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DIFFERENCES IN THE EVOLUTION OF THE ISCHAEMIC PENUMBRA IN THE SHRSP AND WKY RAT.

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**Full Title: DIFFERENCES IN THE EVOLUTION OF THE ISCHAEMIC
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Cover Title: Evolution of the ischaemic penumbra in the SHRSP and WKY rat.

Tables: 1

Figures: 5 graphs

Keywords: Ischaemic penumbra, stroke, hypertension, MRI

Abstract

Background and Purpose: Stroke prone spontaneously hypertensive rats (SHRSP) are a highly pertinent stroke model with increased sensitivity to focal ischaemia compared to the normotensive reference strain (WKY). Study aims were to investigate temporal changes in the ischaemic penumbra in SHRSP compared with WKY.

Methods: Permanent middle cerebral artery occlusion was induced with intraluminal filament. Diffusion (DWI) and perfusion-weighted (PWI) MRI was performed from 1-6hrs post-stroke with PWI/DWI mismatch to define penumbra and thresholded Apparent Diffusion Coefficient maps to define ischaemic damage.

Results: There was significantly more ischaemic damage in SHRSP than WKY from 1-6 hours post-stroke. The perfusion deficit remained unchanged in WKY (39.9 ± 6 at 1hr, 39.6 ± 5.3 mm² at 6hrs) but, surprisingly, increased in SHRSP (43.9 ± 9.2 at 1hr, 48.5 ± 7.4 mm² at 6hrs, P=0.01). One hour post-stroke, SHRSP had significantly less penumbra (3.4 ± 5.8 mm²) than WKY (9.7 ± 3.8 , P=0.03). In WKY, 56% of 1hr penumbra area was incorporated into ADC lesion by 6hrs, while in SHRSP the small penumbra remained static due to the temporal increase in both ADC lesion and perfusion deficit.

Conclusions: Firstly, SHRSP have significantly more ischaemic damage and less penumbra than WKY within 1hr of stroke; Secondly, penumbra is recruited into the ADC abnormality over time in both strains; Thirdly, the expanding perfusion deficit in SHRSP predicts more tissue at risk of infarction. These results have important implications for management of stroke patients with pre-existing hypertension and suggest ischaemic damage could progress at a faster rate and over a longer timeframe in the presence of hypertension.

Introduction

One of the most important considerations when treating acute stroke patients is to establish whether potentially salvageable (penumbral) tissue is still present within the brain. In this manuscript we have used a pertinent rodent model to investigate if recognised stroke risk factors such as hypertension influence the amount of penumbral tissue and the rate at which it is incorporated into the ischaemic lesion.

The ischaemic penumbra is a brain region surrounding the ischaemic core which receives limited blood supply from the collateral circulation [1]. Tissue perfusion lies between the blood flow thresholds for functional impairment and morphological integrity which means the tissue may be capable of recovery, provided perfusion is reinstated within a limited time window, beyond which the tissue becomes irreversibly damaged and incorporated into the infarct. Ischaemic penumbra can be assessed clinically with MRI, identifying the mismatch between the perfusion deficit (perfusion weighted imaging, PWI) and injured tissue in which cell swelling has occurred (diffusion weighted imaging, DWI) [2].

The stroke prone spontaneously hypertensive rat (SHRSP) is a relevant model of human stroke. As its name implies, SHRSP develop hypertension between 8-12 weeks of age and have an increased incidence of spontaneous stroke [3,4]. They are an ideal model for studying ischaemic stroke due to similarities in terms of their cerebrovascular architecture and risk factors compared to stroke in man [5]: SHRSP have an increased sensitivity to experimental stroke compared to normotensive strains [6,7,8,9], thought to be due to genetically determined hypertension and additional genetic factors influencing cerebral vessels and levels of oxidative stress [8,10]. The Wistar Kyoto rat (WKY), the normotensive

strain from which the SHRSP was derived, is used as the control strain for the SHRSP. To our knowledge, differences in the amount of penumbral tissue available between SHRSP and WKY has not previously been investigated, nor has serial *in vivo* evolution of acute ischaemic damage or fate of penumbra.

The aims of the present study were 1. to identify and follow the fate of penumbral tissue 6 hours following permanent MCAO using diffusion and perfusion MRI; and 2. to compare the size and lifespan of penumbral tissue in SHRSP and WKY.

Methods

Animal Preparation

Experiments were carried out under license from the UK Home Office and were subject to the Animals (Scientific Procedures) Act, 1986. SHRSP and WKY rats were obtained from inbred colonies within the University of Glasgow, Division of Cardiovascular and Medical Sciences.

Male WKY and SHRSP rats (220-390g, age; 12-16 weeks, n=8 per strain) were anaesthetised (4-5% isoflurane) in an induction chamber, then intubated and artificially ventilated (with 1.5-2% isoflurane in 70:30 N₂O/O₂). Body temperature was monitored throughout surgery with a rectal thermocouple and maintained at 37⁰C. The femoral artery was cannulated with PE-50 tubing for continuous monitoring of arterial blood pressure and heart rate (Biopac) and for measurement of arterial blood gases (Bayer, Rapidlab 248).

Permanent occlusion of the MCA was performed by intraluminal thread occlusion using a 3-0 nylon monofilament with rounded tip (diameter 0.28-0.3mm for rats 200-400g) as previously described [11]. Following filament insertion, the animal was transferred to the magnet. Anaesthesia was maintained at 1-2% isoflurane in 70:30 N₂O/O₂, temperature was continuously monitored and maintained by an enclosed warm water circuit.

Magnetic Resonance Imaging

Magnetic Resonance Imaging was performed on a Bruker Biospec 7T/30 cm system with a gradient coil (121 mm ID, 400mT/m) and a 72mm birdcage resonator. An actively decoupled linear surface receiver coil (2cm diameter) was used for brain imaging. After surgery, animals were placed prone in a rat cradle, with the head restrained using ear and tooth bars to limit movement, with the surface coil placed above the head .

A pilot sequence was acquired first to determine the correct geometry, followed by a RARE T_2 (effective TE: 47.2ms, TR: 5000 ms, 4 averages, matrix: 256x256, FOV: 25x25 mm, 30 contiguous slices, 0.5 mm thickness) for anatomical reference. For quantitative determination of the apparent diffusion coefficient (ADC), a 4 shots Spin Echo Planar Imaging Diffusion-weighted scan (TE: 22.5 ms, TR: 4000.3 ms, 4 averages, matrix: 96 x 96, FOV: 25 x 25 mm, 3 directions: x, y, z, B values: 0, 1000 s/mm², 8 contiguous slices, 1 mm thickness) was carried out to determine lesion volume.

Non-invasive relative cerebral blood flow (rCBF) measurements were carried out on a single slice within core MCA territory (coronal slice 5 of 8 slices covering MCA territory) using flow alternating inversion recovery (FAIR) [12]. A slice-selective and a non-selective inversion recovery image (M_s & M_{ns}) were acquired with a single shot Spin Echo EPI sequence with a sufficient inversion time (TI) to allow for inflow of labelled spins into brain (TE: 50ms, TR: 12000ms, TI: 2000ms, flip angle: 180°, matrix: 96x96, FOV: 25 x 25 mm, slice thickness, 2mm). For normalisation of the FAIR signal an equilibrium magnetisation (M_0) image was acquired with the same parameters, without inversion. A single shot Spin Echo Planar Diffusion-weighted scan (TE: 22.5 ms, TR: 4000.3 ms, 4 averages, matrix: 96 x 96, FOV: 25 x 25 mm, 3 directions: x, y, z, B values: 0, 200, 600, 1200, 2000 s/mm², slice thickness, 2mm) was also carried out on the same slice as the CBF measurements which had the same image distortion as the CBF maps and allowed us to co-register the ADC and CBF maps for determination of the diffusion/perfusion mismatch area.

DWI and PWI scans were started 1 hour following stroke and repeated every hour until 6 hours after MCAO.

Data Analysis

Quantitative ADC_{av} maps, in units of square millimetres per second, were calculated using the Stejskal-Tanner equation [13]. ADC maps and rCBF maps were generated using Image J software (<http://rsb.info.nih.gov/ij/>). A 23% reduction of mean contralateral ADC was used to determine ischaemic lesion volume from the multi slice ADC maps and lesion area from the single slice ADC map. Perfusion deficit area was calculated based on a 57% reduction of mean contralateral CBF [14]. Diffusion-perfusion mismatch was calculated as the difference between the perfusion deficit minus the ADC lesion area on the corresponding slice. All data analysis was carried out blind as to rat strain.

Statistical Analysis

Data are expressed as mean \pm S.D. A Student's paired t-test was used to determine significance within strains. Sequential changes between strains were compared with two-way ANOVA with a Bonferroni post test to correct for multiple comparisons. An unpaired t-test was used to compare lesion progression between strains. A P value of 0.05 or less was considered statistically significant.

Results

Physiological Variables & 1 hour Perfusion and Diffusion scans

Physiological variables are shown in **Table 1**. All physiological variables were well controlled throughout the 6 hour scanning period in both groups ($P>0.05$). The mean CBF reduction, taken from the ischaemic core in caudate putamen, was not significantly different between strains ($9.7\pm6.7\%$ and $10.5\pm6\%$ of mean contralateral CBF for WKY and SHRSP rats, respectively) indicating a similar severity of ischaemic insult. **Figure 1** highlights the DWI/PWI mismatch at 1 and 6 hours following permanent MCAO in WKY and SHRSP.

Evolution of ADC-derived lesion volume

In both strains the ADC-derived lesion volume increased significantly from 1-6 hours post-MCAO (WKY, 171 ± 30 mm³ at 1hr, 253 ± 43 at 6hr; SHRSP 275 ± 75 mm³ at 1 hr, 330 ± 57 mm³ at 6 hr, **Figure 2**). The ADC lesion was significantly larger in SHRSP than WKY within one hour of stroke and at all subsequent time points ($P<0.05$ for all time points). The 1-6 hour evolution of the ADC lesion was not significantly different between the two strains (increase of 77 and 54 mm³ in WKY and SHRSP, respectively; $P>0.05$, unpaired t-test). However, the SHRSP lesion volume was virtually maximal at 6 hours, covering the entire MCA territory .

Evolution of the Perfusion Deficit

Single slice rCBF maps within the MCA territory revealed the persistence of MCAO over the 6 hours. **Figure 3** shows area of perfusion deficit over the entire time course for WKY and SHRSP. The perfusion deficit area was unchanged in WKY throughout the 6 hour time course (from 39.9 ± 6 at 1hr to 39.6 ± 5.3 mm² at 6 hrs post-MCAO, $P=0.64$, paired t-test). In contrast, SHRSP rats displayed a significant increase in perfusion deficit area over the 6 hours (from 43.9 ± 9.2 at 1 hr to 48.5 ± 7.4 mm², 6 hrs post-MCAO, $P=0.01$, paired t-test). There was little difference in perfusion deficit area at 1hr following MCAO between strains (39.9 ± 6.1 mm² WKY versus 43.6 ± 9.2 SHRSP). However, by 5 hours (40.6 ± 5.6 versus 48.7 ± 6.2 mm²) and 6 hours (39.6 ± 5.3 versus 48.5 ± 7.5 mm²) there was a greater difference in perfusion deficit between strains, although this was not statistically significant.

Evolution of the Diffusion Perfusion Mismatch

The diffusion-weighted image from the same coronal slice as that used for perfusion-weighted imaging was used to calculate the diffusion-perfusion mismatch area. **Figure 4**

illustrates the evolution of the ADC-derived lesion on the single slice used for calculating the diffusion-perfusion mismatch. The ADC derived lesion was significantly larger in SHRSP from as early as 1hour post-stroke (40.6 ± 5.8 versus 29.3 ± 4 mm² at 1hr post-MCAO, respectively; P<0.001) and throughout the 6 hour time course (P<0.001 for all time points). There was no significant difference in the evolution of the ADC lesion area between strains (increase of 7 and 6 mm² in WKY and SHRSP between 1 and 6 hours post-MCAO, respectively; P>0.05, unpaired t-test).

Mismatch was determined as the difference between the ADC derived lesion area and the perfusion deficit area from the same slice. At 1 hour post-MCAO, the diffusion-perfusion mismatch area was significantly smaller in SHRSP (3.4 ± 5.8 versus 9.7 ± 3.8 mm² in WKY, P=0.03, **Figure 5**) even though the perfusion deficit area was not significantly different between strains at this time point. This resulted in 65% less penumbral tissue in SHRSP compared to WKY at 1 hour post-stroke. Over time, there was a significant decrease in mismatch in WKY (from 9.7 ± 3.8 at 1 hr to 3.2 ± 2.7 mm² 6 hrs post-MCAO, P=0.01, paired t-test) resulting in a 66% reduction in penumbra at 6 hours. In SHRSP there was a small but non-significant decrease in the already small mismatch area over the same time course (from 3.4 ± 5.8 at 1 hr to 1.7 ± 4.2 mm², 6 hrs post-MCAO, P=0.23, paired t-test). This lack of change in mismatch area over time in SHRSP was due to the increase in perfusion deficit area alongside the increase in ADC lesion area.

Discussion

This is the first study to investigate the spatiotemporal evolution of ischaemic damage during the acute phase in SHRSP and WKY rats following MCAO. Previous studies have demonstrated that SHRSP display increased sensitivity to stroke and have increased infarct volume following MCAO when compared to their normotensive control the WKY [6,9,15,16,17]. However, none of these studies have investigated the presence of penumbral tissue, nor its fate in SHRSP compared to WKY. The present study demonstrates that the ADC-derived lesion is significantly larger in SHRSP from 1 hour after MCAO and remained so throughout the 6 hour time course. However, there were no significant differences in the rate of lesion evolution between the strains. Parameters such as blood pressure and blood gases which can influence infarct size and development were maintained within normal levels throughout anaesthesia. Therefore it is unlikely that the increased lesion volume can be attributed to physiological variables in the SHRSP. It has been previously shown that when experimental stroke is induced in young SHRSP rats before the onset of hypertension, infarct volume is still significantly larger than age-matched WKY, indicating that factors other than hypertension contribute to increased stroke sensitivity in the SHRSP [8,18]. Increased release of glutamate has previously been demonstrated following stroke in the SHRSP [19] suggesting neuronal damage through glutamate mediated excitotoxicity as one potential mechanism that could contribute to increased lesion volume in SHRSP. Another proposed mechanism is reduced blood flow through collateral vessels linking the MCA with anterior and posterior cerebral arteries. Collateral blood vessels, investigated one month after MCAO in SHRSP and WKY revealed the anastomoses arising from the MCA were significantly narrower and blood flow less than in WKY [20]. A recent study demonstrated that the potent cerebral vasoconstrictor, 20-hydroxyeicosatetraenoic acid (20-HETE), is increased in the cerebral vasculature of SHRSP and inhibition with HET-0016, administered prior to

transient MCAO, resulted in a marked reduction in infarct volume, associated with reduced production of cerebrovascular reactive oxygen species (ROS) and reduced endothelial dysfunction [21]. In the present study the perfusion deficit area increased over 6 hours in SHRSP but remained unchanged in WKY. This increase in size of the perfusion abnormality may be attributed to failure of collateral supply, possibly mediated via increased 20-HETE, and demonstrates the potential for the perfusion deficit to gradually worsen over time. Additionally, this increase in perfusion deficit in SHRSP could further exacerbate ischaemic damage resulting in a greater incorporation of penumbral tissue into the infarct core.

One limitation of the present study was the fact that the perfusion abnormality was assessed on a single coronal slice within MCA territory, due to technical limitations of the current protocol. It is possible that more anterior or posterior perfusion scans within MCA territory would have shown a greater difference in perfusion deficit between strains.

The diffusion perfusion mismatch method [22] has been used to provide an index of penumbra and therefore the amount of potentially salvageable tissue remaining over time. The diffusion abnormality detects the development of cytotoxic oedema due to ischaemic damage [23] while the perfusion abnormality shows the region of reduced cerebral blood flow. Since the DWI/PWI mismatch is characterised as a region of tissue with a cerebral blood flow abnormality but no evidence of cytotoxic oedema, it is assumed to be potentially viable. In the present study, the SHRSP had significantly less mismatch tissue available at 1 and 2 hours following stroke when compared to WKY. This was due to the fact that the diffusion abnormality on the same slice was significantly larger in the SHRSP from 1 hour post-stroke and thereafter throughout the 6 hour time course. The mismatch area gradually became incorporated into the ADC lesion in both strains over time, indicating a reduction in

the amount of potentially salvageable tissue remaining. The insignificant amount of mismatch tissue in SHRSP from as early as 1 hour following stroke indicates that the ischaemic injury occurs very rapidly in this strain and suggests that there is less potentially salvageable tissue available for acute stroke therapies. In fact it has previously been shown that the DWI lesion is maximal at 1 hour after distal MCAO in the related spontaneously hypertensive (SHR) rat and that this correlates well with final infarct at 24 hours [24,25]. Legos and colleagues have also demonstrated that the SHR rat has little mismatch tissue at 1 hour after distal MCAO, which was attributed to poorer collateral flow in these animals.

The increase in perfusion deficit observed in SHRSP with the resulting increase in ADC lesion resulted in little change in the already small mismatch area over time. The expanding perfusion deficit would partly explain why the ADC lesion is larger in SHRSP at later time points. However at earlier time points, there was no significant difference in the perfusion deficit between strains.

In conclusion, the present study demonstrates that genetic hypertension can influence the amount and lifespan of penumbral tissue. SHRSP had significantly more ischaemic damage and less penumbral tissue than WKY within 1 hour of stroke onset. In addition the expanding perfusion deficit in SHRSP could indicate that these rats have a greater volume of tissue at risk of infarction compared to WKY. These results could have important implications for the management of stroke patients with pre-existing risk factors and suggest that ischaemic damage could progress at a faster rate and over a longer timeframe in the presence of hypertension.

References

- [1] Astrup J, Siesjö BK, Symon L. Thresholds in cerebral ischemia - the ischemic penumbra. *Stroke.* 1981;12:723-5.
- [2] Warach S, Dashe JF, Edelman RR. Clinical outcome in ischemic stroke predicted by early diffusion-weighted and perfusion magnetic resonance imaging: a preliminary analysis. *J Cereb Blood Flow Metab.* 1996; 16: 53–59.
- [3] Okamoto K, Yamori Y & Nagaoka A. Establishment of the Stroke-prone Spontaneously Hypertensive Rat (SHR). *Circ Res.* 1974; 34: I-143-I153.
- [4] Davidson AO, Schork N, Jaques BC, Kelman AW, Sutcliffe RG, Reid JL, Dominiczak AF. Blood pressure in genetically hypertensive rats. Influence of the Y chromosome. *Hypertension.* 1995; 26: 452-9.
- [5] Yamori Y, Horie R, Handa H, Sato M, Fukase M. Pathogenetic similarity of strokes in stroke-prone spontaneously hypertensive rats and humans. *Stroke.* 1976; 7: 46-53.
- [6] Jeffs B, Clark J, Anderson N, Gratton J, Brosnan MJ, Gauguier D, Reid J, MacRae IM, Dominiczak AF. Sensitivity to cerebral ischaemic insult in a rat model of stroke is determined by a single genetic locus. *Nature Genet.* 1997; 16: 364–367.
- [7] Gratton JA, Sauter A, Rudin M, Lees KR, McColl J, Reid JL, Dominiczak AF, Macrae IM, Bohr DF. Susceptibility to cerebral infarction in the stroke-prone spontaneously hypertensive rat is inherited as a dominant trait. *Stroke.* 1998; 29: 690-694.

- [8] Coyle P, Jokelainen PT. Differential outcome to middle cerebral artery occlusion in spontaneously hypertensive stroke-prone rats (SHRSP) and Wistar Kyoto (WKY) rats. *Stroke*. 1983;14: 605-11.
- [9] Marks L, Carswell HV, Peters EE, Graham DI, Patterson J, Dominiczak AF, Macrae IM. Characterization of the microglial response to cerebral ischemia in the stroke-prone spontaneously hypertensive rat. *Hypertension*. 2001; 38: 116-22.
- [10] Heistad DD, Mayhan WG, Coyle P, Baumbach GL. Impaired dilatation of cerebral arterioles in chronic hypertension. *Blood Vessels*. 1990; 27: 258-62.
- [11] Longa E.Z., Weinstein P.R., Carlson S., Cummins R. Reversible middle cerebral artery occlusion without craniectomy in rats. *Stroke*. 1989; 20; 84-91.
- [12] Kim SG. Quantification of rCBF change by flow-sensitive alternating inversion recovery (FAIR) technique: application to functional mapping. *Magn Reson Med*. 1995; 34: 293-301.
- [13] Stejskal EO, Tanner JE. Spin diffusion measurements: spin echoes in the presence of a time dependent field gradient. *J Chem Phys*. 1965; 42: 288-292.
- [14] Meng X, Fisher M, Shen Q, Sotak CH, Duong TQ. Characterizing the diffusion/perfusion mismatch in experimental focal cerebral ischemia. *Ann Neurol*. 2004; 55: 207-12.

- [15] Coyle P, Heistad DD. Blood flow through cerebral collateral vessels one month after middle cerebral artery occlusion. *Stroke*. 1987;18:407-11.
- [16] Carswell HV, Anderson NH, Clark JS, Graham D, Jeffs B, Dominiczak AF, Macrae IM. Genetic and gender influences on sensitivity to focal cerebral ischemia in the stroke-prone spontaneously hypertensive rat. *Hypertension*. 1999; 33:681-5.
- [17] McGill JK, Gallagher L, Carswell HV, Irving EA, Dominiczak AF, Macrae IM. Impaired functional recovery after stroke in the stroke-prone spontaneously hypertensive rat. *Stroke*. 2005; 36: 135-41.
- [18] Coyle P. Middle cerebral artery occlusion in the young rat. *Stroke*. 1982;13: 855–859.
- [19] Gemba T, Matsunaga K, Ueda M. Changes in extracellular concentration of amino acids in the hippocampus during cerebral ischemia in stroke-prone SHR, stroke-resistant SHR and normotensive rats. *Neurosci Lett*. 1992; 135: 184-8.
- [20] Coyle P, Heistad DD. Development of collaterals in the cerebral circulation. *Blood Vessels*. 1991; 28:183-9.
- [21] Dunn KM, Renic M, Flasch AK, Harder DR, Falck J, Roman RJ. Elevated production of 20-HETE in the cerebral vasculature contributes to severity of ischemic stroke and oxidative stress in spontaneously hypertensive rats. *Am J Physiol Heart Circ Physiol*. 2008; 295: 2455-65.

[22] Schlaug G, Benfield A, Baird AE, Siewert B, Lövblad KO, Parker RA, Edelman RR, Warach S. The ischemic penumbra: operationally defined by diffusion and perfusion MRI. *Neurology*. 1999; 53: 1528-37.

[23] Abe O, Aoki S, Shirouzu I, Kunimatsu A, Hayashi N, Matsumoto T, Mori H, Yamada H, Watanabe M, Masutani Y, Ohtomo K. MR imaging of ischemic penumbra. *Eur J Radiol*. 2003; 46: 67-78.

[24] Legos JJ, Lenhard SC, Haimbach RE, Schaeffer TR, Bentley RG, McVey MJ, Chandra S, Irving EA, Andrew A Parsons, Barone FC. SB 234551 selective ET(A) receptor antagonism: perfusion/diffusion MRI used to define treatable stroke model, time to treatment and mechanism of protection. *Exp Neurol*. 2008; 212: 53-62.

[25] Chandra S, White RF, Everding D, Feuerstein GZ, Coatney RW, Sarkar SK, Barone FC. Use of diffusion-weighted MRI and neurological deficit scores to demonstrate beneficial effects of isradipine in a rat model of focal ischemia. *Pharmacology*. 1999; 58:292-9.

Figure Legends

Figure 1. Representative thresholded CBF map with ADC abnormality (blue) overlaid highlighting the DWI/PWI mismatch (shown in pink) at 1 and 6 hours following stroke in a WKY (top) and SHRSP (bottom).

Figure 2. Temporal evolution of the ADC-derived lesion volume following permanent MCAO in SHRSP and WKY. Data presented as mean \pm SD * indicates significant statistical difference in ADC lesion volume between strains (Two-Way ANOVA with Bonferroni post test; P<0.05, n=7-8).

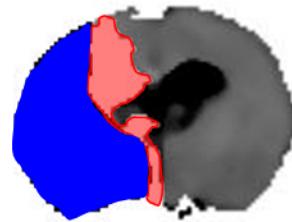
Figure 3. Temporal evolution of the PWI-derived perfusion deficit following permanent MCAO in SHRSP and WKY. Data presented as mean \pm SD. * indicates statistically significant increase in PWI lesion area from 1 – 6 hour time points (paired t-test; P<0.05, n=7-8).

Figure 4. Temporal evolution of the ADC-derived lesion area following permanent MCAO in SHRSP and WKY. Data presented as mean \pm SD * indicates statistically significant difference in ADC lesion area between strains (Two-Way ANOVA with Bonferroni post test; P<0.05, n=7-8).

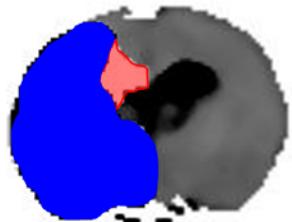
Figure 5. Temporal evolution of DWI-PWI mismatch area following permanent MCAO in SHRSP and WKY. Data presented as mean \pm SD * indicates a statistically significant difference in mismatch area between WKY and SHRSP (Two-Way ANOVA with Bonferroni post test; P<0.05). # indicates statistically significant decrease from 1 -6 hour time points in WKY (P<0.05, paired t-test, n=7-8).

Table 1. Physiological parameters before (Baseline), 1 and 6 hours after permanent MCAO in WKY and SHRSP. Data expressed as mean \pm SD MABP, mean arterial blood pressure; HR, heart rate.

WKY

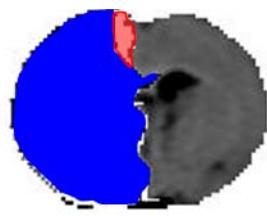


1 hour

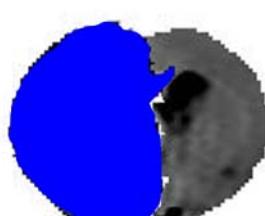


6 hour

SHRSP



1 hour



6 hour

Figure 1.

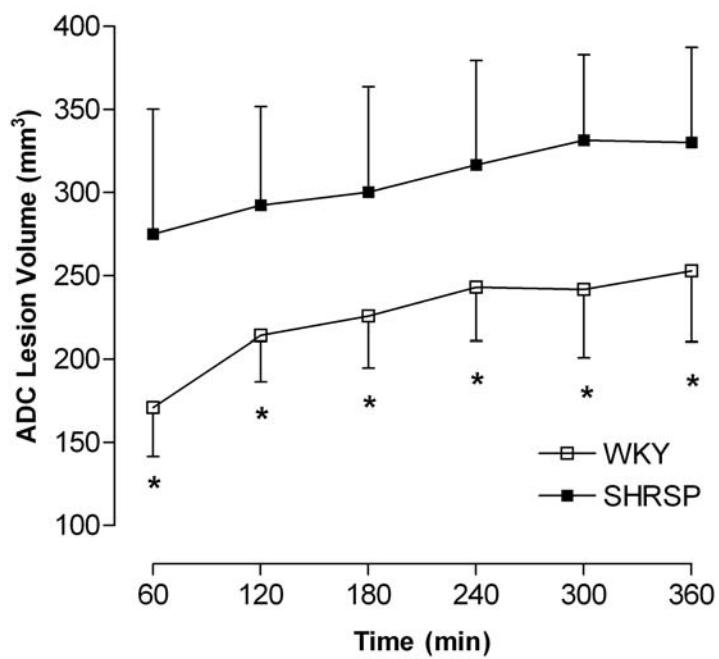


Figure 2.

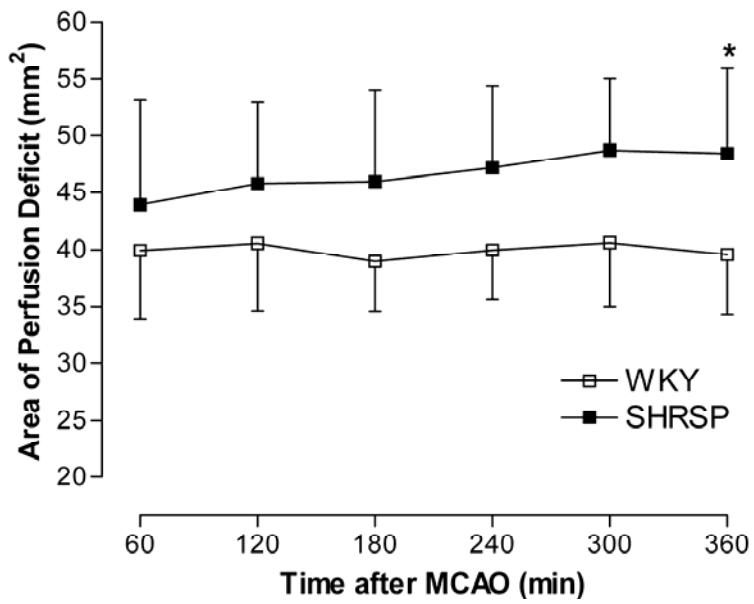


Figure 3.

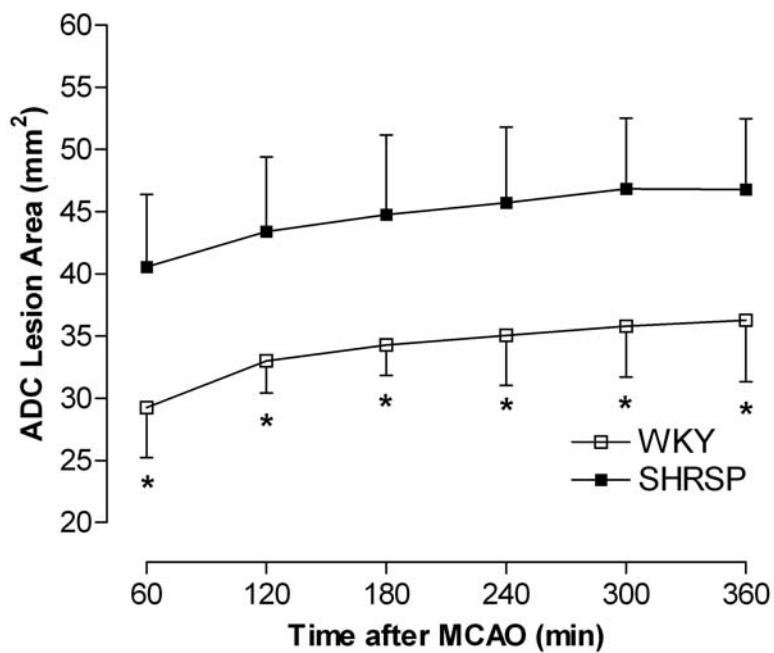


Figure 4.

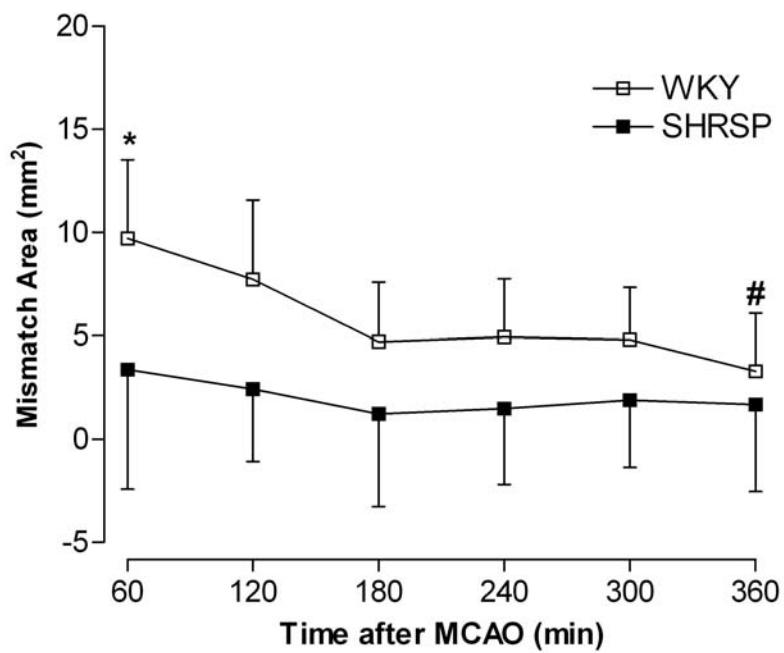


Figure 5.

Table 1: Physiological Data

WKY	Baseline	1 hour	6 hour
Body temperature (°C)	36.7±0.6	37.1±0.3	37±0.1
MABP (mm Hg)	85±5	84±7	84±7
HR (bpm)	332±21	330±24	342±21
Blood pH	7.40±0.07	7.35±0.05	7.35±0.05
PaO ₂ (mm Hg)	112±4	105±23	118±20
PaCO ₂ (mm Hg)	38±5	40±5	40±6
SHRSP	Baseline	1 hour	6 hour
Body temperature (°C)	36.7±0.3	36.8±0.4	36.5±0.1
MABP (mm Hg)	93±4	92±3	85±3
HR (bpm)	317±31	328±30	320±30
Blood pH	7.43±0.4	7.40±0.02	7.39±0.05
PaO ₂ (mm Hg)	114±13	108±17	121±24
PaCO ₂ (mm Hg)	36±7	35±10	34±9