Effects of magnesium treatment in a model of internal capsule lesion in spontaneously hypertensive rats

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ABSTRACT

Background and Purpose - The aim of this study was to assess the effects of magnesium sulphate administration following white matter damage *in vivo* in spontaneously hypertensive rats (SHR).

Methods - We developed a model involving a lesion of the left internal capsule by a local injection of endothelin-1 (ET-1) (200 pmol) in adult SHR. The effects of subcutaneous injections of magnesium sulphate (1x300 mg/kg, 30 minutes before stroke + 4x200 mg/kg every hour after) were evaluated on functional recovery 3 and 10 days after ET-1 injection.

Results – ET-1 induced lesion of the internal capsule resulted in significant motor deficits in the vehicle group on both the forelimb and the hindlimb for 10 days. These impairments were significantly ameliorated by the magnesium treatment in both the cylinder (left forelimb use, $p<0.01$ and both forelimb use, $p<0.03$ versus vehicle) and the walking-ladder (right hindlimb score, $p<0.02$ versus vehicle) tests. Infarct volumes were not significantly different between animals receiving vehicle (median 1.6mm^3; interquartile range 1.2 to 2.1) or magnesium (2.1mm^3; 1.3 to 3.8) treatments using MRI at 2 days and 0.3mm^3 (0.2 to 0.5) and 0.3mm^3 (0.2 to 0.9) respectively from histology at 11 days post-stroke.

Conclusions - We have developed a new model of white matter ischaemia in SHR in which we were able to assess specific behavioral deficits persistent for 10 days. Furthermore, magnesium treatment provides an improvement of motor abilities, suggesting that it remains an appealing protective substance in the case of white matter ischaemic damage in the rat.

Introduction

White matter constitutes a high proportion of the brain tissue involved in ischaemic stroke[1], yet axonal injury following cerebral ischemia has received little attention compared to the abundant literature on the pathophysiology of grey matter. Models of white matter damage in the rat are most commonly used in neonates[2], but are rarely developed in adult rats. Existing models of white matter damage in the adult rat comprise mainly of either *in vitro* studies using the rat optic nerve or spinal cord, or *in vivo* models, such as chronic cerebral hypoperfusion[3] and demyelinating lesions to mimic experimental allergic encephalomyelitis or multiple sclerosis[4]. However, the pathophysiology involved in these cases is not representative of acute stroke pathology.

Despite demonstrating neuroprotective properties in several animal models of ischaemia, a recent large clinical trial, the Intravenous Magnesium Efficacy in Stroke (IMAGES) trial, found no significant effect of magnesium sulphate given within 12 hours of symptom onset in stroke patients. However, pre-planned sub-group analysis revealed significant benefit in patients with lacunar stroke syndromes, and post-hoc analysis additionally suggested benefit in those with higher blood pressures (BP) at presentation[5]. Lacunar strokes account for up to 25% of all ischaemic strokes and are defined as small lesions normally located in sub-cortical regions, often involving white matter fiber tracts, and usually resulting from the occlusion of a single perforating artery[6]. The specific vascular mechanisms may differ from cardioembolic or atherothrombotic strokes[7,8].

Clinical trials of magnesium followed on from animal studies reporting that magnesium sulphate ($MgSO_4$) and magnesium chloride ($MgCl_2$) are neuroprotective in rodent models of both reversible and of permanent middle cerebral artery occlusion (MCAO), with a reduction of total infarct volume of up to 61% (review in [9]). *In vitro* studies[10,11] indicate a potential neuroprotective effect of magnesium on white matter damage, but there are no data

from *in vivo* models. In order to assess the effect of magnesium on both functional recovery and lesion size, we developed a model of white matter damage in the adult rat. The internal capsule (IC) appeared a target structure of choice, first, because lesions of the pyramidal tract in the IC are likely to produce measurable motor deficits, and second, because the IC is one of the larger white matter regions of the rat brain. A model of unilateral section of the pyramidal tract at the brainstem level has been documented[12]; this pyramidotomy lesion results in deficits in voluntary motor functions, and impairment of both forelimb and hindlimb functions[13,14].

In light of the other sub-group analysis from IMAGES demonstrating treatment benefit in those with higher than average BP, we elected to develop the model in the spontaneously hypertensive rat (SHR). If the model proved successful in the SHR, future studies could compare the effects of treatment in non-hypertensive strains. Arterial hypertension is tightly linked to stroke as it represents the most important modifiable risk factor for every sub-types of stroke, including lacunar infarction[15].

Therefore, the aims of this study were first to develop a model of white matter damage in the IC in SHR, in which we would be able to assess both a reproducible size of infarct and a consistent neurological deficit, and second, to use this model to investigate the effects of magnesium on lesion volume and functional recovery.

Materials and Methods

Animals

All experiments were carried out under license from the UK Home Office and were subject to the Animals (Scientific Procedures) Act of 1986. Adult male spontaneously hypertensive rats (SHR) (weight 335±18g, Charles River) were subjected to unilateral endothelin-1 (ET-1) injection into the left IC. Animals were randomly allocated to active (n=15) or control (n=15)

treatment by an independent technician, who prepared injection material accordingly and provided this in masked form to the researcher. Animals that died before 11 days or that failed to demonstrate infarction within the territory of the IC were excluded from analysis. Thus, the analysis was conducted on a 'per-protocol' basis. The investigators who measured infarct size with MRI and histology and scored the behavioral data were blind to treatment allocation. In a separate group of SHRs, magnesium concentration in the blood and BP were determined following repeated injections of either vehicle (n=4) or magnesium (n=4). Magnesium plasma levels were also determined in two adult Sprague-Dawley rats.
A schematic illustration of the experimental protocol is shown in Figure 1A.

Endothelin-1 injection in the Internal Capsule

Animals were anesthetized with halothane (1.5%) in a mixture of oxygen and nitrous oxide (70%/30%) delivered via a facemask. Body temperature was maintained at 37±0.5ºC with the use of a heating pad. Animals were placed in a stereotaxic frame, the skin was carefully opened, and a small portion of the temporal muscle was withdrawn. A craniectomy was carried out with a temperature-controlled drill (to limit underlying brain damage) in order to expose the cortical surface. A 30 gauge dental needle was inserted into the left IC according to the coordinates modified from the Stereotaxic Atlas (Figure 1B) (Posterior: 1.9mm to the Bregma, Lateral to the midline: 7.0mm, Ventral from the surface of the brain: 0.48mm, Angle: 25°)[16]. The 25° angle was used to avoid the needle track passing through the cerebral ventricles and motor cortex. ET-1 was then injected at a concentration of 200 pmol/µL at a rate of 0.05 µL/min. The needle was withdrawn 10 minutes after the end of injection, the hole in the skull was covered with dental cement, and the muscle and skin were sutured.

Despite precautions, one rat exhibited cortical lesion due to the surgical approach. Furthermore, another rat displayed a typically thalamic lesion. These two rats were subsequently excluded. Final group sizes were vehicle (n=15) and magnesium (n=13).

Magnesium administration and dose

The schedule of magnesium injections was determined according to previous neuroprotective studies and analysis of magnesium plasma levels after repeated injections. Blood samples were collected via a cannula inserted in the femoral artery in animals subjected to ET-1 injection and repeated sub-cutaneous injections of magnesium. The arterial cannula was externalized through an incision behind the neck. To determine the plasma levels of magnesium, blood samples were collected at various time points following ET-1 injection (15 min and thereafter every 30 min for 5 hours).

The final schedule of magnesium injections was as follows: a first sub-cutaneous injection of magnesium (300 mg/kg) 30 minutes before ET-1 injection, and then repeated sub-cutaneous injections of 200 mg/kg every hour for 4 hours.

T_2 weighted magnetic resonance imaging (MRI)

MRI scans were carried out 2 days after ET-1 injection on a Bruker Biospec 7-Tesla MRI system. Animals were initially anesthetized with 5% halothane in a 30% O_2: 70% N_2O mix. After intubation, the animals were mechanically ventilated with 1.5% halothane in a mixture of oxygen and nitrous oxide (30%/70%) at respiratory rate of 60 bpm. The heart rate and respiration rate were monitored throughout the procedure by an electrocardiogram and a respiration pad placed on the rat. A heating pad maintained the body temperature at 37ºC. A rapid acquisition relaxation enhancement (RARE) T_2 weighted sequence was used: RARE factor: 16, TR : 5086 ms, TE : 70.1 ms. The in plane resolution was 250 x 250 x 250 microns,

with a total of 15 slices. This initial scan allowed us to determine the precise location of the lesion. Once the lesion was located another T_2-weighted set of images was acquired throughout the lesion: RARE factor: 16, TR: 5086 ms, TE : 70.1 ms. The in plane resolution was 117 x 117 x 500 microns with a total of 25 contiguous slices.

To assess infarct volume, 48 hours after ET-1 injection, the infarct area was manually delineated from each of the MRI slice images using the region of interest tool in the Paravision software program. Volumetric assessment of the infarct was obtained by multiplying each individual area by the interslice distance.

Behavioural Tests

Both Cylinder[17] and Walking-Ladder tests[18] were performed before ET-1 injection for baseline values, and then 3 and 10 days post-surgery. In order to limit variability, all the behavioral tests were performed at the same time of day (between 11:00 and 15:00) by the same investigator.

Cylinder test

In order to assess the asymmetry of forelimb use, we used the cylinder test developed by Schallert[17]. Briefly, the rat is placed inside a perspex cylinder (diameter: 20 cm, height: 30 cm) for either a maximum time of 10 minutes or 20 rears. No habituation to the cylinder prior to the experiment was allowed. All the sessions were video recorded for subsequent analysis. This test was performed twice at each time point for all the rats. The total number of forelimb contacts on the wall of the cylinder was scored. The score was determined as follows: contact with the "left" forelimb, the "right" forelimb or with "both" forelimbs simultaneously. The results are presented as percentage of each type of placement compared to the total number of placements.

Walking-ladder test

This test has been developed to assess fine motor deficits precisely[18]. The test requires rats to cross an elevated horizontal ladder (length: 1 m) of irregularly spaced rungs (diameter: 1 mm). The irregular pattern limits the ability of the rats to learn the rung arrangement. All of the trials were recorded on video and visualized image by image to determine the score for each paw placement.

The score for each rat was assessed as previously described, from 0 to 6: 0: total miss, 1: deep slip, 2: slight slip, 3: replacement, 4: correction, 5: digits, 6: correct placement. An error was defined as a score of either 0, 1 or 2, and a slight error as a score of either 3,4 or 5.

The animals were handled regularly prior to starting the behavioral tests and then trained to cross the ladder before the test sessions. Each rat was tested on 5 different irregular rung patterns during each session.

Histology Analysis

Animals were euthanased and transcardially perfused with heparinised saline followed by 4% paraformaldehyde 11 days after ET-1 injection. Brains were processed and embedded in paraffin wax and 6µm sections were cut throughout the lesion area. The analysis of white matter damage was performed by Luxol-Fast-Blue staining. The damage area was manually assessed on 13 sections analyzed microscopically. The volume of ischaemic damage was calculated by summing the areas assessed for each slide and multiplying by the distance between the measured slices.

Statistical Analysis

Infarct volumes are expressed as median; interquartile range and were compared between treatment groups by two sample t-tests. Behavioral tests were analysed either by repeated-

measures 2-way analysis of variance (ANOVA) to determine the effects of strain, time and treatment or analysis of covariance (ANCOVA) adjusting for baseline values. All behavioral data are presented as mean ±SEM.

Results

Effect of Mg SO_4 on plasma levels of magnesium and on arterial blood pressure

$MgSO_4$ was injected subcutaneously at 300mg/kg 30 minutes before ET-1 injection, and then every hour for 4 hours at 200mg/kg. This dosing regime maintained mean magnesium levels in the plasma above 1.40mmol/L[19] over the course of the entire injection schedule (Table 1). Repeated injections of $MgSO_4$ had a hypotensive effect in SHR compared to control SHR receiving vehicle injection (Figure 2).

ET-1 induced ischaemic damage in the internal capsule

Ischaemic damage induced by ET-1 was measured at day 2 using T2-weighted MRI (Figure 3A). The lesion volume was not significantly different between the vehicle group (median 1.6mm^3; interquartile range 1.2 to 2.1, n=15) and magnesium treated group (2.1mm^3; 1.3 to 3.8, n=13) when measured at day 2 (Figure 3B). The location of the lesion in the IC was confirmed in the same rats at day 11 from histological staining of white matter (Figure 3C). The lesion was significantly decreased compared to day 2 in vehicle group (0.3mm^3; 0.2 to 0.5, $p<0.0001$) and in magnesium treated group (0.3mm^3; 0.2 to 0.9, $p<0.001$) (Figure 3B).

Cylinder assessment of forelimb asymmetry

Before ET-1 injection into the left IC, the distribution of forelimb use was about 50% use of both forelimbs simultaneously and 25 % use of each individual forelimb (left or right forelimb alone), in both the vehicle and the magnesium groups (Figure 4A).

ANOVA with repeated measures revealed a significant modification of forelimb use in the vehicle group after the lesion, with a decrease of “both” forelimb placements at day 3 ($p<0.02$) and day 10 ($p<0.003$) compared to baseline and an increase of left forelimb use at both time points ($p<0.02$), whereas there was no modification of forelimb use following ET-1 injection in animals treated with magnesium (Figure 4A). The magnesium treatment provides a significant improvement of the forelimb use following the lesion compared to the vehicle group on both the left forelimb ($p<0.01$), and both forelimb use simultaneously ($p<0.03$) revealed by the ANCOVA analysis.

Figure 4B shows the variation of the total numbers of forelimb placements in the cylinder. ET-1 lesion induced a decrease of the total numbers of rears in the vehicle group (about 15%, $p<0.03$), while there was no significant change in the magnesium group (about 8%) at day 3. After 10 days, both groups showed a significantly higher number of forelimb contacts in the cylinder compared to baseline ($p<0.05$).

Walking-ladder test

The ladder rung walking test allows assessment of skilled walking, by scoring each limb placement during a crossing of the ladder: at baseline, the most frequent score was 6 for correct placements in both groups (72.5±7 % in the vehicle group and 73.7±7 % in the magnesium group). The lesion of the left IC by ET-1 was significantly associated with the walking ability of the right limbs, as revealed by the calculation of the mean score for each limb during the cross of the ladder. The mean score for the right forelimb was significantly decreased in both groups at day 3 and persisted out to day 10 compared to baseline (Figure 5). At day 3, ET-1 injection induced an impairment of the right hindlimb in the vehicle group compared to baseline ($p<0.02$), while there was no impairment in the magnesium group. This difference between groups at day 3 was statistically significant ($p<0.03$). At day 10, the right

hindlimb score was not different from baseline in the two groups. The specificity of the motor deficit induced by the lesion of the left IC was confirmed by the absence of deficits on the left side, either on fore- or hind-limb placement. The left hindlimb score was increased compared to baseline at day 3 in the vehicle group ($p<0.01$) and at day 10 in the magnesium group ($p<0.003$) (Figure 5).

Discussion

Here we present the first set of experimental data examining specific white matter damage induced by ET-1 injection in spontaneously hypertensive rats, with the associated early MRI imaging, confirmed with late histological evaluation of ischaemic damage, and behavioural assessment of the resulting deficits. We have gone on to test the influence of magnesium sulphate on white matter ischaemia using these outcome measures.

ET-1 is one of the most potent vasoconstrictors and its local administration produces ischaemic injury by prolonged but reversible reduction of local blood flow[20]. ET-1 injection has consequently been used as a tool to induce localised focal cerebral ischaemia in rats[21]. A number of well described models have been developed, including stereotaxic administration adjacent to the middle cerebral artery[22] and topical administration onto the exposed middle cerebral artery[23]. In these models, the extent of the damage is related to the concentration of ET-1 injected[23]. Therefore, ET-1 appeared a promising tool to develop a model of white matter damage, by local administration into the IC, in the rat. Moreover, a similar methodological approach published in 2006, describes an ET-1 induced IC lesion in normotensive Sprague-Dawley rats, resulting in behavioural deficits on placing and sensorimotor tests[24].

To our knowledge, we are the first to present a model of IC lesion with quantifiable and reproducible lesion size. However, the T2-weighted lesion did not allow us to determine

if the damage was located precisely in the white matter as some damage may also extend into neighboring grey matter. Therefore, we also used histological staining specific for white matter (Luxol-fast-blue) to determine the precise location of the ET-1 induced lesion. Lesion volumes were smaller when measured by histology compared to MRI. This is most likely due to a combination of the resorption of acute brain oedema and brain shrinkage during histological processing.

Our lesion-model induced significant motor deficits of both forelimbs and hindlimbs as measured by specific motor tests. The cylinder test shows forelimb use impairments during the 10 days following the lesion. The walking-ladder test provides evidence of hindlimb placement impairments over this period. We demonstrated that magnesium has a beneficial effect on functional outcome measures on both the forelimbs and the right hindlimb compared to the vehicle group. The significant effect of magnesium is more pronounced in the cylinder test, suggesting that the cylinder data are less dependent than the walking-ladder data on minor variability in the placement of lesions, especially in view of the small lesion volume and density of axonal tracts in this region.

In the present study we demonstrated that magnesium has a beneficial effect without inducing a significant reduction in lesion volume, measured on T2-weighted MRI or white matter histology. The mechanisms involved in white matter damage and functional recovery are still inadequately understood, and therefore we can only hypothesize regarding the possible mechanisms for the observed magnesium-associated preservation of motor functions. Magnesium-induced neuroprotection could include both vascular and neuroglial mechanisms. For example, in models of spinal cord injury, magnesium-induced vasodilatation has been proposed to contribute to improved blood flow and a reduction in deleterious vasospasm[25]. Several mechanisms for the vascular effects of magnesium have been proposed, such as the blockade of voltage-dependent calcium channels, the release of nitric oxide[26] and the ability

to inhibit ET-1 vasoconstriction[27]. However, if the severity or duration of ischaemia were reduced by magnesium, you would expect a reduction in infarct volume and this was not apparent in our data. We can also suggest several hypotheses on actions of magnesium on oligodendrocytes which are the main cell type constituting white matter. The first is the voltage-dependent blockade of ion flux through the glutamatergic N-methyl D-aspartate (NMDA) receptor. It has been shown recently that oligodendrocytes expressed several sub-units of the NMDA receptor[28,29]. Therefore, by blocking the activation of one of the major receptors involved in excitotoxic damage, magnesium could contribute to salvaging white matter integrity. Secondly, magnesium inhibits the sodium-calcium ion exchanger that is thought to be a major mediator of calcium influx in ischaemic axons[11]. Thirdly, and more speculatively, magnesium ions are able to potently inhibit the transient receptor potential melastatin (TRPM) TRPM7 channels[30] that have recently been linked to delayed calcium influx in neurons, and therefore excitotoxic damage, independent of the early action of glutamate on NMDA receptors[31]. However, despite being appealing, this hypothesis requires further investigation to confirm the presence of TRPM receptors in white matter and the inhibitory effect of magnesium in *in vivo* models of ischemia.

A beneficial effect of magnesium *via* an action on grey matter could also be involved in our model, as it has been shown that axotomy at the level of the IC induced death of nearly 50% of corticospinal neurons within the first week after axotomy[32]. Therefore, the beneficial effect of magnesium could also be linked to a direct effect on the neuronal cellular body in the cortex with an improvement of their survival.

In conclusion, we have developed a new model of white matter focal ischemia in SHR at the level of the IC by local injection of ET-1. We were able to assess reproducible lesion volumes by both early MRI scan and delayed histological staining. Furthermore, we have been able to identify specific motor deficits on the contralateral side both on the forelimb and

the hindlimb. Finally, we provide new evidence of a potential beneficial effect of magnesium administration on functional recovery in a rodent model with cardiovascular risk factors.

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TABLE 1. Plasma levels of Mg^{2+}(mmol/L) following repeated $MgSO_4$ injections.

Normal range is 0.66-0.95 mmol/L[33]. Statistical differences are indicated for each strain compared to baseline: ***:p<0.001,**:p<0.01,*:p<0.05 (Repeated measurements ANOVA, PLSD Fisher). There is no significant difference in plasma magnesium levels between Sprague-Dawley (SD) and SHR rats.

	1st injection (300mg/kg)	**2nd injection** (200mg/kg)	**3th injection** (200mg/kg)	**4th injection** (200mg/kg)	**5th injection** (200mg/kg)			
	Baseline	**1 hour**	**2 hours**	**3 hours**	**4 hours**	**5 hours**	**6 hours**	**7 hours**
SHR *(n=4)*	0.54 ±0.06	1.75 ±0.02***	1.68 ±0.06***	1.99 ±0.02***	1.85 ±0.30***	2.03 ±0.25***	1.21 ±0.12**	0.92 ±0.08*
SD *(n=2)*	0.62 ±0.02	1.72 ±0.00***	1.71 ±0.13***	1.75 ±0.07***	1.77 ±0.01***	1.67 ±0.17***	1.29 ±0.05**	0.93 ±0.01*

Legends

Figure 1. Experimental protocol.

(A) Rats were handled and habituated before ET-1 induced IC lesion. Testing on the cylinder and the walking-ladder tests was performed before surgery (Baseline) and on 2 days following lesion (Days 3 and 10). Early quantification of ischaemic damage was performed by MRI T2-weighted images on day 2; subsequently, ischaemic damage was assessed from histological staining on day 11. (B) The stereotaxic injection of ET-1 into the IC in SHR rats was performed with an angle of 25° to avoid tracking of ET-1 into the ventricle and damage to the motor cortex (Illustration from [16]).

Figure 2. Effect of $MgSO_4$ on mean arterial blood pressure.

$MgSO_4$ is injected subcutaneously at 300mg/kg (Arrow 1) and then at 200mg/kg (Arrows 2 to 5). Repeated measurements ANOVA, PLSD Fisher, *:$p<0.05$ compared to baseline, and *t*-tests, †:$p<0.05$, ‡:$p<0.01$, #:$p<0.001$, compared to the vehicle group at the same time-point.

Figure 3. ET-1 induced ischaemic damage and effect of $MgSO_4$.

(A) Arrows indicate lesion areas on MRI T2-weighted images from a representative brain from each group (section thickness=0.5mm). (B) There was no significant difference in lesion volume between vehicle and magnesium groups. Within each group, lesions measured by histology at day 11 were significantly smaller than lesions measured by MRI at day 2, paired *t*-test. **:$p<0.01$, ***:$p<0.001$. (C) A representative histological section following luxol-fast-blue staining confirms the location of the lesion in the IC (section thickness=0.6µm), scale bar = 1mm.

Figure 4. Forelimb asymmetry following ET-1 induced damage and effect of $MgSO_4$.

(A) Each type of forelimb contact on the wall of the cylinder is expressed as a percentage of the total number of placements. Data were analyzed by repeated-measurements ANOVA, PLSD Fisher. *:$p<0.05$, **:$p<0.01$ compared to baseline. (B) Data for each forelimb is expressed as a percentage of the total number of placements and were analyzed by repeated-measurements ANOVA, PLSD Fisher. *:$p<0.05$ compared to baseline in vehicle group. †:$p<0.05$; ‡:$p<0.001$ compared to day 3.

Figure 5. Mean score for each limb placement on the walking-ladder test.

Scoring scale ranges from 0 to 6, with 6 points for a correct placement. The mean score represents the average of 5 trials for each time point. Data were analyzed by either ANCOVA: *:$p<0.05$ comparison between vehicle and magnesium groups or ANOVA, PLSD Fisher †:$p<0.05$; ‡:$p<0.01$, #:$p<0.001$ compared to baseline.

FIGURE 1

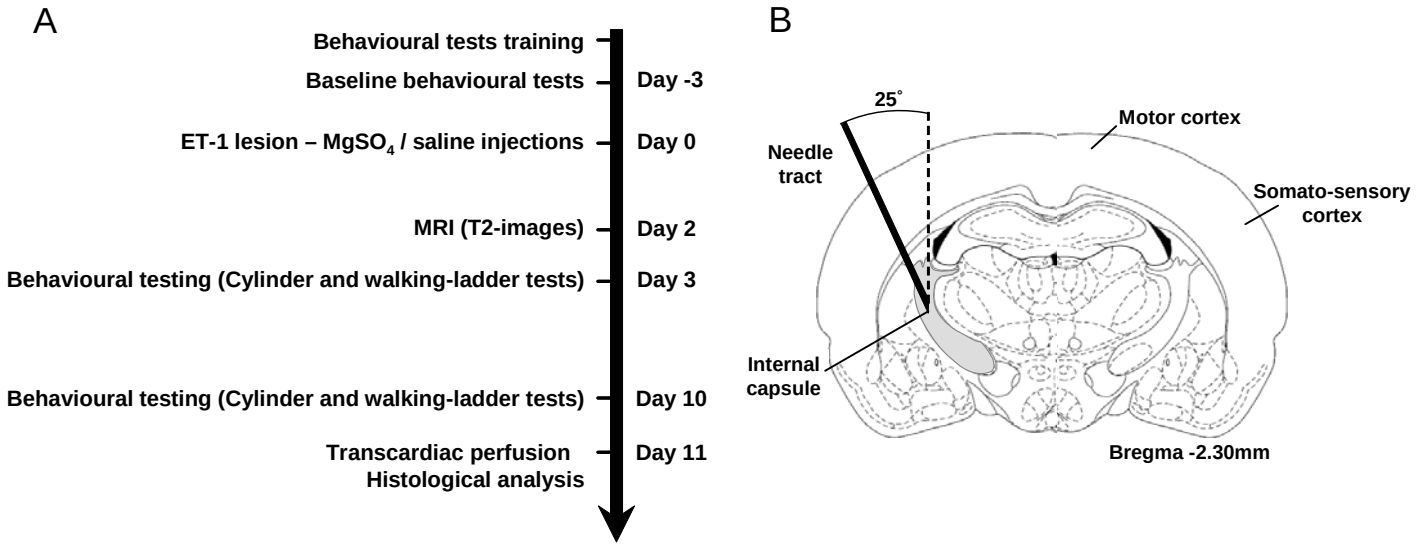

A
Behavioural tests training
Baseline behavioural tests
Day -3
ET-1 lesion – MgSO4 / saline injections
Day 0
MRI (T2-images)
Day 2
Behavioural testing (Cylinder and walking-ladder tests)
Day 3
Behavioural testing (Cylinder and walking-ladder tests)
Day 10
Transcardiac perfusion
Histological analysis
Day 11
B
25°
Needle tract
Motor cortex
Somato-sensory cortex
Internal capsule
Bregma -2.30mm

FIGURE 2

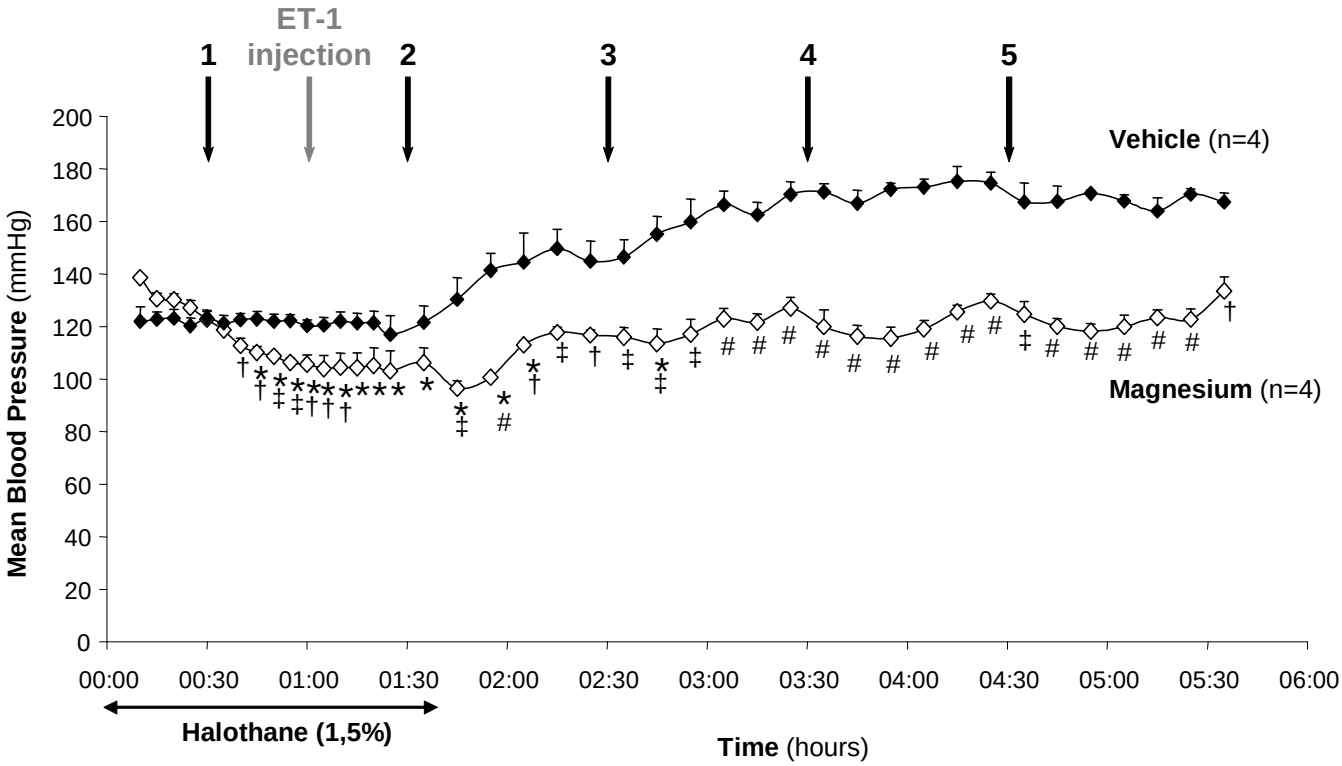

ET-1
injection
1
2
3
4
5
Vehicle (n=4)
Magnesium (n=4)
Mean Blood Pressure (mmHg)
200
180
160
140
120
100
80
60
40
20
0
00:00
00:30
01:00
01:30
02:00
02:30
03:00
03:30
04:00
04:30
05:00
05:30
06:00
Halothane (1,5%)
Time (hours)

FIGURE 3

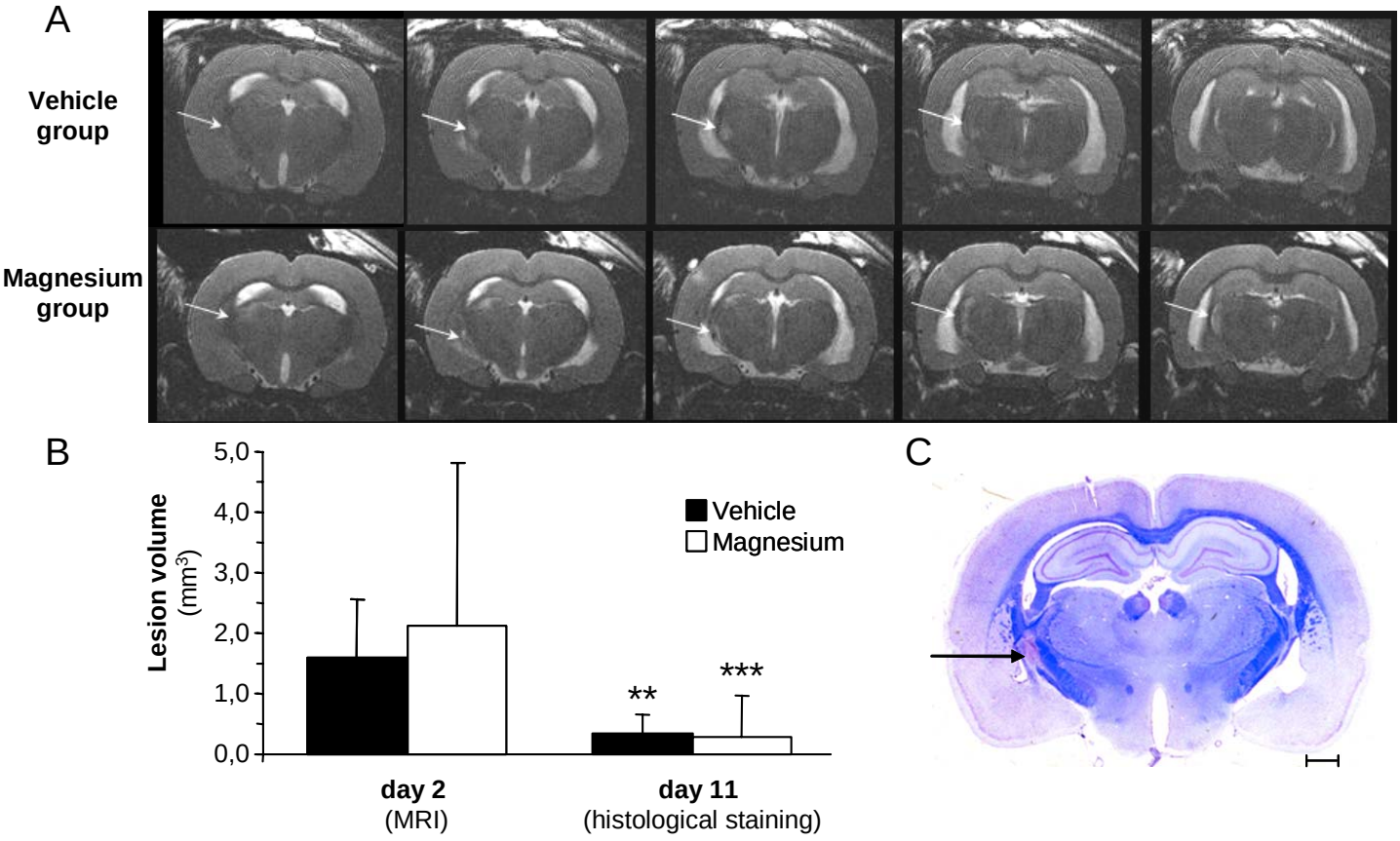

A
Vehicle group
Magnesium group
B
Lesion volume (mm3)
5,0
4,0
3,0
2,0
1,0
0,0
Vehicle
Magnesium
**
***
day 2
(MRI)
day 11
(histological staining)
C

FIGURE 4

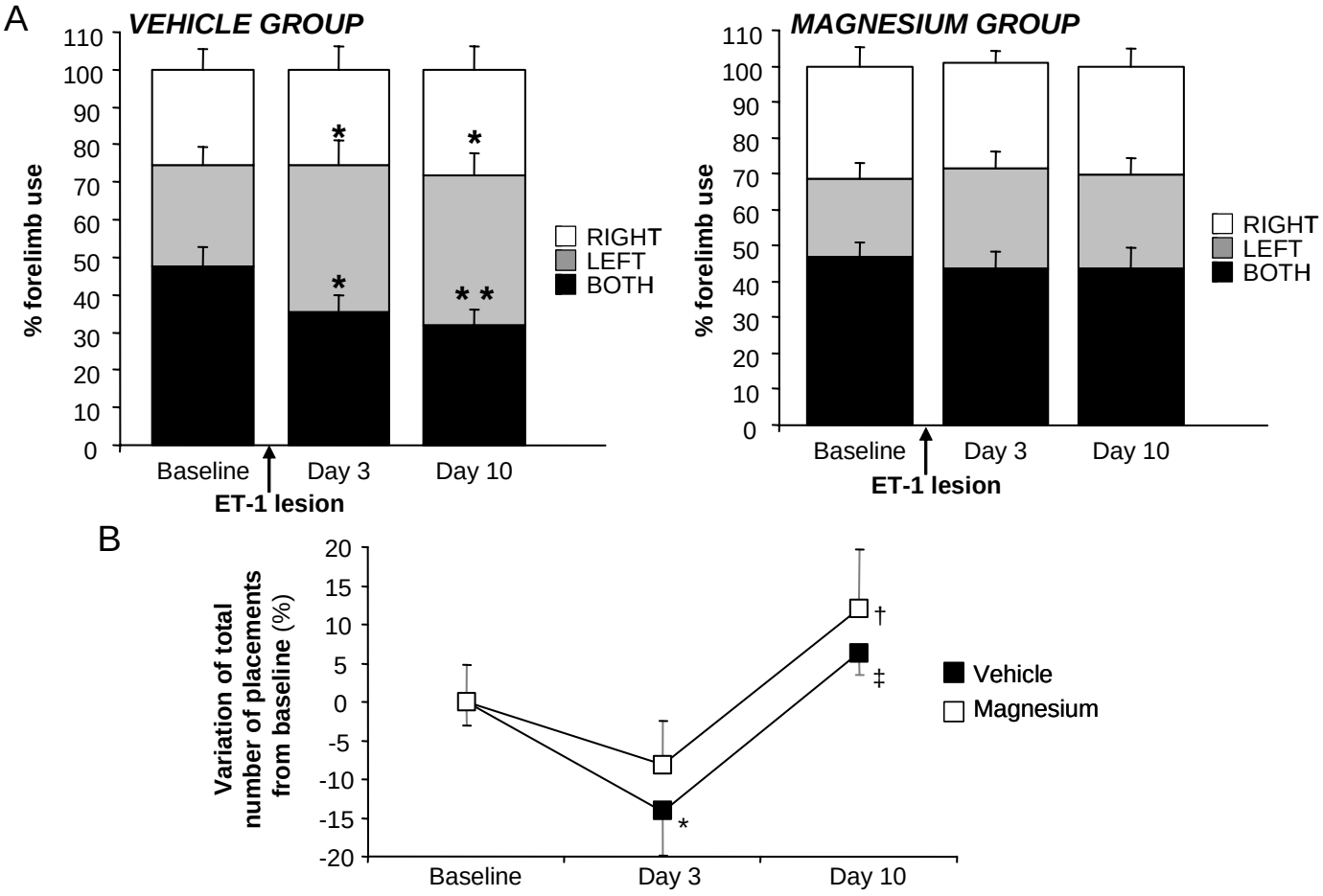
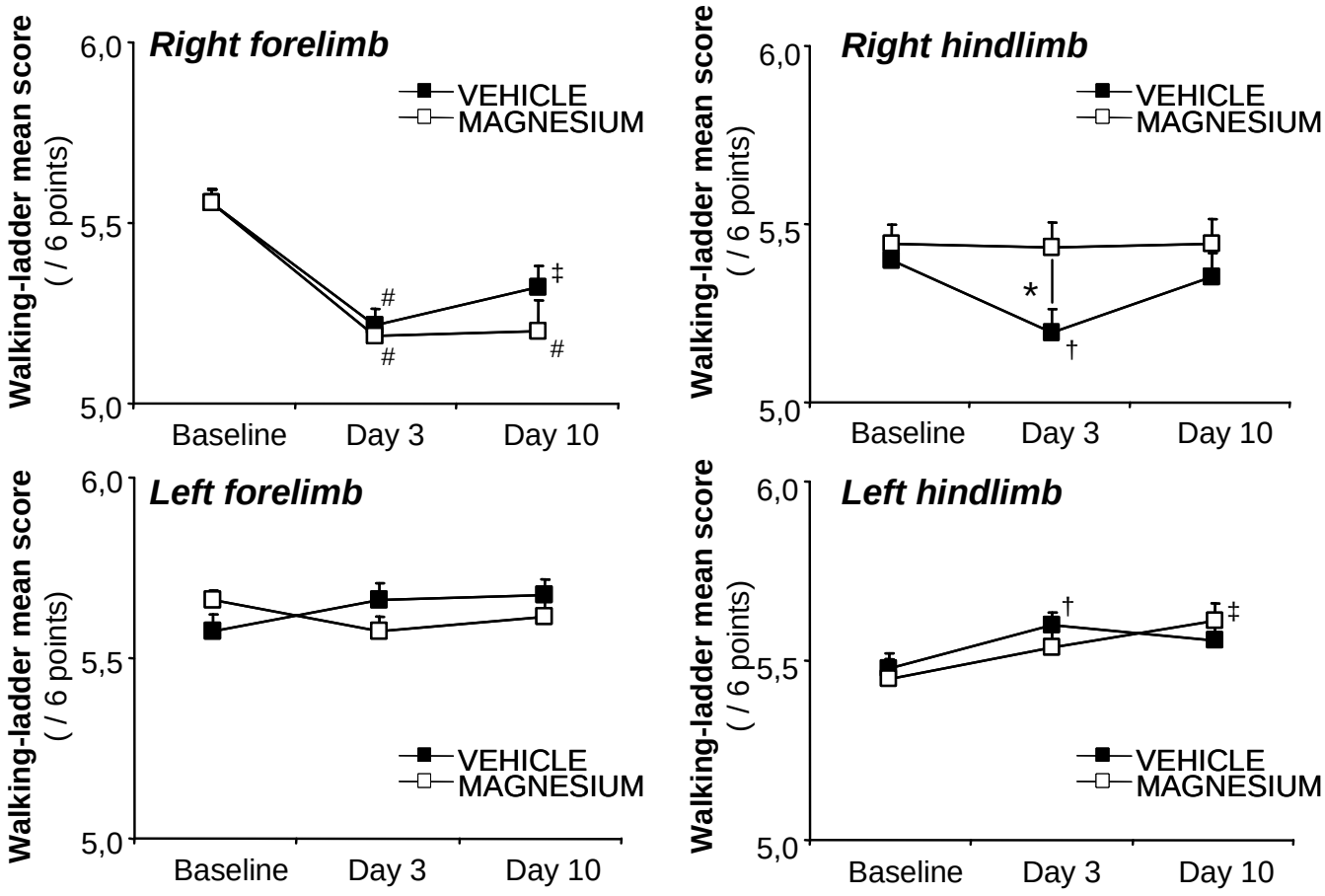

A
VEHICLE GROUP
MAGNESIUM GROUP
% forelimb use
RIGHT
LEFT
BOTH
Baseline
Day 3
Day 10
ET-1 lesion
B
Variation of total number of placements from baseline (%)
Vehicle
Magnesium

FIGURE 5


Right forelimb
Right hindlimb
Left forelimb
Left hindlimb
Walking-ladder mean score
(/ 6 points)
6,0
5,5
5,0
VEHICLE
MAGNESIUM
Baseline
Day 3
Day 10
#
‡
*
†