Regional and temporal variations in tissue sodium concentration during the acute stroke phase

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Quantitative Na MRI during acute stroke

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Abstract

A technique for non-invasively quantifying the concentration of sodium (^{23}Na) ions was applied to the study of ischaemic stroke. ^{23}Na-MRI techniques have shown considerable potential for measuring subtle changes in ischaemic tissue, although studies to-date have suffered primarily from poor signal/noise (SNR). In the current study, accurate quantification of tissue sodium concentration (TSC) was achieved in ^{23}Na images with voxel sizes of 1.2μl acquired in 10mins. The evolution of TSC was investigated from 0.5 to 8h in focal cortical and subcortical ischaemic tissue following permanent middle cerebral artery occlusion in the rat (n=5). Infarct volumes determined from TSC measurements correlated significantly with histology (p=0.0006). A delayed linear model was fitted to the TSC time course data in each voxel, which revealed that the TSC increase was more immediate (0.2±0.1h delay time) in subcortical ischaemic tissue, whereas it was delayed by 1.6±0.5h in ischaemic cortex (p=0.0002). No significant differences (p=0.5) were measured between TSC slope rates in cortical (10.2±1.1mM/h) and subcortical (9.7±1.1mM/h) ischaemic tissue. The data suggest that any TSC increase measured in ischaemic tissue indicates infarction (core) and regions exhibiting a delay to TSC increase indicate potentially salvageable tissue (penumbra).

Keywords: qNa-MRI, tissue sodium concentration, infarct, stroke.

Introduction

Ischaemic stroke is a catastrophic event in which the blood supply to a part of the brain is restricted and oxygen and glucose supply to cells within the affected region is severely limited. The loss of nutrient supply to cells in turn compromises energy metabolism within the stroke-affected area leading to a rapid loss in ATP and ionic homeostasis, including diminished efficiency in the ^{23}Na/^{39}K-pump function. The consequent changes in local ion concentrations may allow for the use of Magnetic Resonance Imaging (MRI) to investigate such states non-invasively. In particular, quantitative ^{23}Na-MRI (qNa-MRI) shows promise for investigating and assessing such tissue changes over time in acute stroke [1].

The accurate identification of hypoperfused potentially salvageable penumbral tissue is critical in identifying stroke patients who will 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 based on an MRI diagnosis [2]. The MR technique, which is currently available for identifying patients likely to benefit from thrombolytic treatment is the so-called “perfusion-diffusion mismatch”, where the mismatch between the ischaemically-injured tissue and hypoperfused tissue is determined from diffusion- and perfusion-weighted ^{1}H-MRI, respectively [3]. This technique endeavors to identify hypoperfused penumbral tissue, which is still viable but at risk of infarction due to compromised energy supply; however, this indirect approach to penumbral tissue identification leads to a lack of precision in the separation of still-viable from irreversibly damaged ischaemic core, or tissue not at risk of infarction (benign oligaemia). Indeed, large deviations have been observed between areas with decreased Apparent Diffusion Coefficient (ADC) compared to infarct determined by the gold standard technique of histology after stroke [4]. Furthermore ADC values have also been observed to normalize in areas of permanently damaged tissue during the subacute phase [5, 6]. Because conventional MR techniques demonstrate such ambiguities, we still lack an early direct and non-invasive marker for irreversibly damaged tissue.

The ability to measure elevated ^{23}Na levels [7, 8] and to accurately quantify the tissue sodium concentration (TSC - a measure related to combined intra- and extra-cellular ^{23}Na concentrations) in humans using ^{23}Na-MRI has demonstrated considerable potential to provide such a direct marker for tissue viability in stroke [1, 9]. However, the clinical role of ^{23}Na-MRI in assessing stroke patients remains unclear, due primarily to the lack of

temporally-resolved ^{23}Na data in these studies coupled with the difficulty in correlating the TSC measurements in man with histological data, and consequently ^{23}Na-MRI is not an established diagnostic technique in hospitals. It is clear that further experimentation is required with experimental *in vivo* stroke models to fully determine the exact time course of TSC changes in the acute phase after stroke.

Global increases in TSC values in the hours after stroke onset have been well established in the literature for many years using flame photometry [10]. However, although this technique allows for the measurement of TSC increase after stroke, its limited spatial resolution has prevented the investigation of TSC variations in different neuroanatomical areas affected by the stroke. Furthermore, this invasive technique is confined to one temporal measurement as it requires dissected brain samples. Recent studies using ^{23}Na-MRI have succeeded in non-invasively measuring an increase in TSC for up to 3 days after stroke induction with moderate spatial-temporal resolution in various animal stroke models [4, 10-14]. However, significant differences were reported in the specific time course of the TSC after cerebral ischaemia in these studies, with measurements varying between a direct increase [11, 14], and a delayed increase with either an initial decrease [15] or with only a slightly increasing TSC [1, 9] during the early phase. Furthermore, to our knowledge no study to-date has succeeded in quantifying the TSC in different ischaemic subregions (i.e. penumbra and core) during the early hours of stroke.

^{23}Na-MRI studies in animal stroke models have made use of a variety of models, including: the intraluminal thread model [13, 14], direct middle cerebral artery occlusion (MCAO) with combined bilateral common carotid artery occlusion (CCAO) [11, 13] in the rat, and autologous embolic MCAO models in rabbit [15] and non-human primate [1, 9]. These models induced different severities of ischaemic insult, depending on the addition of CCAO, the level of collateral blood supply in the model and the neuroanatomical location of the region of interest (e.g. ischaemic core versus penumbra) with resulting differences in TSC. In one study where acute ^{23}Na signal data was measured in one animal (from 20 min post-stroke), an initial decrease in ^{23}Na was detected prior to increasing ^{23}Na in tissue destined to die [15]. A common feature of the majority of these studies was the lack of quantification of the TSC, due to the achieved low SNR values. This low SNR stems from a number of sources, primarily the low *in vivo* concentration of ^{23}Na ions, but also the low gyromagnetic ratio of ^{23}Na, its fast T_2-relaxation component and the need to image with relatively high spatial resolutions of the order of several μl particularly for rat brain applications. Severe demands are thus placed on the measurement technique itself, requiring the use of highly

sensitive detector coils and short Echo Time (< 1 ms) imaging pulse sequences, both of which when unfulfilled have restricted the use of qNa-MRI in animal experiments to date.

This study aimed to test the hypothesis that TSC can be used as a surrogate marker for identifying tissue destined to die. This was tested by accurately quantifying changes in TSC in the rat brain in an established model of permanent focal cerebral ischemia, which allowed for TSC measurements from a very early time-point (0.5 h) out to ~7 h after stroke. Furthermore, a custom-built SNR-optimized detector coil was used in order to quantify the TSC with high temporal and spatial resolution so that subtle TSC changes could be studied in distinct sub-regions of the ischemic tissue.

Methods

Quantitative ^{23}Na- and anatomical ^{1}H-MRI

A newly-developed dual resonator system was used for quantitative ^{23}Na- and anatomical ^{1}H-MRI [16]. The resonator system was composed of a 12-rung double-tuned ^{23}Na/^{1}H birdcage volume resonator and a two-winding single-tuned ^{23}Na receive-only surface coil. The birdcage resonator generated a linearly polarized B_1-field at the respective ^{23}Na and ^{1}H Larmor frequencies (79.4 and 300.3 MHz). A high B_1-field homogeneity of ± 4.5 % for the birdcage ^{23}Na channel was achieved across the central spherical region with 3 cm diameter. The ^{1}H channel was used to acquire survey images and T_2-weighted ^{1}H images for registration with the low resolution ^{23}Na images. Although the volume resonator was double-tuned, the achievable ^{1}H SNR and resolution were insufficient to also acquire ^{1}H Diffusion-Weighted Image (DWI) data in a reasonable time using this resonator, which was optimized for transmit homogeneity rather than detection sensitivity. ^{1}H-sensitivity was consciously traded-off in order to achieve the stated aim of the study, namely to measure the TSC with the highest possible quantification accuracy. The coil windings of the ^{23}Na surface coil had diameters of 20 and 30 mm to closely match the dimensions of the rat brain; its design is described in detail elsewhere [16].

An optimized 2D Ultra-short Echo Time (UTE) sequence was used to acquire ^{23}Na images with high spatial and temporal resolution using a 7T system (Bruker BioSpec 70/30 system, Ettlingen, Germany). Using this technique, a *TE* of 853 μs was achieved, which helped to minimize T_2-weighting effects on the measured signal intensity. T_1-weighting effects were also minimized by a relatively long *TR* of 200 ms, considering the T_1 for ^{23}Na *in vivo* to be in the region of 40 ms [17, 18]. Details of the acquisition parameters are presented in Table 1. The number of slices that could be acquired within the *TR* of

200 ms was restricted to five and hence accurate slice positioning was required in each rat to cover the entire stroke lesion. This was achieved by positioning the ^{23}Na slice stack in the middle of the frontal cortex using the ^{1}H survey images as a guide. The 2D-radial data sets were reconstructed using a nearest neighbor regridding algorithm implemented in Matlab® (The Mathworks, Natick, MA, USA) using code developed in-house. The regridded data were filtered by a Hanning window before inverse Fourier transformation.

To quantify the TSC (combined intracellular and extracellular ^{23}Na) in the rat brain, the approach of Ouwerkerk *et al*. was adapted [19]. In addition to scanning the rat head, a separate scan was acquired of a homogeneous reference phantom of diameter 40 mm containing 120 mM NaCl dissolved in distilled water and suspended in a 1% agarose gel. In both scans, an 8 mm diameter cylindrical fiducial vial, permanently attached to the top of the surface detector coil, was included in the image field of view. Using this approach with code developed in-house in Matlab® (The Mathworks, Natick, MA, USA), the sensitivity profile of the surface coil could be compensated for given the relatively homogeneous B_1-field generated by the birdcage resonator, while also allowing for the quantification of TSC. A quantification accuracy of 10mM was achievable [16].

Stroke Model

In vivo experiments were performed under license from the UK Home Office and were subject to the Animals (Scientific Procedures) Act, 1986. The intraluminal filament model of middle cerebral artery occlusion (MCAO) was used to induce an experimental stroke [20], wherein the right MCA was permanently occluded in five male Sprague Dawley rats and a sham surgical operation performed on a further two (bodyweights 320 ± 21 g). Filaments were prepared in-house as follows: the tip of a 3/0 Dermalon blue nylon monofilament (1744-41, Sherwood, UK) was heated with a low temperature cauterizing pen (AA90, Bovie, UK) to create a bulb of 300 μm diameter for use in animals of the specific weight range 275-350 g. The left common carotid and external carotid artery were exposed and ligated. Branches of the external carotid artery (superior thyroid, occipital, lingual and maxillar arteries) were electrocoagulated and sectioned. Ligatures were also placed on the internal carotid and pterygopalatine arteries to secure and facilitate the advancement of the filament.

An incision was made on the external carotid artery close to the bifurcation of the internal carotid artery; the filament was then introduced and advanced 22 mm (distance for male Sprague Dawley rats within this weight range) along the internal carotid artery to block the origin of the MCA. Once in place the incision in the external artery was electrocoagulated and the ligature around the internal carotid artery was tightened to prevent any movement in

the filament position during transfer of the animal from the operating table to the MRI scanner. Arterial lines were frequently flushed with heparinised sterile saline (1000 units/100ml physiological saline) after blood sample collection to maintain patency of the lines, which also prevented blood clots forming as a consequence of intraluminal filament insertion.

In the sham experiments, the filament was advanced to the origin of the MCA, but immediately withdrawn. 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 (Table 2). Following completion of scanning the animals were killed by transcardial perfusion fixation using 4 % paraformaldehyde in phosphate buffer. The brains were harvested, processed, and embedded in paraffin wax, subsequently sectioned at 6 µm and stained with haematoxylin and eosin for histological analysis. Stained sections revealed the cellular morphology and neuropil pallor associated with irreversible ischaemic damage, which was transcribed onto line diagrams from the rat brain stereotaxic atlas [21] to correct infarct measurements for ischaemia-associated swelling and brain shrinkage associated with histological processing.

The continual real-time monitoring and maintenance of physiological parameters was critically important in maintaining the animals' stability during the long duration (typically ~ 7-8 h) of the experiments. From previous experience with this stroke model, penumbra tissue was expected to occur primarily in the dorsolateral cortical regions of the MCA territory, which receive collateral blood flow from the anterior cerebral artery. End artery subcortical (striatal) tissue within the MCA territory was believed to be permanently damaged from a very early stage (within 1h) post-stroke [22].

Experimental Workflow

In preparation for the MRI experiment, the rat was anaesthetized with 5 % isoflurane in nitrous oxide:oxygen (70:30). A tracheal cannula was surgically implanted for artificial ventilation via a small animal respirator pump (6025 rodent ventilator, Ugo Basile, Italy) and anaesthesia maintained using 2-2.5 % isoflurane. Following induction of cerebral ischaemia [20] the rat was positioned on a support cradle, which could be moved in and out of the magnet bore, and kept warm by a blanket which was connected to warm water circulation. The double-tuned resonator system was tuned and matched *in situ* using the MRI system's facilities. The MRI system's automatic adjustment procedures were performed via the ^{1}H

channel on the volume resonator, following which the survey ^{1}H images were acquired to verify correct sample and coil positioning inside the magnet. Manual adjustments of the reference pulse attenuation value and shimming were then performed for the ^{23}Na surface coil using a ^{23}Na-Free Induction decay (FID) experiment. ^{23}Na-MRI data were acquired for up to 8 hours, followed by one T_2-weighted ^{1}H-RARE anatomical scan. Eight rats were scanned in total: six strokes and two shams. One of the six stroke experiments was prematurely terminated due to an arterial bleed in the rat. The analyzed stroke animals are henceforth called as stroke rat 1 to 5.

TSC time-course analysis

In each rat, and in different brain regions within each rat, a similar evolution of the TSC time-course was observed, characterized by an initial variable period of unchanging TSC levels followed by a linear increase in TSC levels in affected regions. To fit these data, a "delayed-linear" model was developed that reflected these variable delay times to TSC increase in the different brain regions. This model automatically divided the TSC data into two distinct phases: an initial phase where the TSC data exhibited no change from baseline, with the data fitted to a near-horizontal line, and a second phase where the TSC data exhibited an increase, with the data here fitted to an increasing linear function. The algorithm used to compute the model parameters is described in the following section:

First, the pixels belonging to tissue exhibiting TSC increase within the observed time range were determined by pixel-by-pixel linear regression. The criterion for including pixels in the subsequent analyses was based on the slope of the fitted line: pixels were included provided the fitted slope remained above a threshold value of 2.7 mM/h. This threshold value reflected the absolute error of the TSC slope measurement, which was determined as the maximum slope variation measured in pixels in contralateral brain tissue, which was not exposed to MCAO (confirmed by histology).

Second, the initial TSC was estimated as the average of the first three data points in order to estimate whether TSC had begun to increase by the time of the first measurement. If the initial TSC was measured to be > 55 mM, then given the absolute quantification accuracy of ± 10 mM [16] and a nominal healthy TSC of 45 mM [23], the TSC was assumed to have already increased, and consequently the initial TSC was assigned to a value of 45 mM.

Third, an initial estimation was made of the cut-off point between the zero slope and non-zero slope TSC time course data. For pixels with no delay to TSC increase, this parameter was pre-set to 20 min after stroke, which was deemed to be a good estimate since the first data point was acquired at ~30 min after MCAO. For all other pixels with an initial

TSC below 55 mM, the first derivative of the TSC time course data with time, d*(TSC)*/d*(t)*, was computed starting with the initial TSC value and including consecutive sampling points one after the other. In this way, the number of sequentially-measured TSC data points which belonged to the initial zero-slope phase of the delayed-linear model could be determined. As before, once the slope value increased above 2.7 mM/h, the TSC was deemed to have increased from baseline and hence the cut-off point for the first phase was determined. The time interval between the time of stroke induction and the time of acquisition of the last data point included in this initial phase defined the parameter "delay time to TSC increase".

Fourth, once this delay-time parameter for the initial phase was determined, the remaining TSC data were fitted to a linear function according to:

$$TSC_j(t) = c_{init,j} + slope_j\left(t - t_{del,j}\right)$$

where the $TSC_j(t)$ in pixel j at time t depends on the initial TSC $c_{init,j}$, the delay time $t_{del,j}$ and the TSC slope $slope_j$. The parameter boundaries for the least square fit were set to ± 10 mM for the initial TSC, to between 2.7 mM/h and 30 mM/h for the TSC slope, and to ± 20 min for the delay time. The exact delay time was then computed as the time at which the linearly increasing curve intercepted the initial TSC value.

This model was fitted to every pixel in the TSC maps and two further maps were generated: (i) a TSC slope map, reflecting the rate of TSC increase at each pixel location, and (ii) a delay-time map, reflecting the delay before the TSC was observed to increase at each pixel location.

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Infarct size analysis

The final infarct size was determined both from TSC slope maps and from histology line diagrams. The TSC slope maps were used rather than absolute sodium concentration maps, since the former better delineated regions exhibiting TSC changes due to the effective data smoothing resulting from the fitting of the data. All five coronal ^{23}Na slices, each with 2 mm slice thickness, were used for the volume measurement. The error in this measurement was estimated to be 10 %. Histology sections were 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 [21]) with appropriate sections matched up to each of the five ^{23}Na-MRI brain slices using neuroanatomical landmarks. Histology sections were examined by light microscopy and the boundary of the ischaemic lesion 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 [21] as described elsewhere [24]**.** The measurement error for determining the ischaemic lesion volume from the histology slices was given as 5 %. Infarct volumes determined by histology were plotted as a function of those determined by the ^{23}Na-MRI approach and linear regression was performed. Furthermore, the correlation coefficient was computed in order to determine how accurately the lesion can be measured with the ^{23}Na-MRI technique.

Statistical Analyses

An Analysis of Variances (ANOVA) analysis was carried out to check for significant differences in the TSC slopes and delay times between the caudate nucleus and cortex regions. Values are expressed as mean and standard deviation. Statistical significance was assumed when $p < 0.05$.

Results

Physiological variables were stable and within normal limits throughout the experiment (Table 2). The TSC maps for one representative stroke rat are presented in Figure 1, showing five coronal slices across the MCA territory at every hour for up to 8 h after MCAO. Clear increases in the TSC during this period are evident in these maps, which further reveal regional variations in the TSC increase, for example between the ipsilateral caudate nucleus (CN) and cortex (COR). The TSC increased from a normal value of ~ 45 mM to approximately 130 mM over the first 7 h post-MCAO in ipsilateral cortical and subcortical regions of the MCA territory. The TSC was followed up to four hours after surgery in the sham experiments, and was found to remain constant at 45.1 ± 0.2 mM and 47.7 ± 0.3 mM for the contralateral and ipsilateral hemispheres, respectively.

Figure 2 shows the qualitative comparison between the region with increased TSC at 7 h (Figure 2b) after MCAO and the region of pallor and histopathology in the corresponding histology sections (Figure 2d) for one representative rat. A modest ~ 15 mM increase in TSC was evident at 1 h post-stroke in the CN (Figure 2a). To quantitatively determine stroke lesion volume from TSC data, the TSC slope maps were computed from the time series of TSC maps (Figure 2c). Photographs of the corresponding histochemically-processed brain sections and data on ischaemic damage transcribed onto line diagrams are presented in Figure 2d & 2e, respectively. Note the similarity in the topography of the ischaemic lesion between the line diagrams (2e) and TSC slope maps (2c). A graph of lesion volume

determined from TSC and histology averaged across the five rats is presented in Figure 3, demonstrating a statistically significant correlation ($p = 0.0006$).

Further analysis of the time-course of the TSC increase revealed delays in the time at which the TSC was observed to increase within regions of interest (ROIs) placed in the COR and the CN based on the known anatomical locations and the knowledge from the histology about the lesion extend at the end of the experiment. A third ROI was placed in the contralateral normal hemisphere. A rapid increase was identified in the ischaemic CN in comparison to a more delayed increase (by upwards of 2 h post-MCAO) in the ischaemic COR. The TSC time-course data for these ROIs and the fitted delayed-linear model are presented in Figure 4 for one representative rat.

The computed TSC slope and delay time maps for the corresponding coronal brain slice are presented in Figure 5. From this figure, several interesting features can be discerned, which were common to all stroke rats studied. First, the rate of TSC increase, as demonstrated by the TSC slope map, was similar in ischaemic CN and COR. Second, there was a marked difference in the delay times measured for CN and COR. Third, a delayed linear rather than linear fit appeared more appropriate for the TSC increase measured in each pixel, as compared to the linear fit that had been previously suggested in earlier stroke studies [11, 13].

The mean TSC slope and delay times averaged across ROIs placed in the CN and the COR for the five stroke rats are listed in Table 3. Due to variability in the location of ischemic damage from animal to animal, the position of the COR ROI was adjusted to the area of ischaemic cortex in each stroke. Although time constants varied across the group, no significant difference ($p = 0.5$) was detected between TSC slope in COR (10.2±1.1mM/h) and CN (9.7±1.1mM/h) in the ischaemic hemisphere. The mean delay times, presented in Table 2, were significantly different ($p=0.0002$) in CN (0.2±0.1h) and COR (1.6±0.5h) across the five stroke rats. To demonstrate regionally discrete TSC changes, time course data were averaged across each of four new ROIs located in the ischaemic lesion, where each ROI was automatically chosen by thresholding the delay time maps to four different ranges: 0-1 h, 1-2 h, 2-3 h, and 3-4 h (i.e. all pixels exhibiting a delay time from 0 to 1 h were assigned to the first ROI, etc.). The color-coded ROIs thus generated across all five slices in each of the five stroke rats are presented in Figure 6, together with the TSC time course data for each ROI averaged across all slices and all rats. The TSC increased first in the core of the ischaemic lesion comprising the caudate nucleus and parts of the ventral cortex before it extended across the dorsal cortex. This is also clearly reflected in the TSC time course data.

An interesting feature evident in Figure 6 is the initial dip in the TSC time course data for the ROI representing the longest delay times (i.e. 3–4 h).

Discussion

Although the potential of ^{23}Na-MRI to identify injured tissue during the acute phase of stroke has been well known for many years [8], its clinical utility remains somewhat unclear. For example, the concept of a threshold of TSC, below which tissue may remain viable but nevertheless at risk of infarction, was first suggested as far back as 1999 [9]. However, it remains to be clarified whether such a threshold exists or indeed whether a single threshold would be valid across all brain regions. Attempts have also been made to infer tissue status from measurements of the rate of increase in TSC. For example, it has been suggested that higher rates of change in ^{23}Na accumulation in subcortical ischaemic core may reflect the consequences of a more severe ischaemia due to the lack of collateral blood supply [1]. In the current study, the TSC was found to increase at similar rates in affected regions in the caudate nucleus and cortex, although the time at which these increases occurred varied significantly, with the former exhibiting an early increase in TSC levels, while the latter exhibiting a delay of 2-3 hours before a similar increase was measured.

Overall, no consistent pattern for rates of increase in TSC has emerged to-date in the literature, but this most likely reflects different regions of interest (cortex versus sub-cortex, penumbra versus ischaemic core), the range of models and species used, which will have variable severities of ischaemia and collateral circulation. Human studies have been limited by the heterogeneity of the disease in man and the uncertainty of perfusion status in serial studies [7]. Studies where TSC sampling is delayed a number of hours after stroke will also fail to identify any acute delays in ^{23}Na accumulation or transitions from slow to more rapid ^{23}Na accumulation in tissue [11]. Taking these factors into consideration, in human stroke, a lag in the increase in ^{23}Na signal intensity has been identified with increases of less than 10 % in the first 7 hours increasing to 23 % beyond 9 hours and eventually leveling at 69 % [7]. A

recent paper reported no ^{23}Na signal increase in penumbral tissue as defined by the diffusion/perfusion-mismatch within 4 to 7 h after stroke onset [25].

In the ischaemic core (not defined as cortex or sub-cortex) in a rabbit embolic model, an acute (11 %) decrease in signal during the first 40 minutes of ischaemia was followed by a 12 %/h increase, culminating in a 25 % increase over baseline by 4 h post-stroke [15]. The measured TSC slopes of 22 %/h (CN) and 23 %/h (COR) in the current study compared well with 25 %/h [11] and 24 %/hr [13] measured in rat ischaemic cortex following MCAO plus bilateral CCAO and intraluminal filament-induced MCAO, respectively. Reported values in caudate nucleus of 15 %/h were significantly lower than the rate of ^{23}Na increase in cortex [13]. The similarity in the rate of increase of TSC in different tissue types, suggests that the rate of TSC increase in itself does not serve as a marker for tissue viability after MCAO, but rather, any increase in TSC above normal levels (approximately 45 mM for the rat brain) may indicate the likelihood of subsequent infarction.

The delayed-linear model applied to the measured TSC data in this study is a novel approach to analyze TSC data after stroke. Other groups have applied linear [11, 13] or sigmoid [7] models to fit their data, but none have measured a delay before the TSC increased. The nature of the actual increase may deviate from linear, particularly when approaching an assumed upper limit of 140 mM, and indeed previous studies have reported such a leveling-off of the TSC increase during the subacute phase between 24 to 48 h after stroke in humans [7] and rats [4].

The observation of a delay to increase of upwards of 3 h in ischaemic MCA territory cortex, compared to an almost immediate increase in dorsolateral caudate nucleus, together with a linear behavior over the investigated time range once the TSC began to increase, was consistent across all five stroke rats examined. Further, the correlation coefficient between the areas exhibiting TSC increases at 8 h post-MCAO and the areas of histology-defined irreversibly damaged tissue was also high across the five rats. Thus, abnormally high TSC at 8 h after stroke indicated infarcted tissue. A similar correlation between TSC measurements and histology was previously reported at 12 h after MCAO in a study, which also described the difficulty in exactly measuring lesion volumes based upon ADC measurements or T_2-weighted images during this phase due to discrepancies between histology and ^{1}H-MR results [4].

The significant difference in delay time to TSC increase observed between caudate nucleus and cortex may be due to early ischaemic damage in this region as a result of more severe ischaemia, compared to dorsal cortex which will receive collateral blood supply from

the anterior cerebral artery [13] allowing it to remain viable during the initial 2-3 h period post-MCAO. However, without reperfusion, this tissue ultimately becomes infarcted, as indicated by the later increase in TSC in this region. With time, collateral supply fails and waves of spreading depolarization from the core compromise its viability. In this context, the delay time maps effectively represent a retrospective view of the penumbral tissue, which could be defined as brain regions exhibiting a significant delay before TSC increase. A recent study describing a method to identify penumbra based on an oxygen challenge and the resultant changes in T_2^* signal intensity, (based on changes in vascular deoxy/oxyhaemoglobin) revealed a similar spatial pattern of core versus penumbral tissue for this stroke model [22]. The histological analysis of samples from the current study further closely resembled that from reference [22] in both the size and location of lesions, which further supports this hypothesis.

The ability to image sodium with high SNR, which leads to a high TSC quantification accuracy, is crucial in identifying subtle changes in TSC during the acute phase of stroke. The excellent ^{23}Na -sensitivity came at the cost of reduced ^{1}H-sensitivity. The approach adopted was to correlate changes in sodium concentration with histology, which provides a more definitive indication of infarcted tissue than ^{1}H-ADC measurements. While this is not practicable in clinical imaging, our aim was rather to investigate the potential utility of ^{23}Na-MRI for acute stroke imaging by investigating with a high degree of quantification accuracy and temporal resolution the TSC changes which occur in the acute phase. A previous study using a monkey stroke model reported different rates of TSC increase with an initial slow rate of increase (characterized by a linear TSC slope of 1.2 mM/h) measured in the periphery of the stroke in the right basal ganglia for the initial 7 h post-MCAO, followed by a faster rate of increase of > 5 mM/h thereafter (n = 1) [1]. However, the validity of such a low TSC slope value of 1.2 mM/h must be considered in relation to the experimental quantification error for ^{23}Na-MRI in general. Although the authors did not quote the quantification error in their study, it is reasonable to suggest that it may have been strongly degraded by swapping the ^{1}H and ^{23}Na resonators several times during the course of the experiment, which was required in their experiment for interleaved ^{1}H and ^{23}Na imaging. By way of comparison, a similar TSC study reported a zero-slope in normal monkey brain tissue with a variance of ± 2.2 %/h (equivalent to ± 1 mM/h) with no swapping of the resonators [26]. Such results emphasize the importance of strictly controlling all experimental factors which can potentially affect the sodium signal intensity. If the TSC slope for potential penumbra tissue reported in [1] was indeed found to be insignificant, these data could be

re-interpreted as evidence of a delay before the TSC increased, in this case of the order of 7 h. Further evidence of a delay before TSC increase may follow from the observation of a 0.45 h delay (determined for the entire histologically-defined stroke lesion at the end of the experiment) before TSC increase in a study of stroke in a single non-human primate [26], and in a rabbit embolic stroke wherein an initial ^{23}Na signal decrease of 11 ± 8 % was observed for 40 minutes followed by a subsequent ^{23}Na signal increase with a slope of 12 %/h [15].

The linear slope maps proved to be a sensitive method of determining stroke lesion volumes, since it enables one to determine a changing TSC regardless of the normal TSC level at that location, which can differ for instance between cerebral spinal fluid (CSF) and cerebral tissue. Furthermore, regions of interest automatically generated from brain regions exhibiting similar delay times are capable of illustrating regionally-disparate behavior in the TSC evolution. Increasing the size of these ROIs by extending the delay time threshold used to categorize brain regions is thought to reflect the gradually increasing region of irreversibly damaged tissue, spreading from the caudate-nucleus and ventral cortex to more dorsal cortex after MCAO. These data support the hypothesis that a threshold TSC for infarction in fact exists at the extreme of normal physiological levels in the brain tissue, and consequently any increase above this value indicates subsequent infarction where ischaemia is permanent. The apparent initial dip in the TSC time course data for the ROI representing the longest delay times (i.e. 3-4 h, Figure 6) may reflect a shift in compartment volumes (i.e. an increase in the intracellular volume), which compensates for the initial ^{23}Na ion influx into the intracellular space prior to complete rupture of the cellular membrane. However, further work is required to verify this theory.

If optimal perfusion thresholds can be defined for stroke tissue, an alternative approach for identification of penumbral tissue could involve a qNa-MRI / PWI mismatch, where the focus of attention in the TSC maps would be on hypoperfused regions for which the TSC has not yet increased. In this manner, core would be identified by qNa-MRI and penumbra would represent the difference between core and the perfusion deficit, in much the same way as the mismatch between the ADC-defined core and the perfusion deficit is currently used to define penumbra. 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, qNa-MRI may more accurately identify core tissue which is “destined to die”. The ratio of core-to-penumbra tissue could then be used as a time-independent decision criterion for various therapy options after ischaemic stroke. Further work is required to investigate the usefulness of such an approach.

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Table 1: Sequence parameters for *in vivo* ^{23}Na and ^{1}H brain imaging. *

	^{23}Na	^{1}H
Sequence	**2D UTE**	**2D RARE**
FoV	**20 cm x 20 cm**	**8 cm x 8 cm**
MTX	**256 x 256 (100 projections).**	**256 x 256**
ST	**2 mm**	**2 mm**
TA	**10 min**	**5 min**
TE	**853 µs**	**64 ms (RARE-factor 8)**
TR	**200 ms**	**3262 ms**
FA	**90°**	**90°/ 180°**
BW	**15 kHz**	**50 kHz**

*Abbreviations used in table: Field of View (FoV), Matrix Size (MTX), Slice Thickness (ST), Acquisition Time (TA), Echo Time (TE), Repetition Time (TR), Flip Angle (FA), band width (BW).

Table 2: Recorded physiological parameters at three stages of the experiment. *

	Body Temperature [°C]	***MABP [mmHG]***	***HR [bpm]***	***pH***	***paO_2 [mmHg]***	***$paCO_2$ [mmHg]***
Before MCAO	**36.9 ± 0.6**	**83 ± 4**	**397 ± 23**	**7.44 ± 0.002**	**156 ± 1**	**36 ± 3**
MRI start	**36.9 ± 0.5**	**85 ± 4**	**376 ± 1**	**7.41 ± 0.013**	**135 ± 29**	**38 ± 3**
MRI end	**37.1 ± 0.1**	**84 ± 4**	**376 ± 15**	**7.37± 0.013**	**104 ± 10**	**36 ± 6**

*Data are presented as mean±SD (n=5)

Table 3: TSC slope and delay time data in caudate nucleus (CN) and cortex (COR) ipsilateral to the stroke along with the histology-derived lesion volume.

	CN TSC slope [mM/h] [a]	***COR TSC slope [mM/h]*** [a]	***CN TSC delay time [h]***	***COR TSC delay time [h]***	***Lesion Volume from histology [mm³]*** [b]
Stroke 1	**8.5±1.2**	**8.4±1.0**	**0.03±0.1**	**1.4±0.5**	***290 ± 15***
Stroke 2	**8.6±1.1**	**9.9±1.4**	**0.2±0.3**	**2.4±0.5**	***260 ± 13***
Stroke 3	**10.4±1.3**	**10.6±2.0**	**0.3±0.3**	**1.3±0.4**	***340 ± 17***
Stroke 4	**10.3±2.2**	**11.0±1.0**	**0.2±0.2**	**1.4±0.4**	***184 ± 9***
Stroke 5	**10.8±2.0**	**11.2±3.0**	**0.04±0.15**	**1.3±0.4**	***110 ± 6***
Mean	**9.7±1.1**	**10.2±1.1**	**0.15±0.1**	**1.6±0.5**	***217 ± 128***
p-value	**0.5**		**0.0002**		

[a] Data are presented as mean±SD across the ROIs

[b] Errors derive from the lesion volume measurement technique (± 5 %)

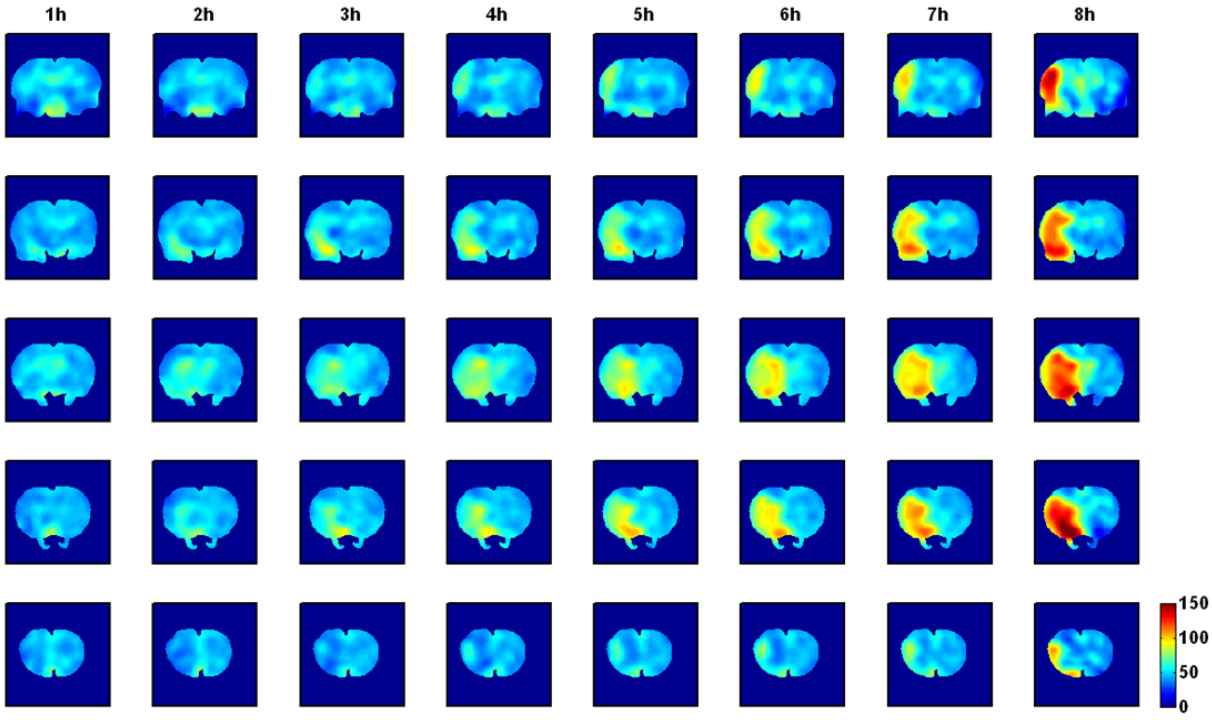

Figure 1: The evolution of TSC after MCAO for 5 coronal slices (rows) across 8 h (columns) in a representative brain (stroke rat 2). Times after MCAO are labeled above each column and the color bar indicates TSC in units of mM. Note the TSC changes in the ischaemic right hemisphere (left side on these TSC maps) after MCAO. Also note the delayed TSC increase in the cortex compared to the caudate nucleus.

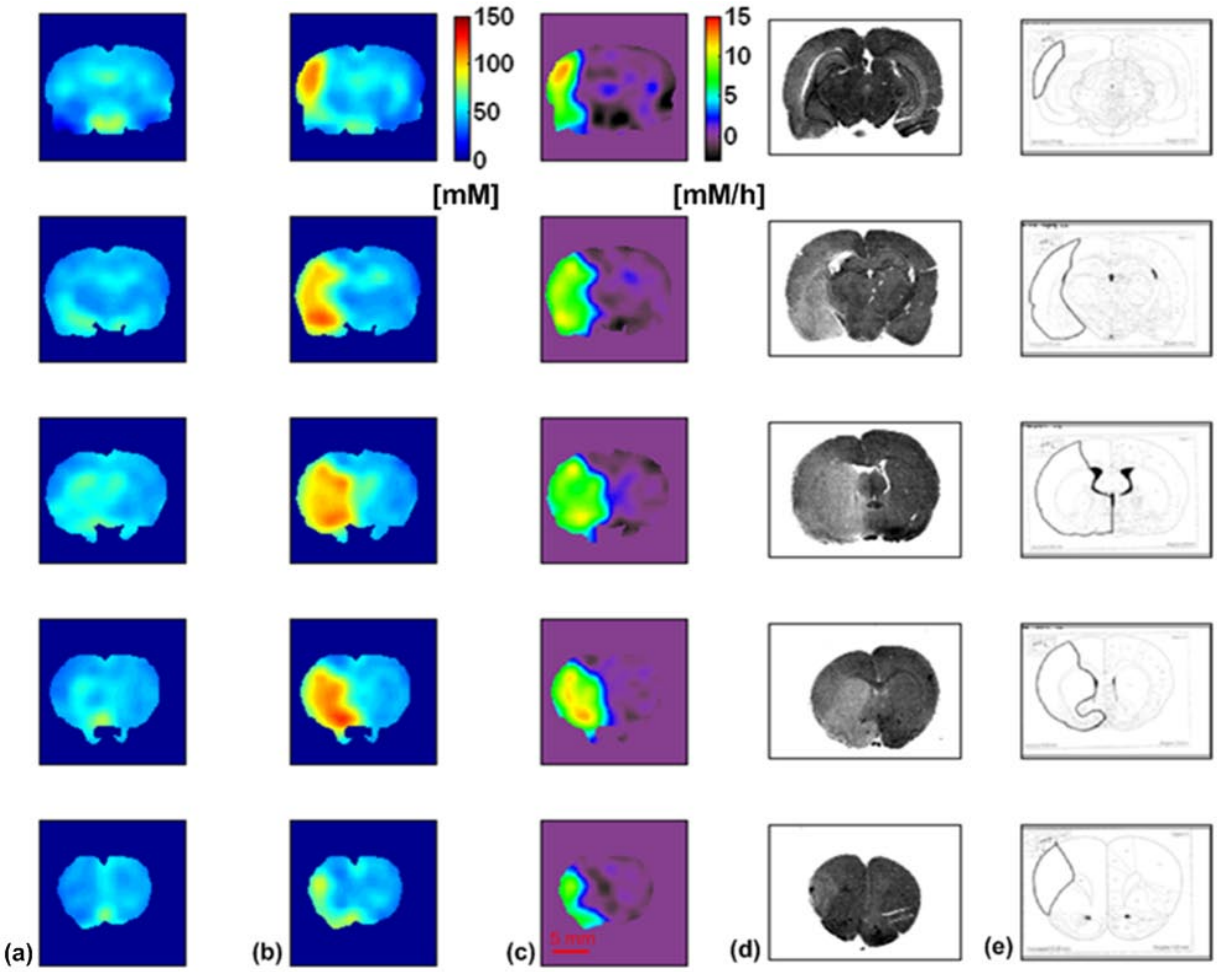

Figure 2: TSC maps at (a) 1 h and (b) 7 h after MCAO for stroke rat 2. (c) The corresponding TSC slope map was used to compute the stroke lesion volumes (threshold 2.7 mM/h). (d) Photographs of the histochemically stained brain sections displaying ischaemic damage at 8 h post-stroke and (e) corresponding line diagrams onto which ischaemic damage was transcribed in order to correct for brain swelling and tissue processing (± 1 mm slice co-registration error). Five of the 8 histology levels measured are displayed.

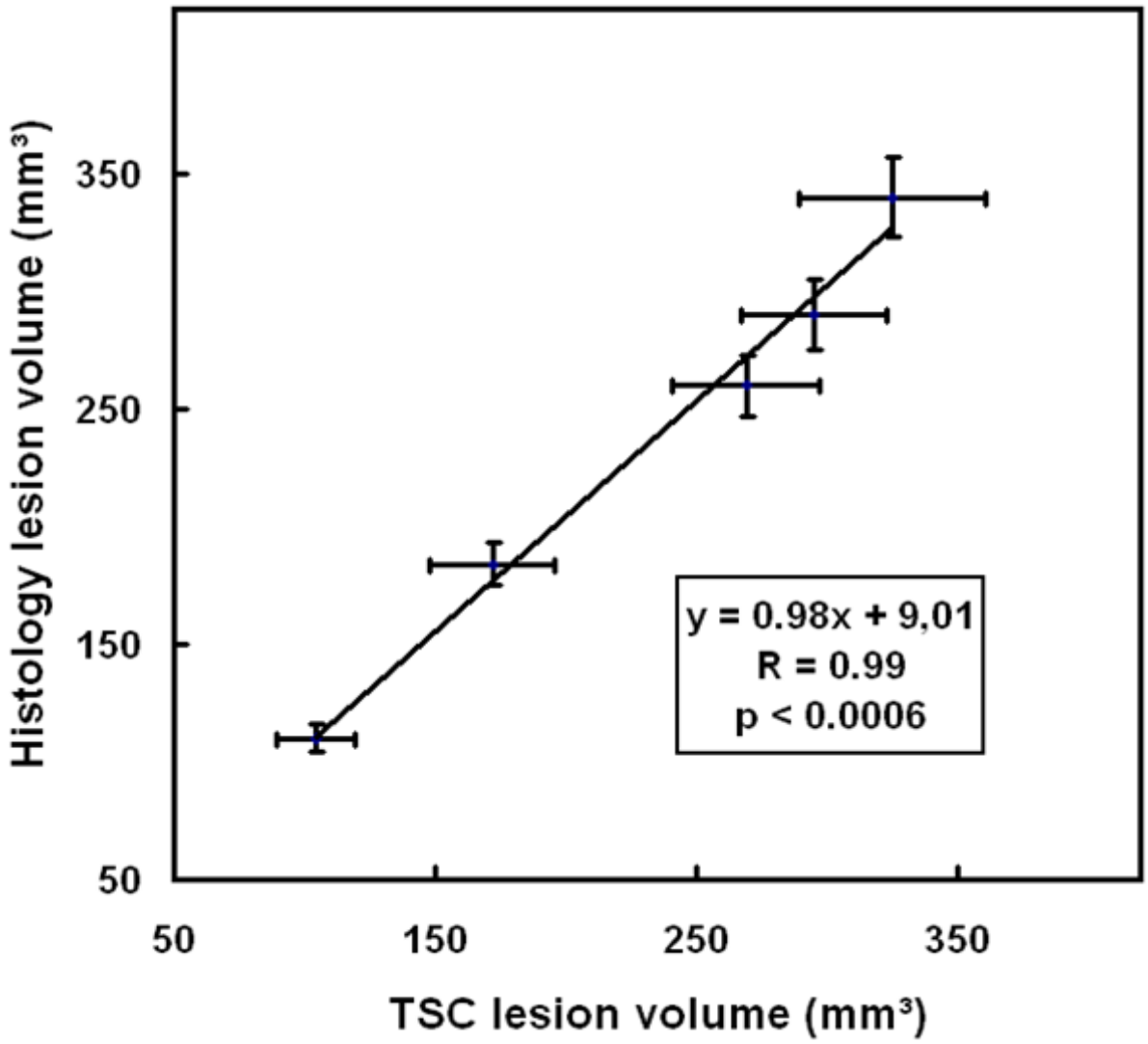

Figure 3: A linear regression correlation between volumes of irreversible ischaemic damage determined from histology transcribed onto line diagrams and threshold TSC slope maps measured at 8 hours after MCAO (n = 5, correlation coefficient = 0.99, $p < 0.0006$).

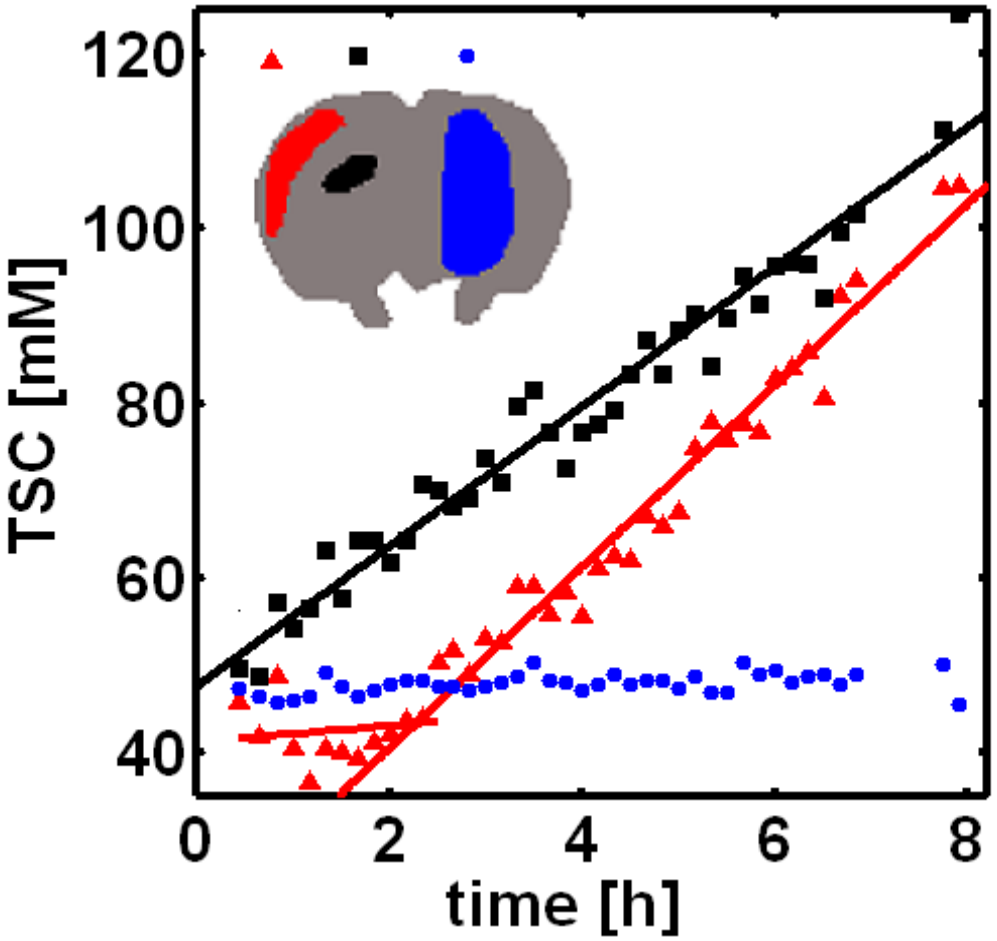

Figure 4: The delayed-linear model fit to the TSC data measured in ROIs located within the ischaemic caudate nucleus (black squares) and cortex (red triangles) together with the TSC data for a contralateral ROI (blue circles) in one representative brain slice (stroke rat 2).

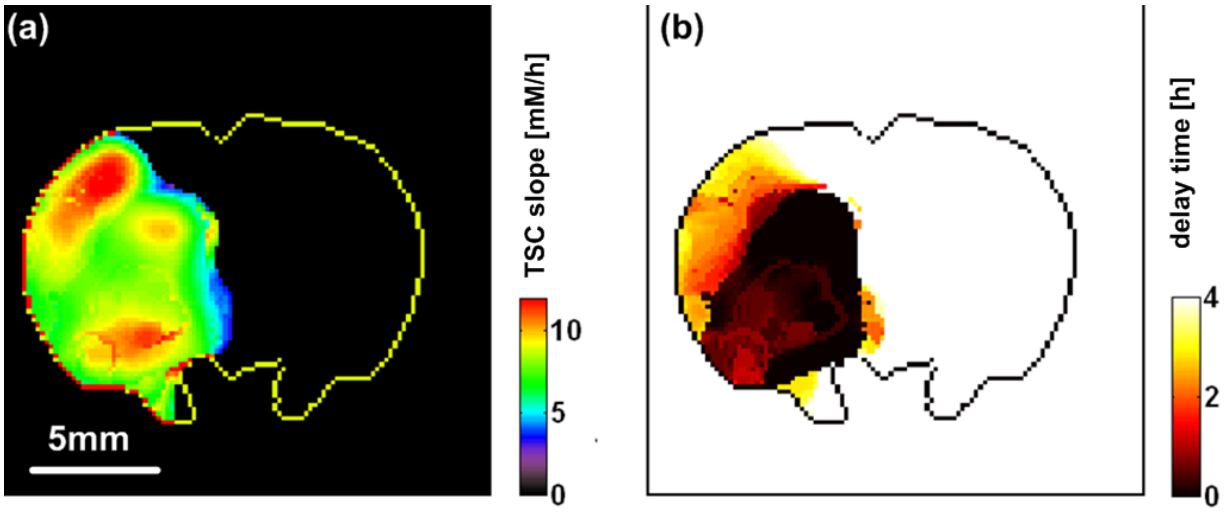

Figure 5: Representative data from stroke rat 2. (a) TSC slope map, illustrating a similar rate of TSC increase in the affected regions in the cortex and caudate nucleus. (b) Delay time map showing the early increase in TSC in the caudate nucleus and ventral cortex, with a delay of > 2-3 h in the more dorsal regions of the cortex.

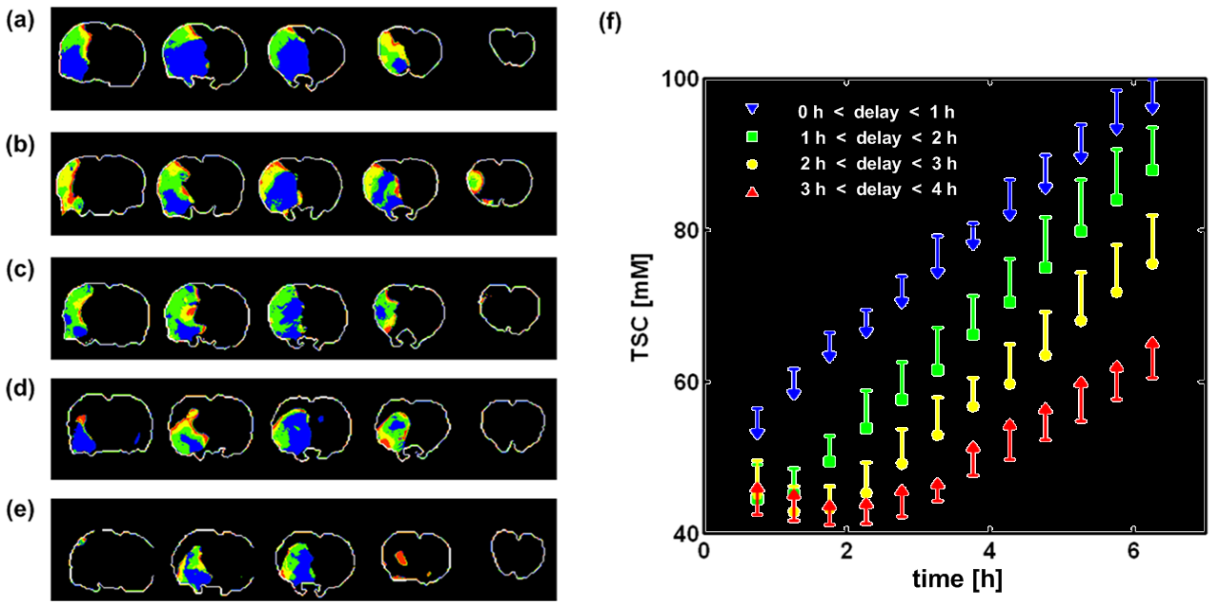

Figure 6: Marked ROIs in five slices of stroke rats 1 to 5 (rows a – e). Four ROIs were automatically selected by thresholding the delay time parameter, to between 0-1 h (blue), 1-2 h (green), 2-3 h (yellow), and 3-4 h (red). (f) TSC time course data for each ROI as a function of time**.**