1H/13C MR spectroscopic imaging of regionally specific metabolic alterations after experimental stroke

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Loss of function and subsequent spontaneous recovery after ischaemic stroke are associated with functional and structural alterations in brain tissue. Acute functional tissue damage involves distortion of key metabolic processes, such as oxidative glycolysis and neurotransmitter metabolism. Nevertheless, initially perturbed metabolism may be restored at later stages, e.g. in perilesional areas, which could play a key role in post-stroke recovery of brain function. The pattern of metabolic recovery in relation to ischaemic tissue damage, however, is basically unknown. The goal of our study was to reveal changes in glycolysis and glutamatergic neurotransmitter metabolism that could underlie post-stroke changes in functional status. We performed in vivo 1H/13C magnetic resonance spectroscopic imaging (MRSI) during 13C-labelled glucose infusion, and MRI, at 24 h (n = 6) and 3 weeks (n = 8) after stroke in a rat model to characterize alterations in baseline metabolite levels, glutamate (Glu) and glutamine (Gln) turnover, and active lactate (Lac) formation in areas with different degrees of ischaemic injury. Inside the lesion, we detected significant reductions in baseline metabolite levels, ongoing Lac formation and seriously diminished Glu and Gln turnover at both time points, indicative of irreversible functional tissue damage. In perilesional areas, significant decrease of N-acetyl aspartate (NAA) levels, and Glu and Gln turnover indicated neuronal dysfunction at 24 h. After 3 weeks, when animals showed significant neurological improvement, anaerobic glycolysis had ceased, NAA levels were normalized, Glu turnover was maintained and Gln turnover had recovered. These findings point out that early metabolic impairment in the lesion borderzone can be restored over time. Alterations in brain metabolism in perilesional areas probably contribute significantly to changes in functional status in stroke subjects, and may provide a gateway for therapeutic strategies directed at improvement of functional recovery after stroke.

Keywords: brain metabolism; brain plasticity; magnetic resonance spectroscopy; neurotransmission; stroke

Abbreviations: MRSI = magnetic resonance spectroscopic imaging; Glu = glutamate; Gln = glutamine; Lac = lactate; NAA = N-acetyl aspartate; MCA = middle cerebral artery; EPI = echo planar imaging; TR = repetition time; TE = echo time; CBV = cerebral blood volume; FE = fractional enrichment; VOI = volume-of-interest; IL = ipsilateral; CL = contralateral; Lip = lipids; Cho = choline; Ins = myo-inositol; Tau = taurine


Introduction

Stroke is the main cause of disability in the Western society. Stroke patients exhibit acute loss of function as a result of neuronal damage, but their functional status can spontaneously recover to at least some extent over time. Acutely after stroke, the ischaemic core is surrounded by an area of affected but potentially viable tissue; the penumbra. Without therapeutic intervention, the penumbra will go into infarction within the first hours after stroke. After the disappearance of the penumbra, later functional recovery must be related to other processes, e.g. brain plasticity. With use of neuroimaging techniques, we and others have demonstrated that initially affected perilesional brain areas can later on regain neuronal function (Dijkhuizen et al., 2001, 2003; Tombari et al., 2004; Jaillard et al., 2005; Weber et al., 2008), which may be associated with remodelling of structural connections (Keyvani and Schallert, 2002; Carmichael, 2003; Nudo, 2006; van der Zijden et al., 2008).
Such brain reorganization is believed to be the key to functional recovery after stroke.

Changes in neuronal and glial metabolism may play a fundamental role in post-stroke loss and recovery of brain function. Ischaemia causes distortion of key metabolic processes in the brain, i.e. glucose oxidation and glutamate (Glu)-glutamine (Gln) cycling between neurons and astrocytes (Pascual et al., 1998; Haberg et al., 1998, 2001, 2006; Thoren et al., 2006), but it is unknown how metabolic activity recovers in relation to restoration of function. It is well conceivable that adaptations in glucose metabolism and glutamatergic neurotransmission underlie structural and functional plasticity in tissue recovering from stroke.

MR spectroscopy (MRS) provides an ideal tool for non-invasive assessment of in vivo brain metabolism. It enables detection of baseline metabolite levels as well as measurement of active metabolic pathways in humans and animal models (Novotny et al., 2003; Choi et al., 2007). Longitudinal in vivo 1H MRS can detect changes in levels of N-acetyl aspartate (NAA), a neuronal marker, and lactate (lact), the endproduct of anaerobic glycolysis (Igarashi et al., 2001). This provides insights into the metabolic status of tissue affected by cerebral ischaemia. Alternatively, 13C-based MRS methods allow measurement of actively formed 13C-labelled metabolic products after infusion of 13C-labelled glucose [for review see de Graaf et al. (2003b)]. With in vivo 1H-observed, 13C-edited (1H/13C) MRS during intravenous infusion of 13C-labelled glucose we have previously shown that penumbral tissue may be distinguished from the lesion core based on dynamics in lactate formation after acute experimental focal cerebral ischaemia (Dijkhuizen et al., 1999). Ex vivo 13C MRS studies have demonstrated that changes in neuronal and glial metabolism can be detected from altered Glu and Gln synthesis after acute focal cerebral ischaemia in rats (Haberg et al., 1998, 2001, 2006; Pascual et al., 1998). Haberg et al. (2001, 2006) showed that preserved astrocytic metabolism in the first 4 h after cerebral ischaemia differentiates the potentially salvageable penumbra zone from the irreversibly damaged lesion core.

The goal of the present study was to characterize alterations in neuronal and glial metabolism in and around an ischaemic lesion in order to elucidate changes in functional tissue status after stroke. To this aim we applied in vivo 1H/13C MRS imaging (MRSI) at ultrahigh magnetic field strength to assess regionally specific alterations in glucose metabolism and glutamatergic neurotransmission, at semi-acute and chronic stages after experimental stroke. We hypothesized that temporary functional deficit of morphologically intact tissue at the border of an ischaemic lesion is reflected in transient dysfunction of neuronal and glial metabolism.

Materials and Methods

Animal preparation

Animals were studied in accordance with the guidelines established by the Yale Animal Care and Use Committee.

Fourteen male Wistar rats (200–250g) were anaesthetized with 1.2% halothane in N2O/O2 (70:30) under spontaneous respiration. Blood oxygen saturation and heart rate were continuously monitored during surgical procedures. Body temperature was maintained at 37.0 ± 0.5°C by means of a heating pad. Additional analgesia was provided by a pre-emptive subcutaneous injection of 1.0 mg/kg Meloxicam (Metacam TM, Sigma Aldrich, St Louis, MO, USA).

Transient focal cerebral ischaemia was induced by 90 min occlusion of the right middle cerebral artery (MCA) with an intraluminal filament (Longa et al., 1989). In brief, a 4.0 silicon-coated polypropylene suture (Ethicon, Piscataway, NJ, USA) was introduced into the right external carotid artery and advanced through the internal carotid artery until a slight resistance was felt, indicating that the MCA was occluded. After 90 min, the filament was withdrawn from the internal carotid artery to allow reperfusion. After surgery, rats received a subcutaneous injection of 5 ml saline to compensate for loss of water and minerals. Post-operative analgesia was provided through Meloxicam (1 mg/kg/day) in drinking water up to 48 h after stroke induction.

Animals were fasted for 12–16 h before MR experiments. At 24 h (24 h group; n = 6) or 3 weeks (3 w group; n = 8) after stroke induction, rats were anaesthetized, tracheotomized and mechanically ventilated with 1.5% halothane in N2O/O2 (70:30). A femoral artery was cannulated for monitoring of blood gases (arterial pO2 and pCO2), pH and blood pressure. Physiological parameters were maintained within normal limits (pCO2: 33–45 mmHg; pO2: >120 mmHg; pH: 7.32–7.57; blood pressure: 90–110 mmHg). Left and right femoral veins were cannulated for infusion of [U-13C]glucose (Cambridge Isotope Laboratories Inc., Andover, MA, USA) and for injection of the intravascular iron oxide contrast agent ferumoxtran-10 (Combidex, Advanced Magnetics, Cambridge, MA, USA). During MR experiments, anaesthesia was maintained with 0.3–0.8% halothane in N2O/O2 (70:30). Rats were restrained using a head-holder and additional immobilization was achieved by i.v. injections of d-tubocurarine chloride (0.25 mg/kg every 60 min). Body temperature was measured with a rectal thermosensor and maintained at 37.0 ± 0.5°C by means of a heating pad. Rats were infused with [U-13C]glucose for 140 min according to a protocol previously described (de Graaf et al., 2003a). Blood samples were taken before and every 35 min after infusion to quantify 13C fractional enrichment of plasma glucose.

Functional examination

Animals were subjected to two behavioural tests to assess sensorimotor function. First, we applied a series of motor, sensory and tactile tests, which provided a neurological score of 0 to 20 points, with 20 as maximal deficit score (van der Zijden et al., 2008). Second, an adhesive removal test was performed (Schallert et al., 2000). A small circular sticky tape was attached to the distal-radial region of the wrist of either the left or right forelimb, and the sticky tape removal time was measured for each forelimb with a maximally allowed removal time of 60 s. Behavioural examination was performed on days 0, 4, 7, 10, 14 and 21 after stroke.

MR acquisition and processing

In vivo MR experiments were performed on an 11.74 T magnet (Magnex Scientific, Oxford, UK) equipped with a 9-cm diameter gradient-set (395 mT/m in 180 μs, Magnex Scientific, Oxford, UK) interfaced to a Bruker Avance console (Bruker, Ettlingen, Germany). Radiofrequency pulse transmission and MR signal detection for 1H
(499.814 MHz) were performed with a 14 mm diameter single-turn surface coil. Radiofrequency pulse transmission on $^{13}$C (125.7 MHz) was achieved with two orthogonal 21 mm diameter surface coils driven in quadrature.

**$T_2$-weighted MRI**

Before MRS experiments, we performed multi-echo, multi-slice $T_2$-weighted echo planar imaging (EPI) of nine slices covering the spectroscopic volume [repetition time (TR)/echo time (TE) spacing = 2500/25 ms; echo train length = 8; acquisition matrix = 64 x 64; voxel resolution = 0.3 x 0.3 x 1.0 mm$^3$].

Quantitative $T_2$ maps were calculated on a voxel-wise basis by weighted linear least-squares-fit of the logarithm of the signal intensity at different echo times versus TE.

**$^{1}H^{13}C$ MRS**

Spatial localization of a 10 x 2 x 5 mm$^3$ volume was achieved with LASER (Garwood and DelaBarre, 2001) using TR/TE = 2500/14 ms. The volume encompassed the ipsilateral as well as the contralateral cortex (Fig. 1). One-dimensional MRSI data were obtained by applying a phase-encoding gradient in the x-direction (16 steps over a 16 mm field-of-view), resulting in a 10 μl nominal voxel size. The magnetic field homogeneity was optimized using MRI-based $B_0$ mapping (Koch et al., 2006) and resulted in water linewidths of 16–20 Hz over the localized volume.

$^{1}H$ and $^{1}H^{13}C$ MR spectra were obtained during infusion of U-$^{13}$C-labelled glucose by applying a $^{13}$C inversion pulse on alternate scans (de Graaf et al., 2003a) and by calculating the difference post-acquisition. With eight averages per phase-encoding increment, the total duration of one $^{1}H^{13}C$ MRSI scan was circa 11 min. Water suppression was achieved with the sequence for water suppression with adiabatic-modulated pulses (SWAMP), an adiabatic analogue of the conventional chemical shift-selective (CHESS) water suppression (de Graaf and Nicolay, 1998).

**Perfusion MRI**

Perfusion MRI was conducted to confirm blood supply to the brain during infusion of $^{13}$C-labelled glucose. Immediately following $^{1}H^{13}C$ MRS experiments, we performed single-slice dynamic susceptibility contrast-enhanced MRI using single-shot gradient recalled EPI in combination with injection of the intravascular contrast agent Combidex (10–30 mg/kg Fe in 10 mg/ml) (TR/TE = 250/14 ms; data matrix = 64 x 64; voxel resolution = 0.3 x 0.3 x 2.0 mm$^3$; number of acquisitions = 180–300). The slice position was adjusted according to the selected MRSI volume. Relative cerebral blood volume (CBV) values were determined from the intravascular contrast-induced change in transverse relaxation rate, $\Delta R_2^*$ (Hamberg et al., 1996).

**Data analysis**

**Ischaemic lesion area**

Brain tissue with significantly prolonged $T_2$ after stroke correlates with infarcted tissue (Palmer et al., 2001; Peeling et al., 2001). Therefore the ischaemic lesion was defined as the ipsilateral area where $T_2 >$ mean $T_2 + 2$ SD in contralateral tissue.

**Metabolite concentrations**

MR spectra were used for quantification of metabolite concentrations and $^{13}$C fractional enrichments (FE) using the LCModel algorithm (Provencher, 1993). In short, the LCModel algorithm models the in vivo MR spectrum as a superposition of a basis set in vitro MR spectra of pure metabolite solutions. To complete the spectral basis set for LCModel fitting, macromolecular baseline was determined from a measurement on a healthy rat (Behar and Ogino, 1993). MRS signals from lipids have been shown to significantly increase after ischaemic brain injury (Gasparovic et al., 2001). Therefore, three macromolecular signals observed at $\sim$0.8, $\sim$1.3 and $\sim$2.7 ppm in ischaemic areas were added to the model. Total metabolite concentrations were determined from individual $^{1}H$-$^{13}C$ MR spectra prior to infusion of [U-$^{13}$C]glucose assuming the concentration of total creatine, as an internal standard, to be 10 mM (Pfeuffer et al., 1999). In the lesion at 24 h and 3 weeks, and in the lesion borderzone at 3 weeks, the concentration of total creatine was significantly reduced and the internal concentration standard total creatine of the contralateral homologues VOI was used for metabolite quantification. The $^{1}H$ surface coil's $B_1$ profile did not show consistent differences between the left and right side. Furthermore, equivalent $B_1$ fields in ipsi- and contralateral homologues regions were ascertained by positioning the coil symmetrically over the brain midline, parallel to the selected MRSI column.

**Metabolite turnover**

To determine the kinetics of [4-$^{13}$C]Glu and [4-$^{13}$C]Gln enrichment, as well as [3-$^{13}$C]Lac formation (overlap of lactate and lipids signals in $^{1}H$ MR spectra precluded reliable FE calculation

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**Fig. 1** MRSI column displaying volumes-of-interest [ipsilateral lesion (lesion); ipsilateral lesion borderzone (border); ipsilateral non-ischaemic tissue (normal); and their contralateral counterparts] overlaid on an average $T_2$ map of five adjacent brain MRI slices (corresponding with the coverage of the MRSI volume) at 3 weeks after stroke. The lesion is characterized by a prolonged $T_2$. Note that voxels outside the lesion, particularly in the borderzone, include part of the lateral ventricle, which was largely equal in size and signal intensity between ipsi- and contralateral voxels.
for Lac), individual spectra across animals at 24 h (n=6) or 3 weeks post-stroke (n=8) were added for each volume-of-interest (VOI) and time-point, and fitted with LCModel. Subsequently, the metabolite turnover time-courses were fitted with a one-phase exponential:

\[ y = y_{\text{max}} [1 - \exp(-kx)] \]

where \( x \) is time after onset of [U-13C]glucose infusion; \( y \) is FE of [4-13C]Glu or [4-13C]Gln or concentration of [3-13C]Lac; \( y_{\text{max}} \) is steady state FE or concentration, and \( k \) is the turnover rate constant. Because Gln synthesis is limited by Glu supply, FE of [4-13C]Gln is unlikely to exceed FE of [4-13C]Glu. Therefore, \( y_{\text{max}} \) was thresholded at the calculated steady state FE of [4-13C]Glu for fitting of the [4-13C]Gln turnover time-courses.

**Region-of-interest analysis**

MRI and MRSI data were analysed in different VOIs that incorporated distinct tissue conditions based on the extent of the lesion. The 16 MRSI voxels were overlaid on the corresponding T2 maps. Circa eight voxels fell within the brain volume and could be used for further analysis. Voxels that included tissue outside the brain were discarded. VOIs included: (i) the ipsilateral lesion area (T2-lesion); (ii) the ipsilateral lesion borderzone (MRSI voxel adjacent to T2-lesion area) (ILborder) and (iii) ipsilateral non-ischaemic tissue [MRSI voxel(s) with normal T2 values, adjacent to lesion borderzone voxel] (ILnormal). Depending on the lesion size, VOIs consisted of 1–3 MRSI voxels. MR spectra of voxels within the same VOI were added for further analysis. Contralateral counterparts served as control VOIs (CLlesion, CLborder and CLnormal). Figure 1 shows location of the VOIs with respect to the T2-lesion area.

**Statistics**

All values are expressed as mean ± SD. The error of the calculated concentration value by LCModel fitting was estimated for each metabolite MR signal by Monte Carlo Simulation. Differences in functional status were analysed with a one-way repeated measures analysis-of-variance (ANOVA) with post hoc multiple comparison t-testing with Bonferroni correction. Differences between ipsi- and contralateral FE (for [4-13C]Glu and [4-13C]Gln) and concentration (for [3-13C]Lac) at the latest time-point were analysed using a one-way repeated measures ANOVA followed by a Student–Newman–Keuls test. Differences between VOIs were analysed using a one-way ANOVA with post hoc multiple comparison t-testing with Bonferroni correction. \( P < 0.05 \) was considered significant.

**Results**

**Neurological status**

All rats demonstrated substantial functional deficits at day 4 after stroke [neurological score: 7.3 ± 1.3 (versus 0.1 ± 0.4 at day 0; \( P < 0.05 \)]; adhesive removal time: 56.0 ± 7.5 s (versus 7.4 ± 6.3 s at day 0; \( P < 0.05 \)], which significantly improved towards day 21 after stroke (neurological score: 5.1 ± 1.3; adhesive removal time: 22.5 ± 13.6 s) \( (P < 0.05 \) versus day 4).

**Ischaemic lesion area**

Unilateral ischaemic lesions were characterized by a significant increase in T2 and were included in part of the MRSI column, e.g. the affected somatosensory cortex (Fig. 1). T2 values in the different VOIs are shown in Table 1. T2 was significantly prolonged in the lesion as compared to contralateral. In the lesion borderzone and in non-ischaemic ipsilateral tissue, T2 values were not significantly different from contralateral values. The coordinates of the lesion borderzone VOI were not significantly different between the 24 h (2.2 ± 0.4 mm lateral from midline) and 3 w groups (2.5 ± 0.9 mm lateral from midline).

Relative CBV values (percentage of contralateral) in the lesion and borderzone VOIs were 126 ± 37% and 139 ± 53%, respectively, at 24 h, and 86 ± 56% and 68 ± 25%, respectively, at 3 weeks post-stroke.

**Metabolic alterations**

**Total metabolite concentrations**

MRS data quality, accuracy of LCModel fitting and the severity of stroke-induced metabolic changes are

| Table 1 | T2 ± SD (ms) in IL and CL VOIs at 24 h (24h) and 3 weeks (3w) after stroke |
|---------|-----------------|-----------------|-----------------|
|         | IL | CL | IL | CL | IL | CL |
| 24h     | 36.9 ± 4.1 | 397 ± 5.5 | 45.2 ± 8.2 | 46.5 ± 8.7 | 55.1 ± 2.9 | 40.6 ± 5.0 |
| 3w      | 471 ± 3.7 | 456 ± 3.9 | 53.6 ± 3.3 | 499 ± 5.0 | 80.0 ± 8.1 | 44.0 ± 3.8 |

\( ^* P < 0.05 \) as compared to contralateral. Note that T2 values in the borderzone (IL and CL) are elevated due to partial inclusion of ventricles.
Table 2 Total metabolite concentrations [mean ± SD (mmol/l)] in ipsi- and contralateral VOIs at 24 h (24h) and 3 weeks (3w) after unilateral stroke

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Borderzone</th>
<th>Lesion</th>
<th>Uncertainty (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IL</td>
<td>CL</td>
<td>IL</td>
<td>CL</td>
</tr>
<tr>
<td>Cho</td>
<td>24 h</td>
<td>6.5 ± 0.8°</td>
<td>9.3 ± 1.3</td>
<td>8.0 ± 2.2°</td>
</tr>
<tr>
<td></td>
<td>3 w</td>
<td>7.2 ± 1.7</td>
<td>7.8 ± 3.2</td>
<td>9.0 ± 4.1</td>
</tr>
<tr>
<td>Gln</td>
<td>24 h</td>
<td>3.9 ± 1.0</td>
<td>3.8 ± 1.1</td>
<td>5.1 ± 0.6°</td>
</tr>
<tr>
<td></td>
<td>3 w</td>
<td>4.8 ± 0.8</td>
<td>4.2 ± 0.7</td>
<td>4.5 ± 1.0</td>
</tr>
<tr>
<td>Glu</td>
<td>24 h</td>
<td>9.2 ± 1.2</td>
<td>9.0 ± 0.5</td>
<td>10.2 ± 2.4</td>
</tr>
<tr>
<td></td>
<td>3 w</td>
<td>8.7 ± 1.0</td>
<td>8.6 ± 1.0</td>
<td>5.7 ± 1.7°</td>
</tr>
<tr>
<td>Ins</td>
<td>24 h</td>
<td>7.0 ± 0.4</td>
<td>7.7 ± 1.0</td>
<td>7.9 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>3 w</td>
<td>11.0 ± 1.0</td>
<td>9.1 ± 0.8</td>
<td>12.3 ± 1.0</td>
</tr>
<tr>
<td>Lac/Lip</td>
<td>24 h</td>
<td>3.0 ± 1.5</td>
<td>2.2 ± 0.8</td>
<td>7.7 ± 4.0°</td>
</tr>
<tr>
<td></td>
<td>3 w</td>
<td>2.4 ± 0.7</td>
<td>1.0 ± 0.5</td>
<td>14.5 ± 9.8°</td>
</tr>
<tr>
<td>NAA</td>
<td>24 h</td>
<td>8.9 ± 1.1</td>
<td>10.3 ± 0.5</td>
<td>7.4 ± 1.0°</td>
</tr>
<tr>
<td></td>
<td>3 w</td>
<td>10.5 ± 2.1</td>
<td>10.8 ± 1.4</td>
<td>9.2 ± 1.1</td>
</tr>
<tr>
<td>Tau</td>
<td>24 h</td>
<td>7.9 ± 0.5</td>
<td>7.7 ± 0.9</td>
<td>9.2 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>3 w</td>
<td>8.8 ± 0.7</td>
<td>8.0 ± 1.2</td>
<td>9.2 ± 0.9</td>
</tr>
</tbody>
</table>

°P < 0.05 as compared to contralateral. The last column represents the error (%) of a typical fit of a MR spectrum from CLnormal calculated by Monte Carlo Simulation.

demonstrated in Fig. 2, which shows representative measured and fitted 1H MR spectra obtained from a healthy brain region (CLnormal at 24 h post-stroke; Fig. 2A) and from a lesioned area at 3 weeks after stroke (ILlesion; Fig. 2B). Most of the metabolite signals, except for the lactate/lipids (Lac/Lip) and lipids peaks at ~1.3 and ~0.8 ppm, respectively, were clearly diminished inside the lesion.

Total concentrations of metabolites as calculated with the LCModel algorithm, are presented in Table 2. The last column in Table 2 presents the uncertainties of a typical fit of a 1H MR spectrum from a non-ischaemic VOI as determined by a Monte Carlo simulation with 100 iterations. Only metabolites fitted with uncertainties <15% were included in our analysis. At 24 h after stroke, we found significant reductions in the concentration of Glu, choline (Cho) and NAA, and an increase in Lac/Lip signal in ILlesion. Cho and NAA levels were significantly reduced in ILborder, while Glu levels were increased in this area. A decrease in Cho signal was detected in ILnormal.

At 3 weeks after stroke, Glu, Gln and NAA levels were significantly decreased, and Lac/Lip was still increased in ILlesion. In ILborder, Glu was decreased and Lac/Lip was increased. There were no significant changes in ILnormal. At both time-points we found no changes in myo-inositol (Ins) and taurine (Tau) concentrations in any of the VOIs.

**Dynamic metabolite formation**

Figure 3 shows localized 1H/13C MR spectra from the different VOIs obtained at 132 ± 2 min after onset of infusion of [U-13C]glucose at 24 h and 3 weeks after stroke. Excellent sensitivity and optimal spectral resolution of the 1H/13C MR spectra allowed separate detection of [4-13C]Glu and [4-13C]Gln signals. Clearly, Glu and Gln formation were equal in ILnormal and CLnormal. Inside the lesion, Glu and Gln signals were considerably reduced, particularly at 3 weeks after stroke. In ILborder, formation of Glu and Gln was moderately decreased as compared to contralateral at 24 h post-stroke, but the [4-13C]Gln signal was normalized after 3 weeks. [3-13C]Lac formation was evident in ILlesion and ILborder after 24 h, which largely diminished after 3 weeks.

FE time-courses and rate constants for Glu and Gln for all VOIs, calculated from group-wise summed spectra, are shown in Figs 4 and 5, respectively. Both for Glu and Gln, turnover curves were comparable in ILlesion and ILborder at both time-points. At 24 h after stroke, FE of Glu and Gln at the latest time-point [FEt(max)] were significantly reduced in ILlesion and ILborder as compared to their contralateral counterparts. Turnover rate constants (k) for Glu and Gln were also clearly decreased in these areas. After 3 weeks, FEt(max) of Glu and Gln was still significantly reduced in ILlesion, but not in ILborder. For Glu, k remained decreased in both these VOIs after 3 weeks. However, k of Gln had recovered in ILborder at this time-point.

Time-courses of [3-13C]Lac formation are shown in Fig. 6. Clear Lac turnover was detected in IL lesion and ILborder at 24 h after stroke, which was still existing after 3 weeks in ILlesion but not in ILborder.
Discussion

In this study we combined ultrahigh-field in vivo $^1$H/$^{13}$C MRSI and MRI to characterize changes in glucose metabolism and glutamatergic neurotransmission in relation to brain tissue status and functional recovery after experimental stroke. Baseline metabolite concentrations, and dynamic formation of Glu, Gln and Lac were measured in lesional, perilesional and unaffected brain areas at 24 h and 3 weeks post-stroke. Our main finding is that (semi-) acutely impaired brain metabolism in perilesional tissue...
recovers at chronic stages, which may play a fundamental role in retrieval of neuronal function after early stroke-induced dysfunction. Below we describe differences in brain tissue condition after cerebral ischaemia and discuss how changes in neuronal and glial metabolism may account for functional loss and subsequent recovery.

Ischaemic lesion

Transient unilateral MCA occlusion resulted in a lesion with highly prolonged T2 and marked decrease in NAA, indicative of severe neuronal injury. Concentrations of most other detectable metabolites were also significantly reduced. Glu levels, on the other hand, were maintained at 24 h post-stroke. This may reflect its well-described increased extracellular release and/or reduced uptake after ischaemia [see Nishizawa (2001) for a review], or its accumulation in glial cells where it is not further metabolized into Gln due to depressed glutamine synthetase (Ottersen et al., 1996). In accordance with the latter, Gln levels were significantly diminished. After 3 weeks, Glu levels were drastically reduced, consistent with severe neuronal death. Both at 24 h and 3 weeks Glu turnover was significantly diminished in the ischaemic core reflective of strongly reduced glycolysis and tricarboxylic acid (TCA) activity in neuronal tissue. Importantly, because ischaemia was temporary, followed by reperfusion, reduced formation of glycolytic products could not be explained by lack of glucose supply. This was confirmed by perfusion MRI, which showed sustained CBV throughout the ipsi- and contralateral brain at both time-points.

Astrocytes play an important role in glutamatergic neurotransmission as these brain cells are responsible for...
the recycling of Glu. Extracellular Glu is taken up by specific transporters and subsequently converted into Gln by the enzyme glutamine synthetase through the Glu–Gln cycle. We detected reduced levels of Gln, along with diminished Gln enrichment and turnover rate at 24 h after stroke, which pointed toward astroglial injury. The partial recovery of Gln levels after 3 weeks may be explained by reactive astrocytosis, which typically arises in chronic, structurally damaged ischaemic tissue (Petito et al., 1990). Yet, our data suggest that such proliferation of glial cells is not accompanied by enhanced Gln synthesis.

Lactate/lipid signals were highly elevated after 24 h and 3 weeks in the lesion area. Significant [3-13C]Lac production, reflective of ongoing anaerobic glycolysis, was particularly evident after 24 h. Considerably reduced lactate formation from infused 13C-glucose at 3 weeks after temporary ischaemia in the largely necrotic lesion core, suggests that MRS-detectable lipids strongly contribute to the 1H MR signals around 1.3 ppm, which is in agreement with Gasparovic et al. (2001) and Harada et al. (2007). High amount of lipids probably reflects accumulation of membrane degradation products inside infiltrated macrophages (Gasparovic et al., 2001).

**Lesion borderzone**

It is not surprising that the above-described cerebral ischaemic damage resulted in significant functional deficits. Nevertheless, despite severe and irreversible tissue impairment in the ischaemic lesion, all animals displayed considerable improvement of neurological function over time. This may be related to recovery of neuronal function, subsequent to initial deficiency, in brain tissue just outside the ischaemic lesion core. At 24 h after stroke we detected clear signs of

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**Fig. 5** [4-13C]glutamine turnover curves for contra- (closed circles, solid lines) and ipsilateral (open circles, dashed lines) VOIs (top: normal; middle: borderzone; bottom: lesion) at 24 h (left) and 3 weeks after stroke (right). Curves were obtained using a single-exponential fitting procedure based on data from group-wise summed spectra. Y error bars represent the estimation error calculated by Monte Carlo Simulation. Turnover rate constants for the ipsi (kI) and contralateral VOIs (kC) are displayed in the graphs. Data points at the right of the graphs represent FE at the latest time-point as calculated from individual spectra (mean ± SD). *P < 0.05 as compared to contralateral.
neuronal dysfunction in perilesional areas: NAA levels were lowered and Glu and Gln turnover were reduced. The reduced fractional enrichment of Glu along with an unaltered baseline level, similar to inside the lesion core, points toward the presence of metabolically active and inactive Glu pools. Yet, the degree of reduction of fractional enrichment and turnover rate of [4-13C]Glu was clearly less in the lesion borderzone as compared to the lesion core, suggestive of preserved oxidative glucose metabolism in surviving neurons. Furthermore, anaerobic glycolysis, evident from active [3-13C]Lac formation, was considerably less than in the lesion core.

Gln formation and Cho levels were significantly reduced in the lesion borderzone after 24 h. However, Gln turnover was still ongoing and we detected elevated baseline Gln levels. This suggests that despite astroglial impairment, cells remained metabolically active. Previous studies in acute rat stroke models have provided evidence that preservation of astrocytic metabolism may be crucial for neuronal survival (Haberg et al., 2001; Thoren et al., 2005). Active neuronal–glial interaction is important for maintenance of brain homeostasis and critical changes in the metabolic coupling between these two compartments may directly affect neuronal viability after stroke (Liu et al., 1999).

After 3 weeks there were clear indications of substantial neuronal and glial recovery in the lesion borderzone. Furthermore, the absence of significant Lac production suggested normal oxidative glycolysis. At this time-point NAA, Cho and Gln levels were normalized, Glu turnover was maintained, and Gln turnover had returned to control levels. The latter reflects recovery of the Glu–Gln neurotransmitter cycle between glutamatergic neurons and astroglia. Functional imaging studies in stroke patients and animal stroke models have reported reinstatement of neuronal activation responses in perilesional sensorimotor cortex, in association with spontaneous recovery of sensorimotor function (Dijkhuizen et al., 2001, 2003; Tombari et al., 2004; Jaillard et al., 2005; Weber et al., 2008). For example, in the same rat model as used in the

Fig. 6 [3-13C]lactate turnover curves for contra- (closed circles, solid lines) and ipsilateral (open circles, dashed lines) VOIs (top: normal; middle: borderzone; bottom: lesion) at 24 h (left) and 3 weeks after stroke (right). Curves were obtained using a single-exponential fitting procedure based on data from group-wise summed spectra. Error bars represent the estimation error calculated by Monte Carlo Simulation. Data points at the right of the graphs represent concentration at the latest time-point as calculated from individual spectra (mean ± SD). *P < 0.05 as compared to contralateral.
current study, Dijkhuizen et al. (2003) have observed that stimulus-induced brain activation in perilesional sensorimotor cortex is lost at 24 h after stroke, but returns after 2 weeks. Morphologically, this region may undergo structural plasticity, including sprouting of neurites and synaptogenesis (Keyvani and Schallert, 2002; Carmichael, 2003; Nudo, 2006). Our current data indicate that (semi-)acute functional ‘silence’ of perilesional tissue is related to deficiency of energy metabolism and Glu–Gln neurotransmitter cycling. More chronically, oxidative glycolysis normalizes and glutamatergic neurotransmission recovers, which is vital to reinstatement of neuronal function and may underlie subsequent behavioural recovery.

In conclusion, we have demonstrated that $^{1}$H/$^{13}$C MRS offers a fine tool to detect regionally specific impairment and recovery of neuronal and glial metabolism after stroke. To the best of our knowledge, this study is the first to show in vivo metabolic alterations in perilesional tissue that may explain post-stroke loss and reinstatement of neuronal function. Future studies with this methodology may enable measurement of absolute metabolic fluxes and further improved regional confinement (de Graaf et al., 2004), as further improvements in MR acquisition methods, e.g. heteronuclear decoupling (de Graaf et al., 2003b), will allow detection of more metabolites with increased signal-to-noise ratio, thereby enabling analysis with advanced mathematical metabolic models (Mason and Rothman, 2004; Patel et al., 2005).

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