ELSEVIER

Journal of Neuroscience Methods 156 (2006) 136–139

JOURNAL OF NEUROSCIENCE METHODS

www.elsevier.com/locate/jneumeth

# Comparison of estimates for volumes of brain ablations derived from structural MRI and classical histology

Anke Deutscher[a], Heiko G. Niessen[c], Frank Angenstein[b,c], Jürgen Goldschmidt[a,b], Henning Scheich[a,b], Holger Schulze[a,*]

[a] *Leibniz Institute for Neurobiology, Department Auditory Learning & Speech, Brenneckestraße 6, 39118 Magdeburg, Germany*
[b] *Leibniz Institute for Neurobiology, Special Lab Non-Invasive Brain Imaging, Brenneckestraße 6, 39118 Magdeburg, Germany*
[c] *Department of Neurology II, Otto-von-Guericke University, Leipziger Strasse 44, 39120 Magdeburg, Germany*

Received 13 January 2006; received in revised form 9 February 2006; accepted 13 February 2006

**Abstract**

Estimates of auditory cortex ablation sizes in a rodent model as derived from classical histology (volume reconstructions from Nissl-stained brain sections) and structural magnetic resonance imaging (MRI) (T1-weighted whole-brain scans from a 4.7 T animal scanner) were compared in the same specimens (Mongolian gerbils). Estimates of lesion volumes obtained with the two methods were very similar, robust, highly correlated and not significantly different from each other. Hence, the general usefulness of structural MRI for the determination of brain lesion size in small animal models is demonstrated. MRI therefore seems to be well suited to determine proper size and location of an experimental brain ablation prior to (potentially extensive) behavioral testing.


*Keywords:* Brain lesion size; Auditory cortex; Cortex ablation; Magnetic resonance imaging; Nissl-staining; Gerbil

## 1. Introduction

A classical method to study brain function is the description of functional deficits associated with brain lesions, either resulting from diseases (for example stroke or injury, e.g. Zatorre, 1989; Nys et al., 2005) brain surgery (e.g. Zatorre and Penhune, 2001; Johnsrude et al., 2000), or experimentally induced in animal models (e.g. Wetzel et al., 1998a, 1998b; Ohl et al., 1999). Crucial to this approach is the exact knowledge of size and location of the brain lesion that is assumed to underlie the observed impairments. Whereas histological preparation has the advantage that size and location of brain lesions can be determined with high spatial resolution, a severe disadvantage of this method is that lesions size and location can only be measured post mortem. Furthermore, this method is prone to artifacts like shrinking or distortion due to histological techniques. On the other hand, it would be advantageous if brain lesions could be precisely characterized already in the living specimen, so that for example in an animal model the proper size and location of an experimental brain ablation could be verified prior to (potentially extensive) behavioral testing. Structural magnetic resonance imaging (MRI) seems to be a suitable method to fulfill these demands, although its in comparison to histological methods low spatial resolution may limit its usefulness in this context, especially in small animal models like rodents.

Here we compare the estimates of brain ablation sizes in a small rodent, i.e. ablations of auditory cortex in Mongolian gerbils (Meriones unguiculatus), as derived from classical histology (volume reconstructions from Nissl-stained brain sections) and structural MRI (T1-weighted whole-brain scans from a 4.7 T animal scanner) in the same specimens. The general usefulness of structural MRI for the described purpose is demonstrated.

## 2. Materials and methods

### 2.1. Cortical ablation

Similar to earlier studies (Wetzel et al., 1998a, 1998b; Ohl et al., 1999), surgery was performed under deep general anesthesia by an intraperitoneal infusion of ketamine (50 mg/ml; Ratiopharm), xylazine (Rompun 2%, BayerVital) and isotonic

* Corresponding author. Tel.: +49 391 6263322; fax: +49 391 6263328.
*E-mail address:* Holger.Schulze@IfN-Magdeburg.de (H. Schulze).

0165-0270/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jneumeth.2006.02.009

sodium chloride solution (mixture 9:1:10) at a rate of 0.06 ml/h (applied every half hour), after an initial doss of 0.2 ml. Body temperature was maintained at 37 °C using a remote-controlled heating blanket. The skin over the skull and over the temporal bone on both sides was cut and retracted, the musculature covering the temporal bones was partly removed. The auditory cortex was then exposed by craniotomy and thermocoagulated. Finally, the skin over the trepanation area was sealed again using Histoacryl (Braun). The whole procedure was usually completed after 1–1.5 h.

### 2.2. Structural MRI

For in vivo MRI experiments, gerbils were anesthetized with 1.5–2% isoflurane (in 70:30 $N_2O$:$O_2$; volume ratio) and secured using a head-holder with bite bar to reduce motion artifacts. MRI experiments were performed on a Bruker Biospec 47/20 scanner at 4.7 T (free bore diameter of 20 cm) equipped with a BGA 12 (200 mT/m) gradient system. A 35 mm Litzcage coil system (DotyScientific Inc., Colombus, SC, USA) was used for RF excitation and signal reception. A continuous 3D data set in horizontal slice orientation was measured using an modified driven equilibrium Fourier transform (MDEFT) sequence with the following parameters: TR 21.18 ms, TE 3.5 ms, flip angle 15°, field of view (FOV) 30 mm × 30 mm × 32 mm, matrix size 256 × 256 × 32, number of averages 10. FOV and matrix size yield an in-plane resolution of 117 μm × 117 μm and a slice thickness of 1 mm. The total scanning time was 115 min for a 3D data set of MR images.

### 2.3. Histology

For histological verification of lesion size, Nissl-stained brain sections were obtained from each animal. Therefore animals were sacrificed by an intrapulmonary injection of T61 (Intervet) and then decapitated. Brains were removed, mounted on object slides with 8% gelatine and frozen (−50 °C isopentane) to minimize spatial distortion or shrinkage of the tissue. Horizontal sections of 40 μm were obtained using an cryostat (−6 °C object temperature; −15 °C blade temperature) and stained with cresyl violet.

### 2.4. Data analysis

Lesion volumes on the MR images were measured using the public domain Java-based imaging processing and analysis program ImageJ (http://rsb.info.nih.gov/ij/). There, the contour of the lesion as visible in the MR images was determined manually (cf. Fig. 1). Size of the resulting plane was computed by the computer program. Estimated volume of the lesion could then be computed by multiplying the total lesion area of all slices with the slice thickness of 1 mm. Depending on lesion size, a lesion typically encompassed 3–5 MR slices.

Analogously, lesion volumes were estimated from Nissl-stained brain sections: series of horizontal sections of each brain containing the complete lesion were camera-scanned (Leica M420, Grundig FA87d, Germany) and mounted using a two-dimensional computer-aided graphic program (N.I.H.-Image program by W. Rasbande, Adobe Photoshop, Macintosh). Again, the contour of the lesion was determined manually (cf. Fig. 2), the lesioned outer cortical border was interpolated from the remaining cortical surface. Lesion volume was again computed by multiplying the total lesion area as calculated by the software with the slice thickness of 40 μm. Depending on lesion size, a lesion typically encompassed 60–80 Nissl sections. Data from MRI and histology were analyzed independently by different experimenters.

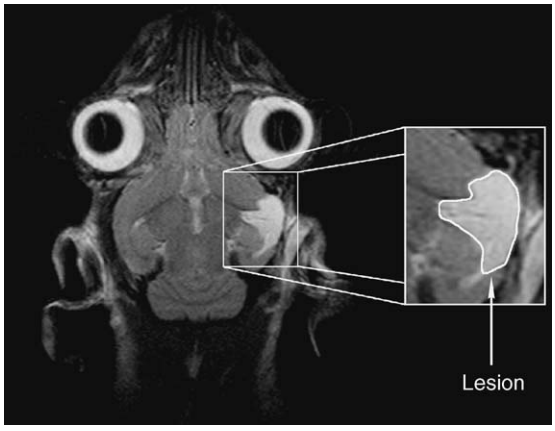


Fig. 1. Example of one horizontal slice from a T1 -weighted whole-brain MRI scan of a gerbil brain obtained with a 4.7 T animal scanner (cf. Section 2). The auditory cortex lesion is visible as a light grey area within the right temporal lobe. An enlarged version of this lesioned area is shown in the inset. The outer contour of the lesion was determined manually (white line in inset). Estimates of the size of the lesioned plane were obtained from these contours by a computer program.

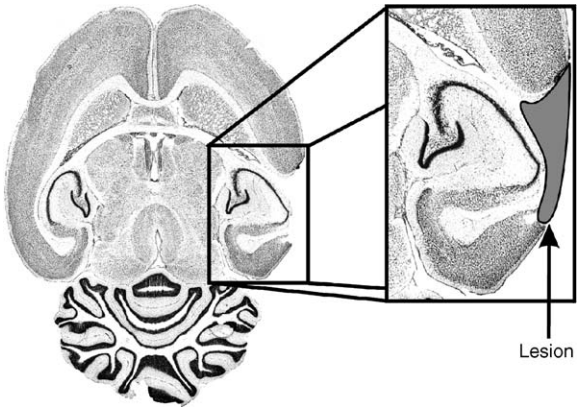


Fig. 2. Example of one horizontal section from a Nissl-stained gerbil brain (cf. Section 2). The auditory cortex lesion is visible in the left temporal lobe. An enlarged version of this lesioned area is shown in the inset. Series of such horizontal sections were camera-scanned and the outer contour of the lesion was determined manually, the lesioned outer cortical border was interpolated from the remaining cortical surface (grey area in inset). Estimates of the size of the lesioned plane were obtained from these contours by a computer program.

## 3. Results

A total of 19 auditory cortex lesions of 19 Mongolian gerbil brain hemispheres were analyzed by both MRI and

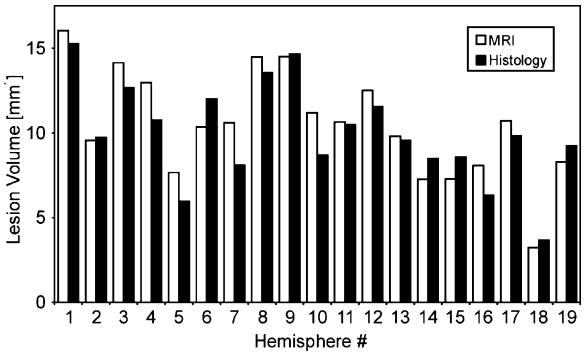


Fig. 3. Estimates of lesion volumes as obtained with the two methods (MRI: open bars; histology: filled bars) from auditory cortex ablations of 19 hemispheres. Volume estimates from MRI could be slightly larger ($N = 12$) or smaller ($N = 7$) than the corresponding estimates from Nissl histology. In general, both methods produced similar results.

histology. In general, the two different methods produced were similar estimates of lesion volumes (Fig. 3). Absolute lesion volumes as estimated from MR images ranged from 3.24 to 16.04 $mm^3$ (mean = 10.49 $mm^3$; S.D. = 3.17 $mm^3$); those estimated from Nissl-stained slices ranged from 3.67 to 15.26 $mm^3$ (mean = 9.96 $mm^3$; S.D. = 2.95 $mm^3$). Volumes estimated from MR images were slightly bigger than those from Nissl analysis in 12 cases, for the remaining 7 cases Nissl analysis gave larger volumes than MR. A paired $t$-test revealed no significant difference between the estimates of the two methods ($p = 0.09$). On the other hand, a significant correlation between the two measures could be demonstrated (Fig. 4; Pearson: $r = 0.912$, $p < 10^{-7}$). Linear regression between the data points from MRI and histology revealed a line close to $y = x$ ($y = 0.98x + 0.73$; with intersections fixed to 0: $y = 1.05x$).

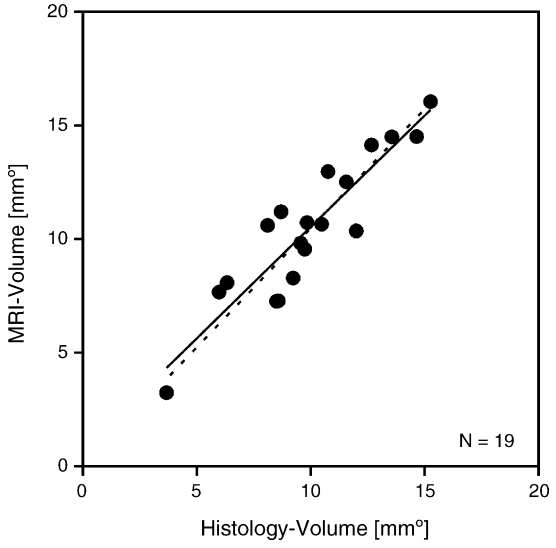


Fig. 4. Correlation of lesion volume estimates obtained from MRI and Nissl histology. Results from the two methods were highly correlated (Pearson: $r = 0.912$, $p < 10^{-7}$). The linear regression line between the data points from MRI and histology was close to $y = x$ ($y = 0.98x + 0.73$ (solid line); with intersections fixed to 0: $y = 1.05x$ (dotted line)).

## 4. Discussion

In this report, we demonstrated that volume estimates for brain lesions in a small rodent derived from classical histology (Nissl-staining) and structural MRI are highly correlated and therefore lead to very similar and robust results, despite a number of methodological problems specific for the two methods used: for example, one might have expected that classical (post-mortem) histology leads to wrong estimations, either over- or underestimations, of the actual lesion volume in vivo because of distortions induced by sectioning of the brain tissue, especially within the lesioned area of each brain section. On the other hand, volume estimates from MR images could be expected to overestimate the actual volume because of the comparably low spatial resolution and the in relation to the total brain size relatively big slice thickness. Nevertheless, when both methods were performed and compared in identical specimen, no such trend could be observed: the results of both methods were very similar, robust, highly correlated and not significantly different. In general, this result is in line with earlier studies comparing brain lesion size estimates of MRI and histology in rat stroke models (Palmer et al., 2001; Kloss et al., 2002; Chen et al., 2004) and comparable studies in other organs (cf. Bremer et al., 2002; Kristoffersen Wiberg et al., 2003), although there are certain differences: for example, MRI may lead to over- or underestimations of lesion size compared to other measures (Kloss et al., 2002; Bremer et al., 2002; Kristoffersen Wiberg et al., 2003). Furthermore, no study on brain lesion size so far demonstrated a linear positive correlation of volume measures obtained from the different methods (MRI and histology) with a slope of the linear regression line close to 1 and a $y$-axial section close to 0, that is, no significant difference between the estimates of the two methods. That is, we demonstrated for the first time in our experiments how exact MRI in vivo analysis with many contrast variables reflects histological organ defects, namely a 1 to 1 correlation and not some (trivial) volume correlation. Our finding is particularly compelling as data from the two measures were analyzed by different experimenters so that a subjective bias that would influence the results of both measures in similar directions can be excluded.

We conclude from these results that structural MR imaging is a useful tool for determining lesion volumes already in living specimen, even in small animals like rodents where the precision of size and location of experimentally induced brain ablations are more critical and at the same time harder to control than in larger animals. In contrast to classical histology, this method therefore enables the investigator to evaluate proper size and location of brain lesions prior to further animal testing. Hence, using MRI to estimate lesion volume time consuming experiments like behavioral training can be avoided in animals where lesion size or location does not match experimental needs. Using MRI in the described way should therefore enable a significant reduction of time and animal number in behavioral lesion studies. With the advancement of system's understanding of the brain and cognitive neuroscience, whole-brain imaging in normal, structurally modified transgenic and selectively lesioned animal brains in conjunction with behavioral testing becomes

more and more important. There is a serious problem today that fewer researchers want to build their career on time consuming behavioral testing necessarily followed by tedious histology. MR structural analysis, especially in conjunction with functional MRI, seems to be an elegant method to preselect relevant living individuals after brain manipulations for further behavioral and other work and finally for selective histology. Our report represents the proof of principle that structural MRI is indeed a well suited method that meets these demands. With the present growth of the market for animal scanners and their falling prices, it seems clear that this will be a widespread approach in the future.

## Acknowledgements

A.D. was supported by grant SCHU 1272/2-1 of the Deutsche Forschungsgemeinschaft (DFG) to Dr. Schulze. H.G.N. and F.A. were supported by the DFG (SFB/TR3, TP A7) and the Center of Advanced Imaging Magdeburg (CAI, BMBF-grant 01GO0202).

We are grateful to Kathrin Gruss for her skillful technical assistance in camera-scanning Nissl sections.

## References

Bremer C, Kreft G, Filler T, Reimer P. Accuracy of non-enhanced MRI to monitor histological lesion size during laser-induced interstitial thermotherapy. Eur Radiol 2002;12:237–44.

Chen F, Suzuki Y, Nagai N, Peeters R, Coenegrachts K, Coudyzer W, Marchal G, Ni Y. Visualization of stroke with clinical MR images in rats: a feasibility study. Radiology 2004;233:905–11.

Johnsrude IS, Penhune VB, Zatorre RJ. Functional specificity in the right human auditory cortex for perceiving pitch direction. Brain 2000;123:155–63.

Kloss CUA, Thomassen N, Fesl G, Martens KH, Yousri TA, Hamann GF. Tissue-saving infarct volumetry using histochemistry validated by MRI in rat focal ischemia. Neurol Res 2002;24:713–8.

Kristoffersen Wiberg M, Aspelin P, Sylvan M, Bone B. Comparison of lesion size estimated by dynamic MR imaging, mammography and histopathology in breast neoplasms. Eur Radiol 2003;13:1207–12.

Nys GMS, van Zandvoort MJE, van der Worp HB, de Haan EHF, de Kort PLM, Kapelle LJ. Early depressive symptoms after stroke: neuropsychological correlates and lesion characteristics. J Neurol Sci 2005;228:27–33.

Ohl FW, Wetzel W, Wagner T, Rech A, Scheich H. Bilateral ablation of auditory cortex in Mongolian gerbil affects discrimination of frequency modulated tones but not of pure tones. Learn Mem 1999;6:347–62.

Palmer GC, Peeling J, Corbett D, Del Bigio MR, Hudzik TJ. T2-weighted MRI correlates with long-term histopathology, neurology scores, and skilled motor behavior in a rat stroke model. Ann N Y Acad Sci 2001;939:283–96.

Wetzel W, Wagner T, Ohl FW, Scheich H. Categorical discrimination of direction in frequency-modulated tones by Mongolian gerbils. Behav Brain Res 1998a;91:29–39.

Wetzel W, Wagner T, Ohl FW, Scheich H. Right auditory cortex lesion in Mongolian gerbils impairs discrimination of rising and falling frequency-modulated tones. Neurosci Lett 1998b;252:115–8.

Zatorre RJ. Intact absolute pitch ability after left temporal lobectomy. Cortex 1989;25:567–80.

Zatorre RJ, Penhune VB. Spatial localization after excision of human auditory cortex. J Neurosci 2001;21:6321–8.