve analysis.

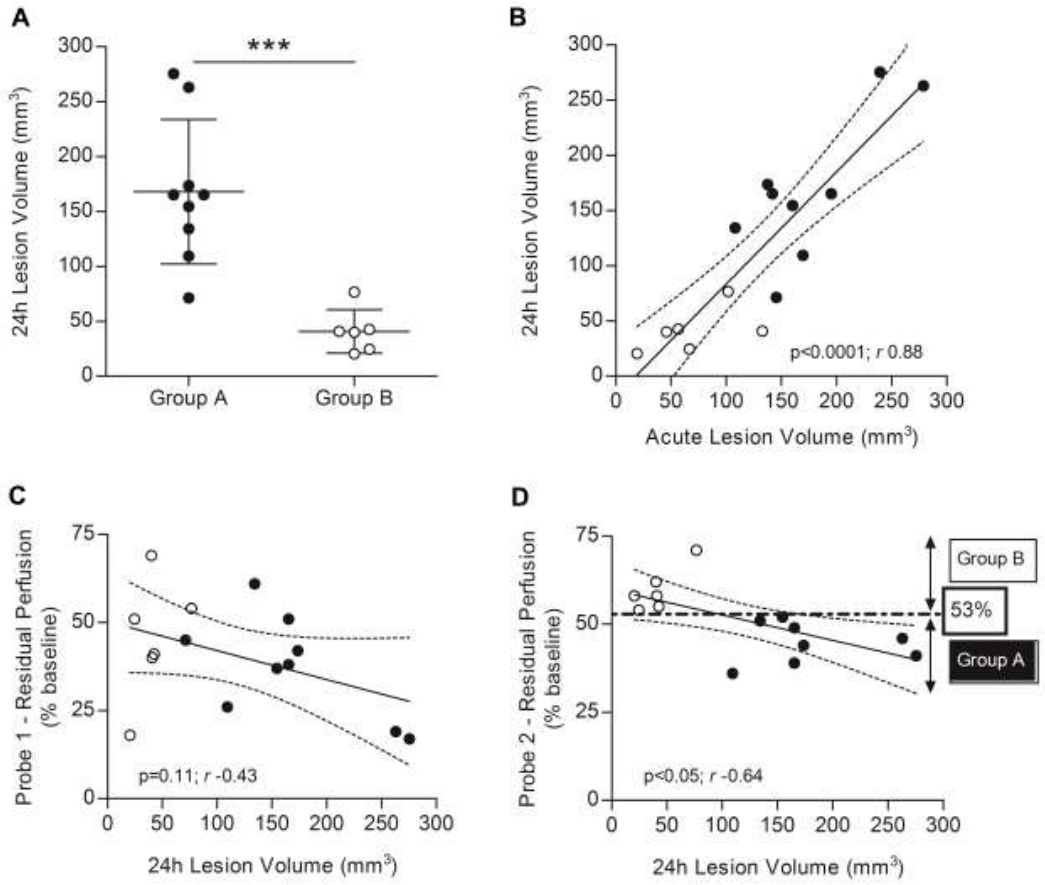
Receiver operator curve (ROC) analysis was performed to identify the optimal LDF (i.e. residual perfusion) threshold values for the prediction of animals developing a large hemispheric infarct at 24 hours. Threshold values maximizing specificity and sensitivity were identified and defined as optimal. The area under curve (AUC) was considered as an indicator of prediction ability (ranging from 1 = best to 0.5 = worst). The positive predictive value (PPV, the probability that animals predicted to develop a large hemispheric infarct, had a large hemispheric infarct at 24 hours) and negative predictive value (NPV, the probability that animals predicted to develop a basal ganglia infarct, had a basal ganglia infarct at 24 hours) were calculated as:

$$PPV\ (\%) = \frac{TP}{(TP + FP)} \times 100$$

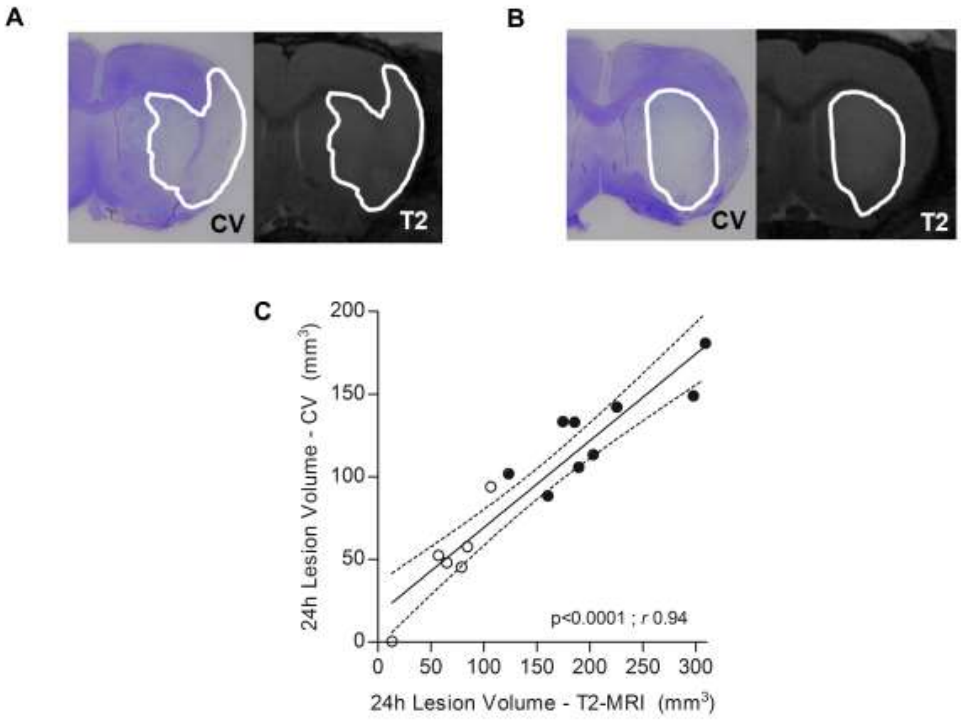
$$NPV\ (\%) = \frac{TN}{(TN + FN)} \times 100$$

Where TP represents the number of true positives, i.e. animals predicted to develop a large hemispheric infarct, and who actually developed large hemispheric infarct at 24h; FP represents the number of false positives, animals predicted to develop a large hemispheric infarct, and who instead developed basal ganglia infarct at 24h; TN represents the number of true negatives, i.e. animals predicted to develop a basal ganglia infarct, and who actually developed a basal ganglia infarct at 24h; FN represents the number of false negatives, i.e. animals predicted to develop a basal ganglia infarct, and who instead developed a large hemispheric infarct at 24h.

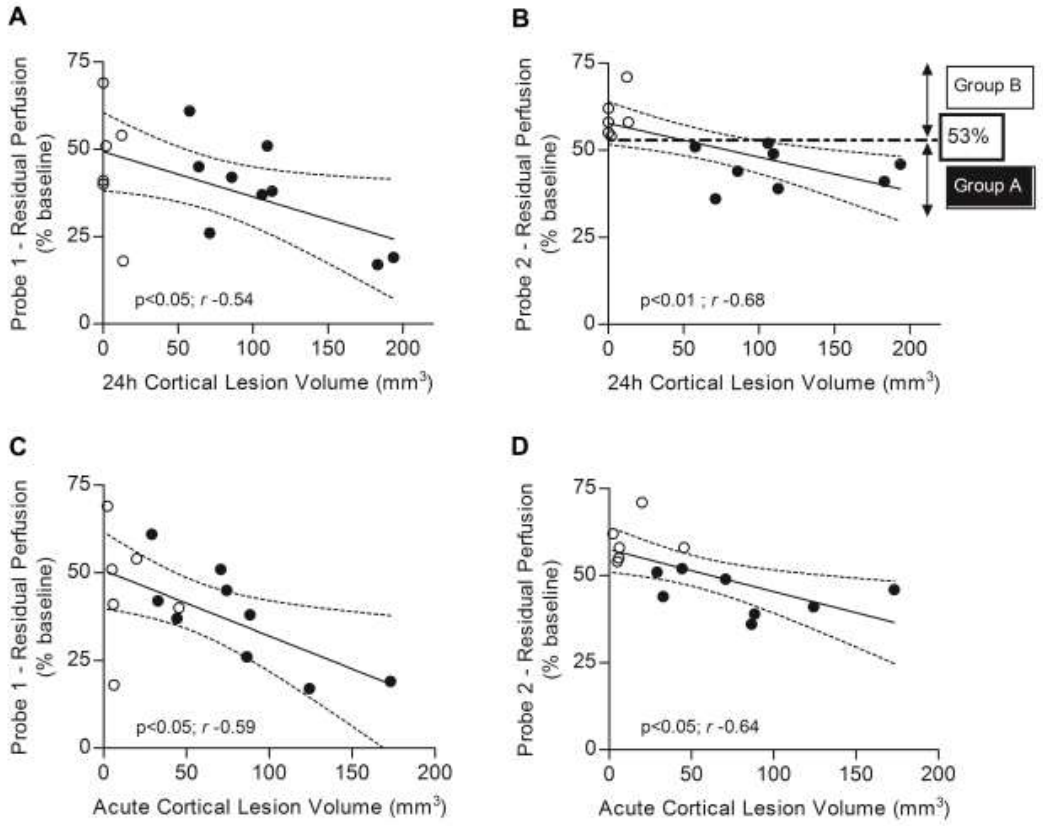
Supplementary Figures



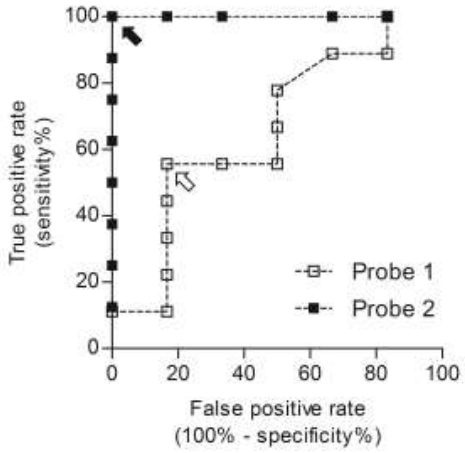
Supplementary Figure 1. Analysis for infarct volumes calculated with an alternative edema correction (Gerriets et al., 2004). **A.** Ischemic lesion volumes of Group A were significantly higher compared to Group B and a slight overlap was present between the two groups. **B.** Acute lesion volumes positively correlate with 24h lesion volumes, with a slope of 1.02 ± 0.15. **C.** No significant correlation was observed between Probe 1 perfusion values and 24h lesion volumes. **D.** A negative correlation was observed between Probe 2 perfusion values and both 24h lesion volumes. The 53% perfusion value threshold discriminating between group A and B in shown in **D** (dot-dashed line). Dotted lines represent the 95% confidence interval of the regression line. Black spots = Group A animals (n=9 in **A**, **B** and **C**; n=8 in **D**); White spots = Group B animals (n=6). *** p<0.001.



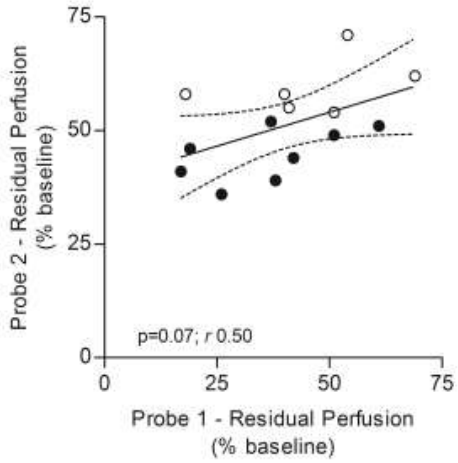
Supplementary Figure 2. Infarct lesion and volume evaluation on cerebral tissue sections stained with Cresyl violet (CV) and comparison with T2-images. Large hemispheric (**A**) and basal ganglia (**B**) infarcts visualized on T2 images and corresponding tissue sections stained with Cresyl violet. **C.** A strong positive correlation is present between infarct volumes calculated with the two methods. Dotted lines represent the 95% confidence interval of the regression line. Black spots = Group A animals (n=9); White spots = Group B animals (n=6).



Supplementary Figure 3. Multi-site LDF perfusion monitoring and correlation with 24h and acute cortical lesion volumes. A significant negative correlation was observed between LDF perfusion values recorded by Probe 1 and 24h (**A**) and acute (**C**) cortical lesion volumes. A stronger significant negative correlation was observed between LDF perfusion values recorded by Probe 2 and 24h (**B**) and acute (**D**) cortical lesion volumes. The 53% perfusion value threshold discriminating between group A and B in shown in **B** (dot-dashed line). Dotted lines represent the 95% confidence interval of the regression line. Black spots = Group A animals (n=9 in **A** and **C**; n=8 in **B** and **D**); White spots = Group B animals (n=6). LDF = laser Doppler flowmetry.



Supplementary Figure 4. ROC curves for the identification of large hemispheric infarcts for LDF residual perfusion for Probe 1 and 2. Values maximizing specificity and sensitivity for our study were identified: <39% residual perfusion for Probe 1 (white harrow) and <53% residual perfusion for Probe 2 (black arrow). AUC values for ROC curves were: 0.66 (for Probe 1) and 1 (for Probe 2). LDF = laser Doppler flowmetry; AUC = area under curve.



Supplementary Figure 5. No significant correlation was observed between Probe 1 and Probe 2 perfusion values monitored in the same animals. Black spots = Group A animals (n=8); White spots = Group B animals (n=6). *** p<0.001. LDF = laser Doppler flowmetry.

Supplementary Table

		Optimal Threshold	TP	TN	FP	FN	Specificity (95% CI)	Sensitivity (95% CI)	PPV	NPV
			(n/total)				(%)			
LDF	Probe 1	<39%	5/15	5/15	1/15	4/15	83 (36-100)	56 (21-86)	83	56
	Probe 2	<53%	8/14	6/14	0/14	0/14	100 (54-100)	100 (63-100)	100	100

Supplementary Table 1. Optimal thresholds for the prediction of large hemispheric infarcts for LDF residual perfusion for Probe 1 and 2 and related: true positive/negative and false positive/negative numbers, specificity (95% CI), sensitivity (95% CI), and positive/negative predictive values. Values maximizing specificity and sensitivity were identified on ROC curves and considered as optimal threshold values. LDF = laser Doppler flowmetry; TP = true positive; TN = true negative; FP = false positive; FN = false negative; CI = confidence interval; PPV = positive predictive value; NPV = negative predictive value.

References

1. Gerriets T, Stolz E, Walberer M, Müller C, Kluge A, Bachmann A *et al.* Noninvasive quantification of brain edema and the space-occupying effect in rat stroke models using magnetic resonance imaging. *Stroke* 2004; 35(2)**:** 566-71.

2. McAuliffe MJ, Lalonde, F.M., McGarry D, Gandler W, Csaky K, Trus BL. Medical Image Processing, Analysis and Visualization in clinical research. In: *14th IEEE Symposium on Computer-Based Medical Systems, 2001. CBMS 2001.* Bethesda, MD, 2001. pp 381 - 386.

3. Leach MJ, Swan JH, Eisenthal D, Dopson M, Nobbs M. BW619C89, a glutamate release inhibitor, protects against focal cerebral ischemic damage. *Stroke* 1993; 24(7)**:** 1063-7.