

# **Sodium-23 Magnetic Resonance Imaging has potential for improving penumbra detection but not for estimating stroke onset time**

## **Authors:**

Friedrich Wetterling, PhD,<sup>1</sup> Lindsay Gallagher, HNC,<sup>2</sup> Jim Mullin, HNC,<sup>2</sup> William M. Holmes, PhD,<sup>2</sup> Chris McCabe, PhD,<sup>2</sup> I. Mhairi Macrae, PhD,<sup>2</sup> Andrew J. Fagan, PhD<sup>3</sup>

<sup>1</sup>Department of Psychiatry, School of Medicine, Trinity College, the University of Dublin, Ireland

<sup>2</sup>Glasgow Experimental MRI Centre, Institute of Neuroscience and Psychology, College of Medicine, Veterinary and Life Sciences, University of Glasgow, United Kingdom.

<sup>3</sup>Centre for Advanced Medical Imaging, St. James's Hospital / Trinity College Dublin, Ireland

## **Abbreviated Title:**

Characterization of tissue sodium in penumbra and core

## **Corresponding Author:**

Dr. Friedrich Wetterling,

Lloyd building

Trinity College, the University of Dublin

College Green,

Dublin 2

Ireland

Email: [wetterlf@tcd.ie](mailto:wetterlf@tcd.ie)

Tel:(+353)85-7227-301

## Abstract

Tissue Sodium Concentration increases in irreversibly-damaged (core) tissue following ischaemic stroke and can potentially help to differentiate core from adjacent hypoperfused but viable penumbra. To test this, multi-nuclear hydrogen-1/sodium-23 MRI was used to measure the changing sodium signal and hydrogen-Apparent Diffusion Coefficient (ADC) in ischaemic core and penumbra after rat middle cerebral artery occlusion (MCAO). Penumbra and core were defined from perfusion imaging and histologically-defined irreversibly damaged tissue. The sodium signal in core increased linearly with time while the ADC rapidly decreased by >30% within 20mins of stroke onset, with very little change thereafter (0.5-6h post-MCAO). Previous reports suggest that the timepoint at which tissue sodium signal starts to rise above normal – (onset of elevated tissue sodium, OETS) - represents stroke onset time (SOT). However, extrapolating core data back in time resulted in a delay of  $72\pm 24$ min in OETS compared to actual SOT. At the OETS in core, penumbra sodium signal was significantly *decreased* ( $88\pm 6\%$ ,  $p=0.0008$ ) while penumbra ADC was not significantly different ( $92\pm 18\%$ ,  $p=0.2$ ) from contralateral tissue. In conclusion, reduced sodium-MRI signal may serve as a viability marker for penumbra detection and can complement hydrogen ADC and perfusion MRI in the time-independent assessment of tissue fate in acute stroke patients.

*Keywords:* sodium MRI, apparent diffusion coefficient, middle cerebral artery occlusion, penumbra, onset of elevated tissue sodium

## INTRODUCTION

Ischaemic stroke is a catastrophic event which causes pathological restrictions in arterial blood flow to a specific brain region. In penumbra, tissue viability can nevertheless be maintained by a mix of aerobic and anaerobic metabolism for several hours after stroke onset. Eventually, if hypoperfusion persists, cells in penumbra become irreversibly damaged and incorporated into the ischaemic core. The term ‘penumbra’ is used in this paper to describe hypoperfused tissue identified on MRI perfusion scans which did not exhibit markers of irreversible damage on subsequent post-mortem histological analysis (i.e. potentially-viable hypoperfused tissue).

The accurate identification of penumbral tissue is critical in identifying stroke patients who could benefit from thrombolysis and in designing future clinical trials of potential neuroprotectants. Indeed, it has been shown that thrombolytic treatment outcomes are improved in patients selected using an MRI diagnosis <sup>1</sup>. Brain tissue viability strongly depends on the severity and duration of hypoperfusion <sup>2</sup>. Nevertheless, while hypoperfusion can be determined non-invasively with MRI, stroke onset time (SOT) and duration of hypoperfusion cannot be accurately determined from acute scans. For instance, in “wake-up” patients, which account for approximately 25 % of strokes <sup>3</sup>, the exact SOT cannot be determined accurately, preventing established acute stroke treatments such as thrombolysis with recombinant tissue plasminogen activator being applied <sup>4</sup>. Thus, an onset time-independent method which could accurately identify core and penumbra tissue in patients would be a valuable addition to MRI protocols. However, the identification of sub-regions within hypoperfused tissue (i.e. core and penumbra) using MRI remains elusive. The most promising approach relies on measuring water mobility in tissue via hydrogen diffusion MRI. The resulting quantitative value, the Apparent Diffusion Coefficient (ADC) drops rapidly within minutes after stroke onset in regions which are initially smaller than the

volume of hypoperfused tissue. Such ADC reduction, however, is not exclusively restricted to irreversibly damaged tissue during acute stroke. Low ADC values have also been measured in still viable stroke tissue<sup>5</sup>, requiring a specific threshold to be defined to support the ADC/core tissue hypothesis. For example, ADC values reduced and maintained below a threshold of 0.53  $\mu\text{m}^2/\text{ms}$  were reported for permanently damaged tissue as determined by TTC staining analysis at 24 h after experimental stroke (middle cerebral artery occlusion, MCAO) in rats<sup>6</sup>. Such ADC thresholds may hence serve as a prospective marker for core tissue during the acute phase. Validation of this hypothesis remains a challenge due to the lack of histological markers that could serve as an independent gold standard to define early tissue damage during the acute phase.

Monitoring changes in local sodium ion concentration may provide an alternative or complementary MRI approach for identification of core and penumbra regions non-invasively via sodium MRI. It is reported that following an ischaemic insult, sodium concentration (intracellular plus extracellular) increases within core tissue at a rate of  $\sim 2\%$ /h in humans<sup>7-9</sup>,  $\sim 5-8\%$ /h in monkeys<sup>10</sup>,  $\sim 12\%$ /h in rabbits<sup>11</sup>, and  $\sim 22-25\%$ /h in rats<sup>12-14</sup>. The core lesion size appears to determine the rate of sodium increase as a function of time, with faster rates in smaller lesions as described recently in a mathematical adaptation of Fick's second law<sup>15</sup>. A study by Wang *et al.* suggested that sodium-MRI may also serve as a ticking clock for determining SOT<sup>16</sup>, while Jones *et al.* reported that SOTs in rat models of middle cerebral artery occlusion (MCAO) can be calculated retrospectively from a linear extrapolation with less than a four minute error<sup>12</sup>.

In a previous rat MCAO study, variable delays were observed in the hemisphere ipsilateral to the stroke before the tissue sodium concentration increased, with an early increase in presumed core and a delayed increase in presumed penumbra (regions of interest (ROIs) estimated from experience with this model)<sup>13, 17</sup>. Furthermore, an initial decrease in

sodium concentration of ~4 mM was observed in presumed penumbra <sup>13</sup>. Bartha *et al.* [11] also reported a decrease of ~8% in stroke tissue (no discrimination made between core and penumbra) during the acute phase in a rabbit stroke model while Tyson *et al.* measured a 5% sodium reduction, acquired spectroscopically, in normoglycemic tissue during ten minutes of transient forebrain ischaemia <sup>18</sup>. However, since neither perfusion nor diffusion values were recorded in these studies, it was impossible to verify whether the tissue was hypoperfused and potentially salvageable. The lack of precise time course measurements and localization of penumbra and core tissue has thus hampered a full evaluation of the use of sodium-MRI in acute stroke.

Recent patient data indicate that the sodium signal remains unchanged in presumed penumbra tissue <sup>8,9</sup> and that sodium signal changes in core do not reverse after reperfusion <sup>19</sup>. During acute stroke it has been reported that the lesion identified from increased sodium concentration can be smaller than the lesion identified from decreased ADC <sup>19</sup>. However, the heterogeneity in stroke progression between individual patients as well as the need for a dedicated ADC threshold to determine lesion size poses a challenge. The lack of sufficient clinical data at different acute time points make it difficult to interpret these results with respect to sodium signal changes in relation to the time after arterial occlusion and the time of tissue viability loss. *In vivo* serial scanning of ischaemic stroke models can deliver the required temporal information in individual animals.

The aim of the current study was to investigate the changes in sodium signal and ADC values in penumbra and core tissue and to check the validity of using sodium image intensity firstly, to predict the SOT and secondly, to identify potentially salvageable penumbral tissue. Using an established model of permanent focal cerebral ischemia (MCAO), alternating sodium and ADC measurements were made from 0.5-6h after stroke with subsequent perfusion imaging for penumbra assessment using perfusion/histology mismatch. To check

the validity of using tissue sodium to predict the SOT, the time point after MCAO at which sodium increases above normal tissue sodium in the affected hemisphere is hereinafter called the Onset of Elevated Tissue Sodium (OETS).

We hypothesize that in penumbra, where cells are still capable of maintaining a membrane potential and a sodium concentration gradient, the sodium signal will be normal or reduced and will remain below or at the sodium signal level from non-ischaemic (contralateral ROI) tissue. As time progresses and the tissue loses its viability - transitioning to core tissue - the sodium signal in this region will increase and will be higher than contralateral sodium levels.

## Methods

### *Sodium- and hydrogen-MRI*

A double-tuned sodium/hydrogen surface resonator was used for sodium- and hydrogen-MRI; the design of this transceiver (TXRX) coil is described in detail elsewhere<sup>17</sup>. A 3D Fast Low Angle SHot (FLASH) sequence was used to acquire sodium images with high spatial and temporal resolution using a 7 T system (Bruker BioSpec 70/30 system, Ettlingen, Germany). A  $TE$  of 2.6 ms was achieved with 10 % partial echo acquisition to minimize  $T_2^*$ -weighting effects on the measured signal intensity. Steady-state imaging was achieved using a short repetition time ( $TR$ ) of 23 ms, which was appropriate considering that the  $T_1$  at 7 T for sodium *in vivo* is ~40 ms<sup>20, 21</sup>. The transmit power was set so that a flip angle equal to the Ernst angle was located in the center of the brain, and the receiver bandwidth was set to 4 kHz. The field of view was 80 mm x 80 mm x 80 mm and the matrix size was 80 x 80 x 20 resulting in a voxel resolution of 1 mm x 1 mm x 4 mm in a 5 min acquisition time (TA) for 8 averages. A nominal voxel resolution of 0.5 mm x 0.5 mm x 2 mm was achieved after two-fold 3D zero-filling. Two 8mm diameter cylindrical fiducial vials, permanently attached to the top of the surface detector coil, were included in the imaged field of view to establish the symmetrical coil positioning relative to the rat head in each experiment.

$^1\text{H}$  Diffusion Weighted Images (DWI) were acquired using an Echo Planar Imaging (EPI) sequence with: TR/TE = 4000/32 ms, a  $b$ -value of  $600 \text{ s/mm}^2$  applied along three different orthogonal directions, voxel size  $(0.25 \times 0.25 \times 1.9) \text{ mm}^3$ , 8 slices with 0.1 mm slice gap, and 8.5 min acquisition time. ADC maps were generated for each slice.

Hydrogen Perfusion Images (PI) were acquired prior to the rats' sacrifice by swapping the sodium coil system with a standard transmit-only receive-only (TORO) resonator system (72 mm diameter linear volume resonator and 20mm diameter receive-only surface coil) in a pseudo-continuous arterial spin labeling (pCASL) EPI-based sequence<sup>22</sup> with repetitive labeling pulse applied every 60 ms (50 pulses) and slice selective labeling once around the neck and once above the head. The read-out EPI parameters were: TR/TE = 4000/22 ms, TA = 2 min per slice, in-plane resolution =  $(0.26 \times 0.26) \text{ mm}^2$ , slice thickness = 2 mm. Five slices were acquired which matched the diffusion slice positions. Perfusion images were computed as the difference between the labeled and unlabeled images and values reported herein are given as a percentage change relative to contralateral cortex.

A final hydrogen DWI data set was then acquired with the same hydrogen TORO resonator system using a 2D-Echo Planar Imaging (EPI) sequence with: TR/TE = 4000/26 ms, voxel size  $(0.25 \times 0.25 \times 1.9) \text{ mm}^3$ , 8 slices with 0.1 mm slice gap, and 2 min 8 s acquisition time. A  $b$ -value of  $600 \text{ s/mm}^2$  was applied along each of the three orthogonal directions in space, and one unweighted  $b = 0 \text{ s/mm}^2$  acquisition was recorded. ADC maps were generated for each slice.

### *Stroke Model*

*In vivo* experiments were performed under license from the UK Home Office and were subject to the Animals (Scientific Procedures) Act, 1986. All research complied with the Declaration of Helsinki. Six male Sprague Dawley rats (bodyweight ~ 300 g, Harlan, Bicester, UK) were anaesthetized with 5 % isoflurane in a ratio of nitrous oxide:oxygen of 70:30. A tracheal cannula was surgically implanted for artificial ventilation via a small animal

respirator pump (6025 rodent ventilator, Ugo Basile, Italy), anaesthesia maintained using 2-2.5 % isoflurane and femoral arteries and veins were cannulated. Experimental stroke was induced by permanently occluding the left middle cerebral artery with an intraluminal filament<sup>23</sup>. Filaments were prepared from 3/0 Dermalon blue nylon monofilament (1744-41, Sherwood, UK), the tip was heated with a low temperature cauterizing pen (AA90, Bovie, UK) to create a bulb of 300 µm diameter for animals of 275-350 g. Data from all animals that entered the study are presented. No animals died under procedure or were excluded from the study. Blood pressure (from femoral artery cannula), heart rate (MRI-compatible ECG electrodes, Red Dot neonatal monitoring electrodes, 2269T, 3M), body temperature (MRI-compatible rectal probe, Bruker, Germany), and blood gases were monitored and maintained within normal limits throughout surgery and MRI scanning.

### *Experimental Workflow*

Following induction of cerebral ischaemia the rat was positioned on a support cradle, for movement in and out of the magnet bore, and kept warm by a warm water circulation jacket. The double-tuned surface resonator was tuned and matched *in situ*. The MRI system's automatic adjustment procedures were performed via the hydrogen channel on the resonator, following which the survey hydrogen images were acquired to verify correct animal and coil positioning inside the magnet. Manual adjustments of the reference pulse attenuation value and shimming were then performed for the sodium surface coil using a sodium-Free Induction decay (FID) experiment. Ten sodium-MRI scans and one hydrogen DWI acquired consecutively were recorded for up to 5.5 h using the TXRX surface resonator, followed by a hydrogen-PI and -DWI scan with the standard hydrogen TORO resonator system. Six stroke rats were scanned in total, referred to herein as stroke 1 to 6. For the last two experiments (stroke 5 and 6) a different sodium surface transceiver resonator with similar detection properties (20 mm inner diameter, planar, double-tuned hydrogen/sodium surface transceiver, Bruker BioSpin GmbH, Ettlingen) was used. The

spacing between ADC acquisitions was 30 min for stroke 1, 5, and 6 and 60 min for stroke 2, 3, and 4.

### *Infarct size analysis*

Following completion of scanning ( $330 \pm 50$  min post-MCAO, Table 1) animals were killed (time of sacrifice =  $378 \pm 42$  min post-SOT, Table 1) by transcardial perfusion fixation using 4 % paraformaldehyde in phosphate buffer and the location and volume of irreversible ischaemic damage was determined from histology sections. The brains were harvested, processed, and embedded in paraffin wax, subsequently sectioned at  $6 \mu\text{m}$  and collected over eight stereotaxic coronal levels covering the same rostro-caudal extent of MCA territory as the MRI slices (2 mm to 12.2 mm from the interaural line <sup>24</sup>) with appropriate sections matched up to each of the sodium-MRI brain slices using neuroanatomical landmarks. Histology sections were stained with haematoxylin and eosin and examined by light microscopy. The boundary of the ischaemic lesion was determined on the basis of neuronal morphology (darkly stained, pyknotic neurones) and vacuolated neuropil. This boundary was then transcribed onto coronal line diagrams from a stereotaxic atlas <sup>24</sup> as described elsewhere <sup>25</sup> to correct infarct measurements for any ischaemia-associated brain swelling and brain shrinkage associated with histological processing.

### *Image co-registration*

The fourth stereotaxic coronal level covering the center slice of the rostro-caudal extent of MCA territory (8.2 mm from the interaural line) was used to carry out noninvasive perfusion, ADC, and sodium measurements on a single slice.

The hydrogen-PI and -DWI images acquired with the hydrogen TORO system and the histology maps were co-registered to the sodium and hydrogen ADC images which were acquired with the double-tuned surface resonator. In order to maintain the high spatial resolution of the histology maps, all images were resized using nearest neighbor interpolation to a matrix size of 1024 x 1024. Image co-registration was done by setting corresponding markers in anatomically identical regions of the brain using the control point selection tool in Matlab<sup>®</sup> (The Mathworks, Natick, MA, USA), with an affine transformation used to register the images. This allowed for the accurate placement of ROIs in the different multimodal images and parametric maps for later computation purposes, some examples of which are shown in Figure 1.

#### *Determination of penumbra and ischaemic core*

In this study the core ROI was defined within the boundary of the histologically-verified, irreversibly damaged tissue, and the penumbra ROI from the mismatch between the area of hypoperfusion and the histology defined area of irreversible tissue damage. The area of perfusion deficit was calculated on the basis of a 57 % reduction of the cerebral blood flow (CBF) relative to the mean contralateral CBF in cortex<sup>25, 26</sup> using a code developed in Matlab<sup>®</sup> (The Mathworks, Natick, MA, USA). ROIs delineating core and penumbra regions (Figure 1d) were manually defined, guided by the location of hypoperfusion and-histology at 5-6 h post-stroke in order to observe the temporal sodium signal and hydrogen ADC evolution in each distinct region. Corresponding ROIs were also manually defined in contralateral tissue homotopic to core and penumbra in order to correct for the slight sodium coil sensitivity profile; an example of all ROIs thus defined for one representative rat are presented in Figure 1d. The sodium values were then computed as the mean and standard deviation of the respective ROIs relative to the contralateral tissue. All images were masked

to the rat brain, wherein the mask was computed from manually-drawn contours drawn around the edge of the brain as visualized in a diffusion-weighted image.

### *<sup>23</sup>Na and ADC data analysis*

For qualitative presentation of the ADC maps and sodium MR images each pixel value was normalized to the mean sodium signal values and ADC in contralateral tissue using a ROI placed over the entire contralateral hemisphere. For quantitative measurements of the sodium signal the mean signal in core and penumbra was normalized to the mean sodium signal values measured in ROIs contralateral to core and penumbra, respectively. Assuming a linear increase in the sodium signal in ischaemic core tissue (as reported in previous studies <sup>12, 13, 17</sup>), the signal in core ROI was fitted to a linear function according to:

$$Signal_{Na}(t) = c + s \cdot t$$

where the line to which the sodium signal was fitted was characterized by the intercept  $c$  (the estimated sodium signal at stroke onset time) and the rate of sodium signal change as a function of time  $s$  (the slope).

The Onset of Elevated Tissue Sodium (OETS) - the time after MCAO at which tissue sodium in the ischaemic core ROI starts to rise above contralateral tissue sodium, was computed from the linear regression results according to:

$$OETS = \frac{100\% - c}{s} .$$

The standard deviation and mean values were computed for the ADC and sodium signal in both the core and penumbra ROIs at OETS and at the end of the experiment.

### *Statistical analysis*

Data are presented as mean  $\pm$  standard deviation (SD). The paired samples *t* test was used for comparison of ipsilateral sodium and ADC signals with contralateral values. Statistical significance was assumed when  $p < 0.05$ .

## **Results**

MCAO induced a region of reduced ADC with a larger perfusion deficit and an increase in tissue sodium signal within MCA territory. The spatial change in the ADC and the normalised sodium signal as a function of time after MCAO, are presented for a representative rat in Figure 2. The quantitative sodium values for the group, together with experimental timings, histology-defined lesion size and OETS, are listed in Table 1. The corresponding ADC values are listed in Table 2.

In the contralateral hemisphere, the ADC values were  $0.83 \pm 0.06 \mu\text{m}^2/\text{ms}$  and  $0.87 \pm 0.04 \mu\text{m}^2/\text{ms}$ , respectively for ROIs homotopic to penumbra and core. The ipsilateral ADC data were thresholded at  $0.53 \mu\text{m}^2/\text{ms}$ <sup>6</sup>, with values below this threshold shaded black. The qualitative data in Figure 2 show that tissue with ADC values below this threshold appeared rapidly within the first 20 minutes in a large fraction of the (later defined) core ROI. Thereafter there was a slight increase in ADC-defined lesion size in this and other animals in the group (Figure 2 & 3)

A linear increase in the sodium signal was observed in core tissue (Figure 3). By the end of the experiment ( $330 \pm 50$  mins after MCAO), the sodium signal in core tissue averaged  $155 \pm 29\%$  of contralateral values. The OETS, averaged across the group, deviated by  $72 \pm 24$  min from the actual time of arterial occlusion.

When sodium in the core starts to rise above contralateral values (OETS =  $72 \pm 24$  mins after MCAO), the penumbra sodium signal was significantly decreased ( $88 \pm 6$  % of contralateral,  $p = 0.0008$ ) gradually rising to  $101 \pm 16$  % of contralateral tissue ( $p = 0.83$ ) by the end of the experiment. Using sodium data from OETS to the end of the experiment, the rate of rise in tissue sodium was  $2.9 \pm 2.3$  %/h, in penumbra and  $12.5 \pm 3.6$  %/h in core tissue.

At the OETS, the ADC value in histologically-defined core was already significantly lower than in the contralateral ROI while the ADC value in penumbra was not-significantly different from the contralateral ROI (Table 2). By the end of the experiment, the penumbra ADC value was significantly lower than the contralateral ROI but was still above the viability threshold (group data  $0.71 \pm 0.10 \mu\text{m}^2/\text{ms}$ ,  $p = 0.026$ ).

## Discussion

Since viability of brain tissue requires maintenance of a strong sodium concentration gradient between the intra- and extracellular space, the ischemic penumbra may be defined as a region of constrained blood supply in which the sodium concentration gradient is maintained. If perfusion is not restored, energy stores become exhausted, membranes depolarize and the concentration gradient is lost as sodium levels increase in the intracellular space. When this occurs, the tissue takes on the characteristics of the ischaemic core, which also includes a significant reduction in ADC. We have explored two potential uses for MR-defined tissue sodium concentration: 1. to predict stroke onset time; and 2. to identify penumbral tissue.

### *1. Tissue sodium signal to predict stroke onset time*

The time point at which tissue sodium, in the affected hemisphere, increases above contralateral levels (i.e. the OETS) has been reported to provide an estimation of the stroke

onset time (SOT); the present study highlights the limitations of such an approach given the heterogeneity in ischaemic stroke patients.

*Defining the onset of elevated tissue sodium from linear regression data*

Jones *et al.* reported a linearly-extrapolated sodium signal equal to normal sodium signal levels at the time of arterial occlusion, resulting in an estimated SOT accuracy of  $\pm 4$  min<sup>12</sup>. From the current study, it is clear that SOT estimation via extrapolation of sodium time course data is prone to significant errors. In all rats investigated, the backwards extrapolation of the linearly-increasing sodium signal resulted in a consistent underestimation of the SOT (Table 1). The tissue sodium signal in the core ROI rose above normal contralateral levels at approximately 70 mins after arterial occlusion. The linear-extrapolation method is based on the hypothesis that the tissue within the analysed core ROI becomes irreversibly damaged immediately following the arterial occlusion. However, tissue included in the core region, as determined by PI and post-mortem histology analysis at 7 h after MCAO, may not have been irreversibly damaged at early time points, resulting in the significant error in the SOT estimation. Indeed, in a previous study carried out using this experimental stroke model, time delays varying from 0 to 4 h after MCAO were observed before the sodium concentration increased in tissue which subsequently displayed as infarct on histology<sup>13</sup>. An earlier study in rats estimated SOT with an error of 41 to 52 mins using this method<sup>16</sup>, while in monkeys a study reported estimated SOTs which were out by 27 mins<sup>10</sup>. Recent studies involving human sodium MRI in the acute stroke period showed that sodium signal levels measured in core tissue, defined by visibly increased DWI signal, remained normal up to 7 h after arterial occlusion before eventually increasing<sup>8,9</sup>. This is consistent with the current and previous<sup>13,17</sup> rat stroke studies, where variable delay times were measured before Tissue Sodium Concentration (TSC) increased, coupled with early small decreases in sodium levels in penumbra, suggesting that any increase above normal levels (we propose this as a viability

threshold level) would indicate irreversible damage. In this case, permanent tissue damage would not be directly correlated with SOT, that is, the time of arterial occlusion, but rather the time at which sodium signal begins to increase above normal levels. Hence, given a viability threshold at non-pathological tissue sodium concentration values <sup>13</sup>, a pixel-wise linear extrapolation back in time (for any regions exhibiting an increase in Na signal above normal values) may indicate the time of tissue viability loss at that specific location, rather than the time of arterial occlusion. Indeed, the very concept of a “stroke onset time” is not particularly useful from a sodium MRI perspective, given the impossibility of uniquely defining “core” tissue (i.e. tissue which was definitively irreversibly damaged immediately after the stroke occurred) and hence the impossibility of estimating the onset time with any degree of accuracy by extrapolating sodium time-course data back in time. From a clinical perspective, it is also unlikely that repeat measurements on patients in the acute phase would be justifiable before treatment is initiated, and hence the minimum of two time-point measurements required to determine a slope and thereby extrapolate back to estimate an onset time, are unlikely to ever be realised in practice.

## *2. TSC to identify penumbral tissue - <sup>23</sup>Na decrease in penumbra tissue*

At the OETS, in comparison to core, the penumbra displays a significant decrease in sodium signal (88±6% compared to contralateral sodium signal) coincident with a small non-significant decrease in ADC value (Tables 1& 2). A similar-sized decrease in sodium signal was observed in a previous study <sup>11</sup>, although the authors did not differentiate between penumbra and core tissue. We have previously reported a 4mM decrease in sodium at various times after MCAO in presumed penumbra tissue <sup>13</sup>, while an NMR study reported a 5% sodium signal decrease after artery occlusion in normo-glycemic tissue, with no change in

hypo- and hyper-glycemic tissue <sup>18</sup>. Factors which could conceivably contribute to the observed decrease in tissue sodium in penumbra include:

***A perfusion effect:*** Immediately after arterial occlusion, blood flow reduces in the territory of the blocked blood vessel while the intravascular volume remains constant, which will have no effect on the overall sodium content in the intravascular space of hypoperfused voxels. Consequently, perfusion effects are not thought to contribute significantly to the observed decrease in tissue sodium in penumbra.

***Relaxation-time Effect:*** The relaxation time differences between intra- and extracellular sodium nuclei can have a strong effect on the measured sodium signal, particularly considering that intra- and extra-cellular  $T_2^*$  relaxation times are quite different <sup>27</sup> and that the compartmental sodium content changes during non-homeostasis. During the acute stroke phase a strong sodium influx into cells has been observed in isolated heart muscle after coronary artery occlusion and reperfusion using chemical shift reagents to separate the intra- and extra-cellular sodium components <sup>28</sup>. Despite no change in the overall sodium content, the measurable relaxation time-weighted signal hence can change dramatically due to faster relaxation effects on sodium ions in the intracellular compartment. Furthermore, during the chronic phase, at 24 h after ischaemic stroke, a recent mouse study reported increasing  $T_2^*$  relaxation time in core tissue <sup>29</sup>. Although, a decrease in  $T_2^*$  relaxation time remains to be experimentally verified during the acute phase, it must be considered as one potential reason for the sodium signal reduction measured in the current study, for instance as an effect of increased intracellular sodium concentration.

Considering that the intravascular compartment (with a 5 % volume fraction) and the extracellular compartment (20 % volume fraction) both contain a 140 mM concentration of sodium, while the intracellular compartment (75 % volume fraction) has a lower concentration of 10 mM, the net density-weighted sodium signal originating from the

extracellular space is ~ 82 %. At 9.4 T the transversal relaxation time for the sodium nuclei  $T_{2 \text{ brain}}^*$  was measured to be 4.8 ms in mouse brain <sup>29</sup>. However, there is a lack of further data in the published literature reporting specific compartmental  $T_2^*$  - relaxation times, and hence we estimated the extracellular component  $T_{2 \text{ e}}^*$  to be in the range of 6 ms, with an intracellular component  $T_{2 \text{ i}}^*$  of approximately 2.1 ms, using the fact that the extracellular  $T_2^*$  component is longer than the intracellular  $T_2^*$  component and the assumption of a monoexponential relaxation behaviour in each compartment.

At 8.5 T, the intracellular longitudinal relaxation time,  $T_{1i}$ , has been determined to be monoexponential with a value of 23 ms <sup>30</sup> and  $T_{1e}$  is assumed to be similar to measured brain CSF of 50 ms <sup>31</sup>. For further computational purposes the following sequence parameters were extracted from the current study: TR=23 ms, TE=2.6 ms, and flip angle = 60°.

The relative sodium signal for each compartment ( $S_{\text{rel}}$ ) can then be computed according to the following equation <sup>32</sup>:

$$S_{\text{rel}}(TE, TR) = \frac{1 - e^{-\frac{TR}{T_1}}}{1 - \cos \alpha \cdot e^{-\frac{TR}{T_1}}} \cdot \sin \alpha \cdot e^{-\frac{TE}{T_2^*}}$$

The signal levels measured for different sodium concentrations, [Na], and volumes, V, can be computed as follows:

$$S(Na_i, Na_e) = [Na_i] \cdot V_i \cdot S_{\text{rel},i} + [Na_e] \cdot V_e \cdot S_{\text{rel},e}$$

To give a numerical estimation, it is further assumed that the influx of sodium ions into still viable penumbra cells leads to an increase in intracellular sodium concentration to 20 mM <sup>33</sup> because the Na/K ATPase pump is still active and the extracellular sodium compartment is much smaller volumetrically so that it would be hard to increase the intracellular concentration drastically from 10 to 140 mM within minutes. We further assume then that

the net amount of sodium ions contributing to the MR signal measured from a single voxel in such tissue remains constant and that the volumetric ratios are unchanged during the acute stroke phase. To raise the intracellular sodium concentration by 10mM through an influx of ions from the extracellular compartment, the extracellular sodium concentration must decrease from 140 mM to 110 mM. This would result in a 4 % signal reduction. If the extracellular sodium concentration were to be re-established at 140 mM, a 14 % signal increase would thus be expected, in obvious contradiction to the measured values in penumbra in the current study. Although present, the relaxation time effect on the measured sodium reduction may thus be negligible when a sufficiently short TE is used in the data acquisition, as in this study.

***Cell Swelling effect:*** A decrease in TSC of 12 % has been measured in presumed penumbra in a rat permanent MCAO study in which there was no relaxation time weighting<sup>13</sup>. It is thus assumed that the sodium signal reduction observed in the current study most likely results from an actual TSC reduction in observed penumbra tissue. An actual TSC decrease can be caused by an increase in intracellular sodium concentration, resulting in disturbed homeostasis and subsequent water influx, which in turn increases the intracellular compartmental volume fraction. Since the measured voxel size is constant during the experiment, the extracellular space thus decreases and consequently the total sodium concentration reduces. Assuming an increase of the intracellular volume normally comprising 75 % of a tissue voxel to 79 % and an intracellular sodium concentration that hence is diluted to the normal content of 10 mM with 140 mM extracellular sodium concentration, a 13 % signal decrease is computed using the above mentioned equations. This is in good agreement with the measured reduction of 12 % in this study. Cell swelling will be accompanied by an ADC decrease, as measured in penumbra in the current study where an ADC decrease of approximately 15 % was evident by 6 h post-stroke. Indeed, the reduction in ADC values due to cellular oedema during the acute stroke phase is a well-documented

phenomenon <sup>5</sup>. The specific time at which the ADC decreases below the viability threshold appears to occur later in tissue which remains viable for longer <sup>5</sup>, while the sodium signal was observed to be decreased in penumbra tissue long before ADC values reduced significantly below contralateral values in the same region (Figure 3).

Reduced sodium signal may hence serve as a measure for cell swelling in still-viable tissue. A decreased sodium signal in hypoperfused tissue was also previously reported in a study involving stroke in rabbits <sup>11</sup>. However, no pathophysiological interpretation was proffered since separate penumbra and core identification was not possible.

### *3. Temporal variations in sodium signal in core and penumbra tissue*

The observed sodium slope in core was measured to be  $12.5 \pm 3.5$  %/h which is below the rate reported in previous rat studies ( $\sim 22 - 25$  %/h) <sup>12-14</sup>. However, considering the  $T_2^*$ - and  $T_1$ -weighting of the sequence used in this study, a lower overall effect of the sodium accumulation was expected and hence these values are within the expected slope range for rat MCAO experiments. The sodium signal difference between brain tissue and CSF is normally a factor of three, but for the sequence used herein this difference reduced to a factor of two (see Figure 2). Furthermore, the maximum sodium signal that can be reached with the imaging pulse sequence is also reduced. As an explanation serves that the sequence has been set to achieve the Ernst angle for brain tissue and its respective longitudinal relaxation parameters. In permanently damaged tissue one can assume that the longitudinal relaxation time becomes more like in CSF and hence a reduced slope is measured.

After the OETS, the subsequent gradual increase of 2.5 %/h observed in the penumbra sodium signal could potentially be explained by the gradual rupturing of cellular membranes within the affected region, that is, as the tissue gradually becomes irreversibly damaged. The resulting increase in TSC this would engender has been described previously <sup>7</sup>; in the model proposed here, the occurrence of an intermediary phase in tissue at risk, wherein cellular

swelling results in a reduction in TSC, is added. Further studies are required to verify this hypothesis.

It remains unclear, however, whether the slow increase in sodium signal observed in penumbra tissue is occurring in still-viable tissue, or whether it is due to the loss of cellular integrity for progressively large numbers of individual cells within the penumbra tissue, with the rest remaining intact. It should be noted that the observed increase could be influenced by a partial volume effect from the neighbouring core area, given the relatively small penumbral area in these animals, or diffusion in extracellular  $\text{Na}^+$  along the concentration gradient from the core, to the neighbouring penumbral tissue. This question could be answered by chemical shift imaging experiments. Shift reagents have acute toxicity issues in vivo, but allow a discrimination of intracellular and extracellular sodium in each voxel.

#### *4. Prolonged tissue survival in penumbra*

By 6 h post-MCAO it had been expected, from previous experience with this model, that no penumbra would remain. Over the first 4-6 h after MCAO, the ADC lesion normally grows until it encompasses the whole of the hypoperfused region. The results in this study show that this was the case for none of the six investigated strokes (suggesting that penumbra can survive for longer than previously thought). We assume that the tight control and maintenance of the animals' physiology within normal limits (e.g.  $\text{PaO}_2$ ,  $\text{PaCO}_2$ , blood pressure, and temperature) during MRI scanning may have resulted in the prolonged tissue survival.

#### *5. Sodium MRI for clinical acute stroke diagnostic imaging*

Current treatment guidelines for stroke patients recommend a time window of 4.5 hours from stroke onset for thrombolytic therapy. In cases where stroke onset time is unknown, a

technique which could determine SOT, would clearly be of benefit within this framework. However, a technique which could reveal the extent of penumbral tissue is arguably of more utility to stroke clinicians. Thrombolytic therapy could benefit some patients who still have penumbra beyond 4.5 hours, but put patients lacking penumbra within the 4.5hour window at increased risk of haemorrhage. It may be here that sodium MRI has most to offer, through identification of the presence of penumbral tissue, leading to a more patient-specific treatment regime. A combination of sodium MRI and DWI, for example, could represent an optimal protocol for guiding patient management: the former indicating whether at-risk but potentially salvageable tissue is still present in the patient, with the latter giving an overall sense of the extent of damage in the brain, and the combination used to determine whether the patient is likely to benefit from the treatment.

## **Conclusion**

A significant decrease in the sodium signal in the acute phase of stroke has been measured for the first time in penumbra tissue identified using a combination of perfusion and histology data. It is clear from the current and previous studies that increased tissue sodium levels indicate irreversibly damaged tissue, suggesting that a threshold for tissue viability lies close to normal values. Given that it is now accepted that the DWI (or ADC) lesion is likely to include some penumbral tissue at early time points after stroke, sodium-MRI may more accurately identify both potentially viable penumbra and non-viable core tissue, although its potential to accurately determine a patient's SOT seems doubtful. Nevertheless, by serving as a marker for tissue viability, sodium-MRI may obviate the need to estimate SOT, opening the way from a more patient-specific treatment regime, perhaps via a combination of sodium and diffusion MRI. Normal and reduced sodium levels are a good indicator for still viable tissue.

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## **Disclosures**

The authors have nothing to declare.

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## Tables

**Table 1: Timings and sodium data in penumbra and core**

<i>Rat Stroke Number</i>	1	2	3	4	5	6	<i>Mean±SD</i>	<i>p-value</i>
<i>Onset of elevated tissue sodium (OETS) [min after actual stroke onset]</i>	75	61	99	57	98	39	72±24	0.00003 <sup>a</sup>
<i>Final MR measurement time post-stroke [min]</i>	290	351	330	419	280	315	330±50	
<i>Time of sacrifice post-stroke [min]</i>	325	370	340	440	400	390	378±42	
<i>Na in penumbra @ OETS [%contr.]</i>	91	83	88	99	83	86	88±6	0.0008 <sup>b</sup>
<i>Na in penumbra @ end[%contr.]</i>	95	109	102	127	93	82	101±16	0.83 <sup>b</sup>
<i>Na in core @ end[%contr.]</i>	147	152	138	214	140	142	155±29	0.0009 <sup>b</sup>
<i>Na slope in core [%contr/h.]</i>	13.1	10.8	9.9	18.9	13.2	9.1	12.5±3.5	
<i>Histology defined lesion size [mm<sup>3</sup>]</i>	292	146	119	364	298	208	238±96	

**Timings and sodium data in penumbra and core for each rat (n=6). The measurement time point “end” corresponds to the final MR measurement time post-stroke given in this table.** <sup>a</sup>with respect to actual SOT; <sup>b</sup>with respect to contralateral tissue

**Table 2: Hydrogen ADC results in penumbra and core**

<i>Rat Stroke Number</i>	1	2	3	4	5	6	<i>Mean±SD</i>	<i>p-value</i>
<i>ADC in penumbra @ OETS [<math>\mu\text{m}^2/\text{ms}</math>]</i>	0.68	0.71	0.78	0.79	0.82	0.86	0.77±0.07	0.180
<i>ADC in ROI contralateral to penumbra @ OETS [<math>\mu\text{m}^2/\text{ms}</math>]</i>	0.70	0.81	0.83	0.88	0.94	0.85	0.84±0.08	
<i>ADC in penumbra @ end [<math>\mu\text{m}^2/\text{ms}</math>]</i>	0.61	0.69	0.77	0.59	0.77	0.84	0.71±0.10	0.026
<i>ADC in ROI contralateral to penumbra @ end [<math>\mu\text{m}^2/\text{ms}</math>]</i>	0.78	0.80	0.80	0.81	0.90	0.91	0.83±0.06	
<i>ADC in core @ OETS [<math>\mu\text{m}^2/\text{ms}</math>]</i>	0.54	0.51	0.57	0.52	0.49	0.59	0.54±0.04	5x10 <sup>-9</sup>
<i>ADC in ROI contralateral to core @ OETS [<math>\mu\text{m}^2/\text{ms}</math>]</i>	0.84	0.88	0.90	0.90	0.90	0.86	0.88±0.03	
<i>ADC in core @ end [<math>\mu\text{m}^2/\text{ms}</math>]</i>	0.52	0.54	0.54	0.51	0.52	0.60	0.54±0.03	2x10 <sup>-8</sup>
<i>ADC in ROI contralateral to core @ end [<math>\mu\text{m}^2/\text{ms}</math>]</i>	0.80	0.90	0.87	0.91	0.87	0.85	0.87±0.04	

**Hydrogen ADC results in penumbra and core for each rat (n = 6). The measurement time point “end” corresponds to the final MR measurement time post-stroke given in Table 1**

## Figure Captions

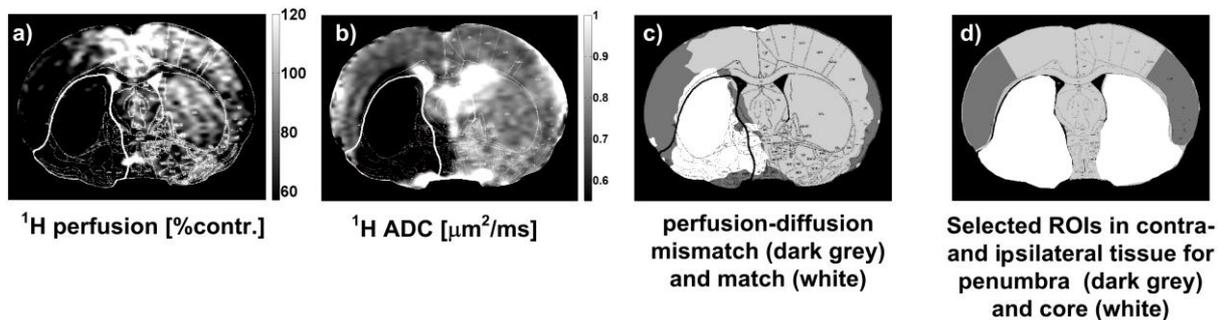


Figure 1: Region of interest (ROI) selection for penumbra and core tissue for one representative rat (stroke 2) using imaging data acquired at the end of the experiment (5 h after MCAO) and histology maps. (a) Relative perfusion-weighted images and (b) ADC maps were used to determine (c) the perfusion-diffusion mismatch area, where the perfusion deficit was defined as regions exhibiting perfusion level  $< 57\%$  of contralateral cortex, while ADC values below a threshold of  $0.53 \mu\text{m}^2/\text{ms}$  defined the ADC lesion. Dark grey regions indicate the diffusion/perfusion mismatch, and white the hypoperfused core with low ADC. (d) Core (white) and penumbra (dark grey) ROIs defined with reference to the histology and PI data. Mirror ROIs in contralateral hemisphere are also shown. The histology defined outline, delineating permanently damaged core tissue is superimposed on each of the four images/maps.

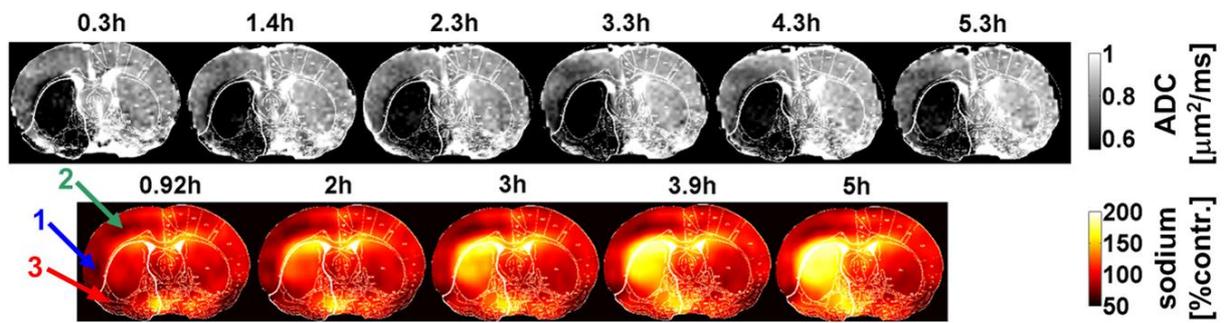


Figure 2: ADC maps (upper row) and sodium images (lower row) for one representative rat (stroke 2), where the signal in the latter was normalized to the contralateral hemisphere. Numbers above each image indicate the time post-MCAO. The lower boundary of the grey scale on the ADC maps represents the  $0.53 \mu\text{m}^2/\text{ms}$  core threshold, and delineates the ADC-defined lesion. The histology defined white outline, delineating permanently damaged core tissue is superimposed on each of the ADC and sodium images. Note the low sodium signal in the ipsilateral cortex during the early time points (arrow 1) and during the entire experiment (arrow 2). Sodium signal change is highly heterogeneous in core and penumbra. Within the core territory, ipsilateral ventral cortex (arrow 3) presenting with initially low sodium exhibits a delayed onset of tissue sodium elevation at 3h after stroke. Note that the sodium signal is approximately two-fold higher in voxels containing cerebrospinal fluid (i.e. within the ventricles) compared to voxels containing brain tissue<sup>34</sup>.

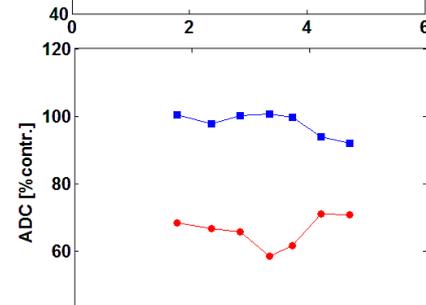
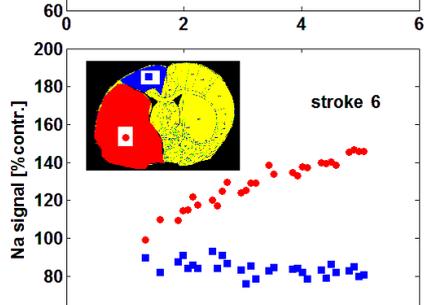
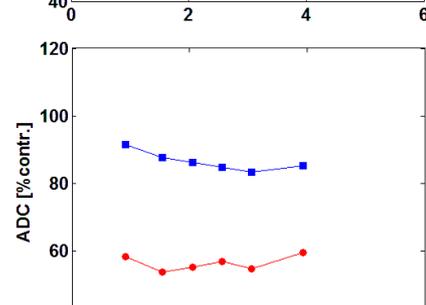
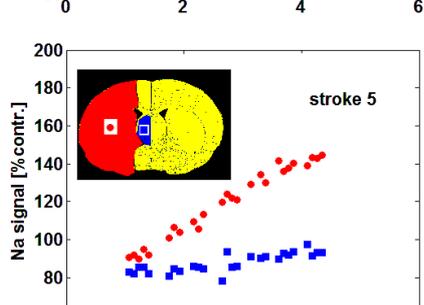
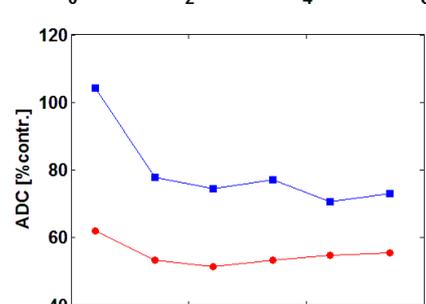
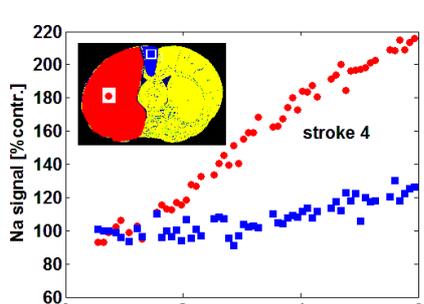
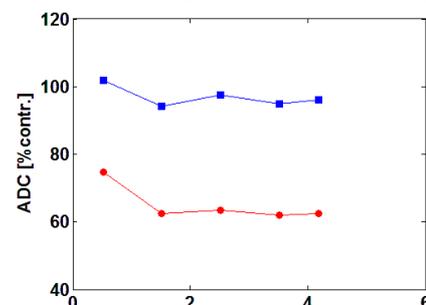
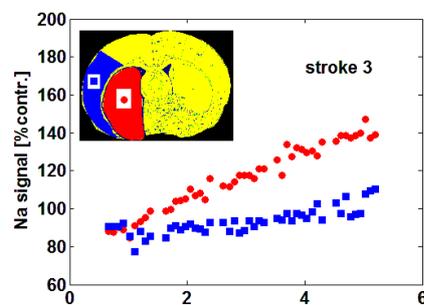
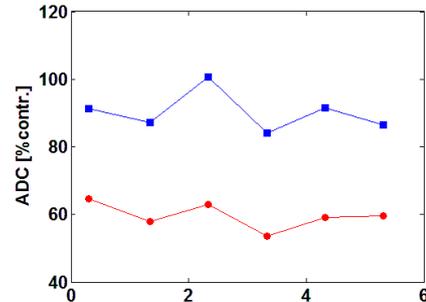
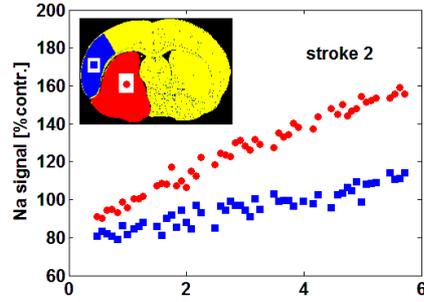
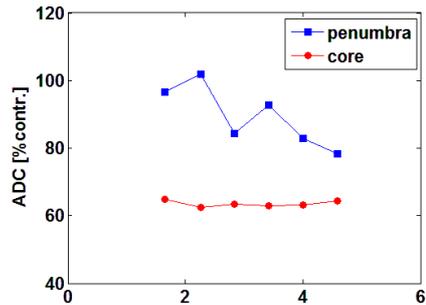
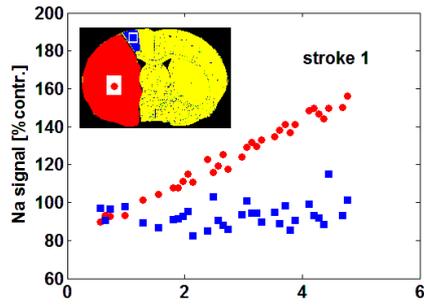


Figure 3: Relative sodium signal and ADC profiles in each animal. Data are averaged across ROIs in penumbra and core tissue (shown in the inset stereotaxic atlas plates), expressed as a % of the contralateral ROI's ADC and sodium values and plotted as a function of time after MCAO. Note that linear-extrapolation of the core sodium signal (red circles) back to the y-axis intersects at a value well below 100 % of contralateral ROI sodium, leading to an underestimation of stroke onset times in all cases (averaging at  $72 \pm 24$  min).