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ORIGINAL RESEARCH

Mice and Rats Exhibit Striking Inter-species Differences in Gene Response to Acute Stroke

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Abstract
Neuroprotection in acute stroke has not been successfully translated from animals to humans. Animal research on promising agents continues largely in rats and mice which are commonly available to researchers. However, controversies continue on the most suitable species to model the human situation. Generally, putative agents seem less effective in mice as compared with rats. We hypothesized that this may be due to inter-species differences in stroke response and that this might be manifest at a genetic level. Here we used whole-genome microarrays to examine the differential gene regulation in the ischemic penumbra of mice and rats at 2 and 6 h after permanent middle cerebral artery occlusion (pMCAO; Raw microarray CEL data files are available in the GEO database with an accession number GSE163654). Differentially expressed genes (adj. $p \leq 0.05$) were organized by hierarchical clustering, correlation plots, Venn diagrams and pathway analyses in each species and at each time-point. Emphasis was placed on genes already known to be associated with stroke, including validation by RT-PCR. Gene expression patterns in the ischemic penumbra differed strikingly between the species at both 2 h and 6 h. Nearly 90% of significantly regulated genes and most pathways modulated by ischemia differed between mice and rats. These differences were evident globally, among stroke-associated genes, immediate early genes, genes implicated in stress response, inflammation, neuroprotection, ion channels, and signal transduction. The findings of this study may have significant implications for the choice of species for screening putative stroke therapies.

Keywords Animal stroke models · Gene expression and regulation · Microarray analysis · Pathophysiology

Introduction

Pharmacological neuroprotection in stroke has to date been difficult to achieve in humans. Putative neuroprotectants are screened in animal models of stroke, and the results inform go-no-go decisions for advancement to clinical studies. However, the failures of translation from animals to humans raise concerns as to whether the animal models are representative enough to predict the human situation. For this reason, the STAIR committee recommended that studies to screen neuroprotectants be conducted in multiple species (Fisher et al. 2009; Stroke Therapy Academic Industry 1999) so that a beneficial effect of an agent in one species be confirmed in others prior to its advancement to the clinic. However, when a beneficial effect is not found in initial studies performed in one species, there are no recommendations for how to proceed. In the scenario of initial negative studies, should development toward human trials cease, or should an agent be tested in another species? Little is known about how inter-species differences in biology, physiology or anatomy impact the ability of stroke studies conducted in a given species to predict success in humans.

This issue has become of interest because of our development of the peptide neuroprotectant Tat-NR2B9c, also termed NA-1 or nerinetide. The preclinical effects of Tat-NR2B9c as a stroke neuroprotectant have been validated by us and others in several stroke models in rats (Aarts et al. 2002; Bratane et al. 2011; Zhou et al. 2015) and in comprehensive studies in non-human primates (Cook et al. 2012a, b). These led to the "ENACT" trial (Hill et al. 2012), an international, multi-center, randomized placebo-controlled

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trial that provided encouraging human data substantially similar to those seen in primates. Additionally, nerinetide has now completed a global phase 3 study (Hill et al. 2020), suggesting that it may improve functional outcome, reduce mortality, and lessen infarction volumes in patients undergoing endovascular thrombectomy without prior thrombolysis. However, despite its advanced stage of development, we and others have found nerinetide to be less effective in mice as compared with rat models of stroke (Bach et al. 2012; Kleinschnitz et al. 2016; Teves et al. 2016). Our observations from the development of nerinetide raise the caution that if mouse experiments are used to gate further development, then potentially beneficial agents might be unnecessarily eliminated.

Originally, mice became popular in stroke research owing to their capacity for facile genetic manipulation. Yet increasingly they are used in studies that do not capitalize on genetic modifications to screen stroke therapies. This shift to using mice may come at a cost because mice may exhibit a greater sensitivity to infarction over just minutes of middle cerebral artery occlusion (MCAO), as well as greater variability of response (Carmichael 2005). The cost of increased variability, leading to reduced power to detect a treatment effect, is only warranted if mice are the most appropriate species commonly available to stroke researchers to predict the human response. This key issue is distinct from questions related to methodological quality that have also affected stroke research (Tymianski 2015). For example, in a mouse study conducted with the rigor of a randomized controlled trial by Llovera and colleagues (Llovera et al. 2015), the evaluation of neuroprotection by anti-CD49d antibodies suggested a benefit in a permanent MCAO producing smaller lesions, but not in transient MCAO producing a larger lesion. Much effort was expended on rigorous studies that produced inconclusive recommendations about whether the agent tested merited further development. However, the question of whether negative data in mice are sufficient to halt the development of a promising agent, or whether screening of promising agents should continue in other species, was unanswered.

Given that rats and mice are distinct species, it would not be surprising if they did not react to stroke identically. Differences may be genetic, anatomical, or physiological and, alone or in combination, could lead to different post-stroke responses. Here we focused on the acute gene responses observed in rats and mice when they are subjected to the experimental stroke models commonly employed in these species when evaluating a putative stroke drug. Specifically, we compared differential gene expression profiles in rats and mice subjected to permanent MCAO (pMCAO). This is a model in widespread use for making go-no-go decisions about treatment development. pMCAO produces similar infarctions and profound neurological deficits in both species. However, we found that the gene responses underlying these similar phenotypic responses were strikingly different.

Materials and Methods

Experimental Animals

All procedures performed involving animals were approved by the University Health Network animal care committee, conformed to Canadian Council of Animal Care guidelines and ARRIVE guidelines (Kilkenny et al. 2010). Adult male Sprague–Dawley rats (270–300 g; age 8–12 weeks; Charles River, Canada) and adult male C57 Black 6 mice (21.9–29.7 g; age 8–10 weeks; Charles River, Canada) were kept at 21 °C, 65% humidity, and a regulated 12-h light/dark cycle with free access to food and water. Male-only animals were used to reduce variability.

Study Design

Animals were randomly assigned to permanent MCAO (pMCAO) or sham treatment ($n = 3$/group). In all animals 3×3 mm tissue samples were obtained from the penumbras of rat and mouse brains at 2 h (2 h) or 6 h (6 h) after pMCAO (Fig. 1a). Control samples were obtained from similar ipsilateral regions of sham-operated brains of each species. The times selected for sampling were intended to capture ischemic changes early (2 h) in the infarction process as well as in near-complete (6 h) infarction in the pMCAO model (Cook et al. 2012b; Lu et al. 2012). For microarray analysis, 12 rats and 12 mice were used [1 animal/microarray, $n = 3$/group, 4 groups per species (sham 2 h; MCAO 2 h; sham 6 h; MCAO 6 h)]. The sample size was calculated at a power of 0.95 using an online calculator for microarray studies (https://sph.umd.edu/department/epib/sample-size-and-power-calculations-microarray-studies. In a completely randomized treatment–control design, the parameters for power calculation were estimated as follows: false discovery rate = 0.05, anticipated mean Log fold difference between treated and control samples = 1; standard deviation = 0.5; sample size $n = 3$). The personnel (TCAG) performing the microarray analyses were blinded to group assignments.

Defining the Penumbra for Tissue Collection

A preliminary study was performed using a separate group of animals to define the penumbra for tissue collection. A total of 9 animals per species underwent pMCAO for

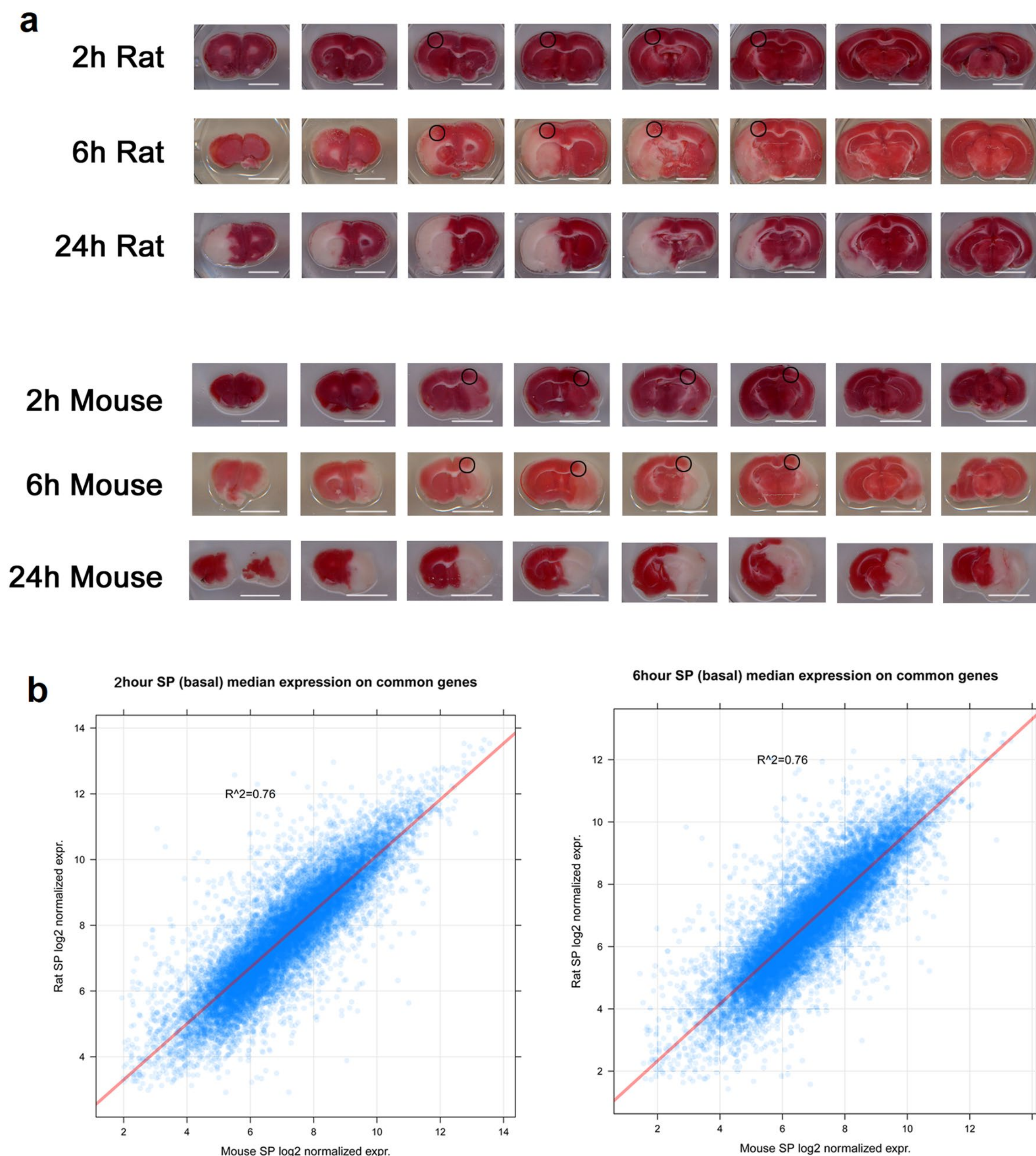

Fig. 1 Penumbra of pMCAO animals and similar ipsilateral areas of sham animals were collected for microarray analysis. **a** A pilot study was conducted to establish the penumbra and area for tissue collection. The penumbra tissue in pMCAO animals at 2 h or 6 h was defined as non-infarcted (red) tissue that progressed to infarction (white) at 24 h by TTC staining. The black circles indicate the 4 samples (3mmx3mm) that were isolated from each MCAO animal in the actual experiment at 2 h or 6 h after the onset of stroke. Similar areas in the ipsilateral cortex were also collected from sham animals for comparison at 2 h or 6 h (images not shown). Scale bar indicates 0.5 cm. **b** Scatter plot of normalized gene expression at 2 h or 6 h after sham ischemia in mice vs. rats ($R^2=0.76$)

2 h ($n=3$), 6 h ($n=3$) or 24 h ($n=3$), sacrificed, and the brains were isolated, frozen, sliced into 8×1 mm coronal sections and stained for 20 min at 37 °C in 0.9% saline with 2% TTC (2,3,5-triphenyltetrazolium chloride) (Teves et al. 2016). Penumbral tissue was defined as that which was not yet infarcted at the time of sampling at 2 h or 6 h but that goes on to infarction by 24 h as visualized by TTC staining (Fig. 1a; Supplementary Fig. S1).

Permanent MCAO

Rats

The left MCA (Middle Cerebral Artery) was occluded by the intraluminal suture method of Longa et al. (1989). In brief, rats were anesthetized with isoflurane (5% for induction and 2% for surgery) mixed with oxygen. Rectal body temperature was maintained at 36.8 ± 0.18 °C with a heating pad. MCAO was achieved by introducing a 0.39 ± 0.02 mm width silicone-coated filament (403934PK10, Doccol Corporation, MA, USA) into the external carotid artery (ECA) and advancing through the Internal Carotid Artery (ICA) to occlude the left MCA origin. The filament was then ligated in situ. Laser Doppler flowmetry (Perimed, Stockholm, Sweden) was used to monitor occlusion success as previously described (Harada et al. 2005; Henninger et al. 2009). After recording a stable baseline for 5 min, stroke was induced, and regional cerebral blood flow (rCBF) was continuously monitored for 15 min after MCAO. If the rCBF change between baseline and occlusion was less than 60%, the animal was excluded from use. Sham animals underwent identical procedures but without the occlusion of MCA. Animals were awakened after surgery and recovered in their cages.

Mice

The right MCA was occluded using the intraluminal suture method of Koizumi et al. (1986) and adapted by Clark et al. (1997) to the mouse (Teves et al. 2016). The mice were anesthetized with isoflurane (3% for induction and 2% for surgery) mixed with oxygen. Rectal body temperature was maintained at 36.9 ± 0.17 °C with a heating pad. MCAO was achieved by inserting a standard 0.21 ± 0.02 mm width filament (602123PK10, Doccol Corporation, MA, USA) in the ECA and advancing through the ICA to occlude the right MCA origin. The filament was left ligated in situ. rCBF was monitored throughout the surgery using the laser Doppler flowmetry (Perimed, Stockholm, Sweden). Successful occlusion was identified by > 80% drop in blood flow comparing to baseline. Sham animals underwent identical procedures but without the occlusion of MCA. Animals were recovered in a chamber held at 26 °C for 2 h after surgery and returned to their cages.

For both mice and rats, at 2 h after the MCAO surgery, successful MCAO was further confirmed through the Bederson neuroscore (Bederson et al. 1986) (≥ 11; including failure to extend left forepaw, circling to the contralateral side, lack of coordination, or no spontaneous motor activity).

RNA Isolation and Microarray

Total RNA was isolated from tissue samples using a commercial kit following the manufacturer's instructions (RNeasy Mini Kit, QIAGEN, Hilden, Germany). Integrity and quantity of isolated RNA were checked with Agilent BioAnalyzer. The microarray analysis was performed at the Center for Applied Genomics (TCAG, Research Institute of Hospital for Sick Children, Ontario, Canada) using Affymetrix GeneChip probe arrays (Mouse Gene 2.0 ST and Rat Gene 2.0 ST, ThermoFisher Scientific, MA, USA). Controls were implemented at each step to ensure data quality. Raw microarray CEL data files are available in the GEO database with an accession number (GSE163654).

Statistical Analysis

Raw microarray data were analyzed at Network Biology Collaborative Centre (NBCC, Lunenfeld-Tanenbaum Research Institute, Ontario, Canada). In brief, raw data were normalized using the Oligo R package. Gene expression levels were compared between MCAO and sham animals at 2 h or 6 h using the Limma R package and presented in the Log2 fold change scale. For each comparison, genes with a Benjamini–Hochberg adjusted p value (adj. p) equal to or less than 0.05 were considered to be differentially expressed. Hierarchical clustering, correlation plots and Venn diagrams were generated based on differentially expressed genes using the lattice R package and Venn Diagram R package. Functional and pathway analyses of the differentially expressed genes were performed using the online STRING tool for functional protein association predictions (STRING version 10.0 available at: http://string-db.org/).

Quantitative Real-Time PCR (RT-PCR)

One microgram of total RNA was reverse transcribed to cDNA by MuLV reverse transcriptase with random hexamer (Applied Biosystems, CA, USA). RT-PCR was performed using the ABI SDS 7900 sequence detection system (Applied Biosystems). Gene targets were amplified with TaqMan MGB probes and specific primer sets (Assay on demand, Applied Biosystems):

Hprt1 (hypoxanthine phosphoribosyltransferase 1): Rn01527840_m1 (Rat); Mm00446968_m1 (Mouse)

Plat (tissue plasminogen activator): Rn01482578_m1 (Rat); Mm00476931_m1 (Mouse)
Dusp1 (dual specificity phosphatase 1): Rn00678341_g1 (Rat); Mm00457274_g1 (Mouse)
Mmp12 (matrix metalloproteinase 12): Rn00588640_m1 (Rat); Mm00500554_m1 (Mouse)
Timp1 (tissue inhibitor metalloproteinase I): Rn00587558_m1 (Rat); Mm01341361_m1 (Mouse)

To quantify gene targets, the delta Ct method was used according to the manufacturer's protocol. HPRT1 was used as an endogenous control for normalization. The PCR reaction for each gene was performed in triplicates.

Results

Cerebral Ischemia Induces Distinct Patterns of Transcriptional Regulation in Rats and Mice

First, we determined whether gene expression patterns in the mouse and rat sham control groups were similar at basal conditions. The normalized expression levels in rats at 2 h or 6 h were plotted against those in sham mice. These were highly correlated ($R^2=0.76$), suggesting that the two animal models had comparable gene expression patterns at basal conditions (Fig. 1b).

Next, differential gene expression patterns from the penumbra regions were compared between ischemic and sham-operated animals. Expression patterns in the ischemic penumbra varied dramatically between the two animal species. Specifically, hierarchical clustering of differentially expressed genes (adj. $p \le 0.05$) comparing pMCAO to sham at either 2 h or 6 h showed that ischemia stimulated virtually completely different gene clusters in rats as compared with mice, with very little overlap between species at either time-point (Fig. 2a). The numbers of differentially expressed genes (adj. $p \le 0.05$) are summarized in Venn diagrams based on the type of regulation (up- or downregulation), species (mouse or rat) and time after pMCAO (Fig. 2b, c). At 2 h after pMCAO, 29 of 30 (97%) of upregulated genes in mice were different from rats; similarly, 14 downregulated genes (88%) in mice and 8 genes in rats (80%) were different from each other. At 6 h after pMCAO, 119 upregulated rat genes (74%) were not significant in mice and 203 mouse genes (83%) were different from those in rats. Rats had 93 downregulated genes (93%) that were not significant in mice, while mice had 159 genes (96%) that were different from the rat gene expression profile. These striking differences in gene expression between rats and mice indicated that cerebral ischemia in these two species triggers extremely distinct gene responses to ischemic insults.

Regulation of Previously Reported Ischemia-Related Genes in Mice and Rats After pMCAO

To further probe these gene expression profiles in mice and rats, we focused on the following categories of genes associated with cerebral ischemia (Cox-Limpens et al. 2014): immediate early genes, stress response, inflammation, neuroprotection, apoptosis, ion channels and signal transduction (Table 1). Full names of the ischemia-related genes are described in Table 1.

Immediate Early Genes

Several immediate early genes (IEG) were upregulated after pMCAO and rats generally exhibited a greater but slower IEG augmentation than mice. Fos, Fosb and Jun were upregulated in mice after both 2 h and 6 h pMCAO. By contrast, IEG upregulation was much slower in rats, with none upregulated at 2 h. By 6 h, Fos, Fosb, Jun, Junb, Egr1, Egr2, Egr3, and Egr4 were all upregulated in rats. Ultimately, the expression of Fos was much higher in rats (Log2 FC = 13) than in mice (Log2 FC = 2.6) at 6 h (Table 1).

Stress Response

Heat shock protein (Hsp) genes were upregulated after pMCAO in both species with an exceptionally high expression of Hsp70 in mice, and a slower and more attenuated response in rats. Expression of Hspa1a and Hspa1b showed a 20-fold increase in mice by 2 h. Hsps were only significantly upregulated in rats at 6 h with a much lower change of 2.54-fold (Table 1).

Inflammation

Inflammatory chemokines and interleukins were upregulated differentially, again with a generally slower response in rats. Ccl3, Ccrl2, and Il1A were upregulated at 2 h, in mice but not rats. Tnfrsf11b was increased in rats at 2 h. Ccl2 was upregulated in mice at 6 h, while Ccl4 and Il4r were only upregulated in rats at 6 h (Table 1).

Neuroprotection

Several previously reported neuroprotective genes were significantly regulated in rats and mice, with generally greater expression in rats. The Angiogenic inducer Cyr61 and heme oxygenase Hmox1 were upregulated in both species.

a
6
4
2
0
-2
-4
-6
2h Mouse
2h Rat
6h Mouse
6h Rat
b
Up-regulated
2h
Rat
Mouse
1
29
6h
Rat
Mouse
119
42
203
c
Down-regulated
2h
Rat
Mouse
8
2
14
6h
Rat
Mouse
93
7
159

◂**Fig. 2** Rats and mice exhibited distinct gene expression patterns induced by permanent focal cerebral ischemia. **a** The heatmap showed the significantly regulated genes (adj. $p \leq 0.05$) in at least one comparison of pMCAO vs. sham animals at 2 h or 6 h. Each comparison was labeled at the bottom of each column: 2 h Mouse MCAO; 2 h Rat MCAO; 6 h Mouse MCAO; 6 h Rat MCAO. The vertical Y axis shows individual genes. Yellow–Red = upregulation; White = no change; Blue = Downregulation. **b** and **c** Venn diagrams of upregulated and downregulated genes showing differences between rats and mice at 2 h or 6 h of ischemia compared with sham controls

However, there were several notable differences in the regulation of other neuroprotective genes. At 6 h, the expression of angiogenic factor Angpt2 and neurotrophic BDNF were stimulated in mice, whereas the angiogenesis stimulator Apold1 and cAMP response element modulator Crem were upregulated in rats. In addition, the insulin-induced gene Insig1 (Taghibiglou et al. 2009) and the metallopeptidase inhibitor Timp1 (Tejima et al. 2009) associated with attenuation of ischemic cell death were only elevated in rats. Neuroprotective Npas4 (Choy et al. 2016) gene was highly regulated in mice (Log2 FC = 8.51) at first but declined with time (Log2 FC = 3.99). By contrast, a very high expression of Npas4 remained at 6 h in rats (Log2 FC = 15.12). It was also noticed that the Plat gene coding for endogenous tissue plasminogen activator was upregulated with a higher expression level in rats (Log2 FC = 3.46) than mice (Log2 FC = 1.39).

Apoptosis

The regulation of apoptotic genes also differed in rats and mice. At 6 h after pMCAO, apoptotic genes bag3, Fas, Mapkapk2, and Mmp12 were all upregulated in mice. On the other hand, Bag4 was downregulated, and Map3k14 was upregulated in rats.

Ion Channels

Downregulation of ion channels and receptors was only observed in mice. They included Cacnb2(calcium channel, voltage-dependent), Grik2(Kainate glutamate receptor), serotonin receptors Htr1f and Htr2a, potassium voltage-gated channel kcna2, kcnc2 and kcnq3, and potassium inwardly-rectifying channel kcnj3.

Signal Transduction

Neither species showed significant changes in the interrogated signal transduction genes at 2 h. By 6 h, rats and mice showed almost completely different patterns of gene regulation, with only Rgs2 being in common. Among G-protein signaling genes, mice showed changes in Gem, Gpr12, Gpr52, Gpr84, P2ry12, Rgs1 and Rgs2, whereas rats showed changes in Gpr34 and P2ry13 and Rgs2 (Table 1).

Comparison of Ischemia-Induced Top Gene Expression and Functional Pathways in Rats and Mice

We next evaluated the top regulated genes and functional pathways in the two animal species subjected to stroke.

The differentially expressed genes (adj. $p \leq 0.05$) induced by pMCAO in each species were sorted by their fold changes in expression levels. As shown in Table 2, the top 10 most up- and downregulated genes in response to pMCAO differed substantially between rats and mice at both 2 h and 6 h. Full names of the ischemia-induced genes are described in Table 2.

At 2 h after pMCAO, the top 10 upregulated genes in mice were (listed from highest to lowest fold change): Hspa1a, Hspa1b, Npas4, Ccl3, Fosb, Fos, Cyr61, Dnajb1, Jun and Gadd45g (Table 2). Among these, the expression of heat shock proteins (Hsp70) Hspa1a and Hspa1b was highest (FC = 19.5, 19.03). By contrast, permanent ischemia only upregulated two genes in rat: Cyr61 and Tnfrsf11b. Additionally, at 2 h after pMCAO, the 10 most downregulated genes mainly were microRNAs in mice (Table 2), which differ from the set of microRNAs observed in rats.

At 6 h after pMCAO, the top 10 upregulated genes in mice were (listed from highest to lowest fold change): Hspa1a, Hspa1b, Ccl3, Lilrb4a, Snora75, Gadd45b, Snord14e, Fosb, Gadd45g, and Ptgs2 (Table 2). In rats, a different list of top upregulated genes was induced: Ccl3, Npas4, Fos, Gadd45g, Egr2, Fosb, Ccl4, Gadd45b, Junb and Olr1. Additionally, ischemia mostly downregulated microRNAs in mice at 6 h, but in rats the most downregulated genes were completely different from those observed in mice (Table 2).

We next examined the KEGG (Kyoto Encyclopedia of Genes and Genomes) functional pathways of the differentially expressed genes induced by ischemia in the two stroke animal models. The functional grouping of all differentially expressed genes (adj. $p \leq 0.05$) at 2 h or 6 h after pMCAO in mouse or rat was analyzed using the online STRING tool. The functional pathways (FDR < 0.05) were ranked based on the number of genes involved.

In mice, at 2 h after MCAO, significantly regulated pathways (FDR < 0.05) included MAPK signaling pathway, estrogen signaling pathway, toll-like receptor signaling and oxytocin signaling pathway among others. (Table 3). In rats, 2 h MCAO did not significantly regulate previously known pathways due to the few significantly regulated genes and microRNAs with unknown functions.

At 6 h after MCAO, mouse significantly regulated pathways include MAPK signaling pathway, oxytocin signaling pathway, estrogen signaling pathway, p53 signaling pathway and others (Table 3). In contrast, at 6 h in rats MCAO induced several pathways involved in inflammatory and immune response including MAPK signaling pathway, TNF

Table 1 Regulation of ischemia-related genes in mice and rats after 2 h or 6 h of permanent focal cerebral ischemia

Function	Gene name	Gene description	2 h pMCAO*		6 h pMCAO	
			Mouse	Rat	Mouse	Rat
Immediate early genes	Arc	Activity-regulated cytoskeleton-associated protein	–	–	–	3.40
	Fos	FBJ osteosarcoma oncogene	3.47	–	2.67	13.07
	Fosb	FBJ osteosarcoma oncogene B	3.62	–	4.07	7.12
	Jun	Jun proto-oncogene	2.55	–	2.68	3.29
	Junb	Jun B proto-oncogene	–	–	–	5.49
	Egr1	Early growth response 1	–	–	–	2.76
	Egr2	Early growth response 2	–	–	–	7.46
	Egr3	Early growth response 3	–	–	–	1.79
	Egr4	Early growth response 4	–	–	–	3.83
Stress response	Dnaja1	DnaJ (Hsp40) homolog, subfamily A, member 1	–	–	1.36	–
	Dnajb1	DnaJ (Hsp40) homolog, subfamily B, member 1	2.70	–	2.73	2.40
	Dnajb4	DnaJ (Hsp40) homolog, subfamily B, member 4	–	–	–	2.21
	Dnajc28	DnaJ (Hsp40) homolog, subfamily C, member 28	–	–	–	− 1.49
	Hspa1a	Heat shock 70kD protein 1A	19.50	-	20.46	2.54
	Hspa1b	Heat shock protein 1B	19.03	–	18.47	–
	Hspa5	Heat shock protein 5	–	–	–	1.55
	Hspb1	Heat shock protein B1	–	–	2.73	2.52
	Hspe1	Heat shock protein 1 (chaperonin 10)	–	–	1.92	1.37
	Hsph1	Heat shock 105 kDa/110 kDa protein 1	–	–	2.09	1.96
	Gadd45b	Growth arrest and DNA-damage-inducible 45 beta	–	–	5.01	5.51
	Gadd45g	Growth arrest and DNA-damage-inducible 45 gamma	2.36	–	4.05	9.77
Inflammation	Ccl2	Chemokine (C–C motif) ligand 2	–	–	1.71	–
	Ccl3	Chemokine (C–C motif) ligand 3	5.15	–	15.93	17.48
	Ccl4	Chemokine (C–C motif) ligand 4	–	–	–	6.88
	Ccrl2	Chemokine (C–C motif) receptor-like 2	1.63	–	–	–
	Cxcl1	Chemokine (C-X-C motif) ligand 1	–	–	–	2.03
	Il1a	Interleukin 1 alpha	1.73	–	2.72	2.25
	Il4r	Interleukin 4 receptor	–	–	–	1.81
	Ptgs2	Prostaglandin-endoperoxide synthase 2	–	–	3.99	4.09
	Tnfrsf11b	Tumor necrosis factor receptor superfamily, member 11b	–	2.06	–	–
	Tnfrsf12a	Tumor necrosis factor receptor superfamily, member 12a	–	–	1.55	–
Neuroprotection	Angpt2	Angiopoietin 2	–	–	1.82	–
	Apold1	Apolipoprotein L domain containing 1	–	–	–	3.09
	Bdnf	Brain derived neurotrophic factor	–	–	1.80	–
	Crem	cAMP responsive element modulator	–	–	–	1.89
	Cyr61	Cysteine-rich, angiogenic inducer, 61	3.11	2.86	2.98	3.09
	Hmox1	Heme oxygenase 1	–	–	2.58	3.14
	Insig1	Insulin induced gene 1	–	–	–	1.93
	Npas4	Neuronal PAS domain protein 4	8.51	–	3.99	15.12
	Plat	Plasminogen activator, tissue	–	–	1.39	3.46
	Timp1	TIMP metallopeptidase inhibitor 1	–	–	–	4.98
Apoptosis	Bag3	BCL2-associated athanogene 3	–	–	1.93	–
	Bag4	BCL2-associated athanogene 4	–	–	–	− 1.34
	Fas	Fas (TNF receptor superfamily member 6)	–	–	1.78	–
	Map3k14	Mitogen-activated protein kinase kinase kinase 14	–	–	–	1.53
	Mapkapk2	MAP kinase-activated protein kinase 2	–	–	1.50	–
	Mmp12	Matrix metallopeptidase 12	–	–	1.94	–

Table 1 (continued)

Function	Gene name	Gene description	2 h pMCAO*		6 h pMCAO	
			Mouse	Rat	Mouse	Rat
Ion channels	Cacnb2	Calcium channel, voltage-dependent, beta 2 subunit	–	–	− 1.27	–
	Grik2	Glutamate receptor, ionotropic, kainate 2 (beta 2)	–	–	− 1.35	–
	Htr1f	5-Hydroxytryptamine (serotonin) receptor 1F	–	–	− 1.43	–
	Htr2a	5-Hydroxytryptamine (serotonin) receptor 2A	–	–	− 1.39	–
	Kcna2	Potassium voltage-gated channel, shaker-related subfamily, member 2	–	–	− 1.27	–
	Kcnc2	Potassium voltage-gated channel, Shaw-related subfamily, member 2	–	–	− 1.35	–
	Kcnj3	Potassium inwardly-rectifying channel, subfamily J, member 3	–	–	− 1.30	–
	Kcnq3	potassium voltage-gated channel, subfamily Q, member 3	–	–	− 1.40	–
Signal transduction	Dusp1	Dual specificity phosphatase 1	–	–	–	2.78
	Dusp6	Dual specificity phosphatase 6	–	–	–	2.83
	Dusp16	Dual specificity phosphatase 16	–	–	1.49	–
	Gem	GTP binding protein (gene overexpressed in skeletal muscle)	–	–	1.52	–
	Gpr12	G-protein-coupled receptor 12	–	–	− 1.44	–
	Gpr34	G-protein-coupled receptor 34	–	–	–	− 2.83
	Gpr52	G-protein-coupled receptor 52	–	–	− 2.47	–
	Gpr84	G-protein-coupled receptor 84	–	–	2.00	–
	P2ry12	Purinergic receptor P2Y, G-protein-coupled 12	–	–	− 1.71	–
	P2ry13	Purinergic receptor P2Y, G-protein-coupled, 13	–	–	–	1.64
	Rgs1	Regulator of G-protein signaling 1	–	–	1.53	–
	Rgs2	Regulator of G-protein signaling 2	–	–	1.59	2.97

*Gene expression levels are presented in Log2 fold change comparing between pMCAO and sham controls. A positive value indicates upregulation, a negative value indicates downregulation. All listed genes have adjusted $p \leq 0.05$

signaling pathway, toll-like receptor signaling pathway, NF-kappa B signaling pathway, chemokine signaling pathway, T cell and B cell receptor signaling pathways. In addition, oxytocin signaling pathway involved in cardiovascular regulation, and HIF-1 and FoxO signaling pathways important for oxidative stress response were also stimulated in rats.

Validation of Microarray Data by qRT-PCR

To validate our microarray results, 4 ischemia-related genes with Log2 FC > 1.5 (adj. $p \leq 0.05$) were analyzed by Real-Time (RT)-PCR and compared between rats and mice. In general, the relative fold changes of genes between rats and mice by RT-PCR were in agreement with their relative expression levels in the microarray (Table 4).

Discussion

Here we showed that rats and mice have very different regulated groups of genes in response to a widely used experimental ischemia model of permanent middle cerebral artery occlusion. Overall, about 90% of genes induced by ischemia differed between the two species and more genes were regulated in mice than rats at both 2 h and 6 h after ischemia. The stroke model we used produced large hemispheric infarctions in each species, accompanied by profound hemiplegia. The striking differences in the observed gene responses occurred despite the similar phenotypic responses of rats and mice to pMCAO. This may be because the acute stroke phenotype (histological and/or behavioral) is independent of the interrogated genes, and the observed differences in gene responses despite phenotypically similar stroke syndromes are geared to govern differences in stroke recovery or response to treatment. Alternatively, the differences we observed may simply be biomarkers for a differential vulnerability of rats and mice to experimental stroke parameters such as severity or duration of ischemia (Carmichael 2005). For all these potential reasons, it is important to highlight these inter-species' differences in gene regulation in order to raise a note of caution about making go-no-go decisions based on results from a single species.

Our study is the first to compare mouse and rat responses to stroke directly but concurs or expands previous findings in single species. For example, our 6 h mouse data concur

Table 2 Top 10 up- and downregulated genes in mice and rats after 2 h or 6 h ischemia

Top 10 up- and downregulated genes by pMCAO

2 h

Mouse			Rat		
Gene name	Gene description	Gene expression*	Gene name	Gene description	Gene expression
Hspa1a	Heat shock protein 1A	19.50	Cyr61	Cysteine-rich, angiogenic inducer, 61	2.86
Hspa1b	Heat shock protein 1B	19.03	Tnfrsf11b	Tumor necrosis factor receptor superfamily, member 11b	2.06
Npas4	Neuronal PAS domain protein 4	8.51	Mir29c	MicroRNA 29c	− 2.96
Ccl3	Chemokine (C–C motif) ligand 3	5.15	Mirlet7f-1	MicroRNA let-7f-1	− 3.45
Fosb	FBJ osteosarcoma oncogene B	3.62	Mir27b	MicroRNA 27b	− 3.60
Fos	FBJ osteosarcoma oncogene	3.47	Mir382	MicroRNA 382	− 3.64
Cyr61	Cysteine-rich protein 61	3.11	Mir543	MicroRNA 543	− 3.76
Dnajb1	DnaJ (Hsp40) homolog, subfamily B, member 1	2.70	Mir154	MicroRNA 154	− 4.13
Jun	Jun proto-oncogene	2.55	Mir421	MicroRNA 421	− 4.31
Gadd45g	Growth arrest and DNA-damage-inducible 45 gamma	2.36	Mir300	MicroRNA 300	− 4.61
2010111I01Rik	RIKEN cDNA 2010111I01 gene	− 2.02	Mir374	MicroRNA 374	− 5.32
Mir667	MicroRNA 667	− 2.12	Mir181b-1	MicroRNA 181b-1	− 6.21
Mir382	MicroRNA 382	− 2.20			
Mir323	MicroRNA 323	− 2.31			
Mir485	MicroRNA 485	− 2.38			
Mir136	MicroRNA 136	− 2.47			
Mir434	MicroRNA 434	− 2.54			
Mir379	MicroRNA 379	− 2.56			
Mir154	MicroRNA 154	− 2.96			
Mir543	MicroRNA 543	− 4.33			

6 h

Mouse			Rat		
Gene name	Gene description	Gene expression	Gene name	Gene description	Gene expression
Hspa1a	Heat shock protein 1A	20.46	Ccl3	Chemokine (C–C motif) ligand 3	17.48
Hspa1b	Heat shock protein 1B	18.47	Npas4	Neuronal PAS domain protein 4	15.12
Ccl3	Chemokine (C–C motif) ligand 3	15.93	Fos	FBJ osteosarcoma oncogene	13.07
Lilrb4a	Leukocyte immunoglobulin-like receptor, subfamily B, member 4A	7.38	Gadd45g	Growth arrest and DNA-damage-inducible, gamma	9.77
Snora75	Small nucleolar RNA, H/ACA box 75	6.05	Egr2	Early growth response 2	7.46
Gadd45b	Growth arrest and DNA-damage-inducible 45 beta	5.01	Fosb	FBJ osteosarcoma oncogene B	7.12
Snord14e	Small nucleolar RNA, C/D box 14E	4.69	Ccl4	Chemokine (C–C motif) ligand 4	6.88
Fosb	FBJ osteosarcoma oncogene B	4.07	Gadd45b	Growth arrest and DNA-damage-inducible, beta	5.51
Gadd45g	Growth arrest and DNA-damage-inducible 45 gamma	4.05	Junb	Jun B proto-oncogene	5.49
Ptgs2	Prostaglandin-endoperoxide synthase 2	3.99	Olr1	Oxidized low density lipoprotein (lectin-like) receptor 1	5.19
Mir128-2	MicroRNA 128-2	− 1.82	Pkp2	Plakophilin 2	− 2.23

Table 2 (continued)

6 h					
Mouse			Rat		
Gene name	Gene description	Gene expression	Gene name	Gene description	Gene expression
Mir154	MicroRNA 154	− 1.84	Ndst4	*N*-deacetylase/*N*-sulfotransferase (heparan glucosaminyl) 4	− 2.36
Mir495	MicroRNA 495	− 1.89	Mirlet7c-1	MicroRNA let7c-1	− 2.43
Mirlet7f-1	MicroRNA let7f-1	− 2.00	Tmem74	Transmembrane protein 74	− 2.44
6330415B21Rik	RIKEN cDNA 6330415B21 gene	− 2.02	Bmp4	Bone morphogenetic protein 4	− 2.55
Mir374b	MicroRNA 374b	− 2.04	Gpr34	G-protein-coupled receptor 34	− 2.83
Mir181b-1	MicroRNA 181b-1	− 2.13	Abcg2	ATP-binding cassette, subfamily G (WHITE), member 2	− 2.93
Gpr52	G-protein-coupled receptor 52	− 2.47	RGD1359158	Similar to RIKEN cDNA 1110059E24	− 3.22
Mir876	MicroRNA 876	− 2.75	Slco1a2	Solute carrier organic anion transporter family, member 1A2	− 3.73
Mir128-1	MicroRNA 128-1	− 2.96	Nts	Neurotensin	− 4.29

*Gene expression levels are presented in Log2 fold change comparing between pMCAO and sham controls. A positive value indicates upregulation, a negative value indicates downregulation. All listed genes have adjusted $p \leq 0.05$

with those of Hori et al. (Hori et al. 2012) showing that mice subjected to pMCAO upregulate the expression of matrix metalloproteinases, chemokines, interleukins and heat shock proteins (Hsp) in the same time frame. Ramos-Cejudo et al. (2012) compared delayed gene responses in the core and peri-infarct areas of rats at 24 h and 3 days post-stroke, to demonstrate the upregulation of Hsp genes and additional genes that may have impacts on stress response or recovery after stroke. To date the only direct inter-species comparison was performed in a study of chemokine/cytokine expression in cultured neurons, astrocytes and microglia exposed to oxygen–glucose deprivation. Human and rat neurons showed similar changes with a downregulation in many chemokines, whereas mouse neurons showed a mixed response with up- and downregulated genes (Du et al. 2017).

Our study has provided findings suggestive of more similarities between rat and human responses as compared with mice. For example, MMPs (matrix metalloproteinases) are implicated in the degradation and remodeling of the extracellular matrix and play an important role in ischemic injury (Cunningham et al. 2005). MMP activity is regulated by specific tissue inhibitors of matrix metalloproteinases (TIMPs). Timp1 is a gene associated with neuroprotection by reducing blood–brain barrier destruction and infarction volume by inhibiting MMPs (Chelluboina et al. 2015; Tejima et al. 2009). We found that the genes metallopeptidase MMP12 were significantly increased in mice by 6 h, but not rats, which upregulate MMP12 over days, not hours (Tables 1 and 4) (Chelluboina et al. 2015). We also found that Timp1 genes were significantly upregulated in rats, but not in mice, at 6 h after pMCAO (Tables 1 and 4). The responses we observed in rats have also been observed in infarcted brain tissue after human ischemic stroke (Cuadrado et al. 2009), strengthening the possibility that rats may share more gene responses to ischemia with humans. In further support of similarities between rats and humans was our finding of the upregulation of TNF signaling pathways in rats, but not mice (Table 3). This is similar to observations noted in human studies. In humans, acute stroke has been shown to induce increased levels of TNF in cerebral spinal fluid and blood (Lambertsen et al. 2012; Maas and Furie 2009) as well as within 1 day in postmortem studies of stroke patients, most prominent in neuronal processes of the infarction core and peri-infarct area during the first day of infarction (Dziewulska and Mossakowski 2003; Sairanen et al. 2001).

We observed a global upregulation of heat shock protein (Hsp) genes. Notably, the genes Hspa1a and Hspa1b of the Hsp70 family were upregulated earlier and to greater levels in mice. The Hsp70 proteins act as molecular chaperones and protect against cellular stress by promoting protein repair by refolding and trafficking damaged proteins in the cells (Mayer and Bukau 2005). The rapid and robust increase of Hsp expression in mice following permanent ischemia not only implicates an early protective mechanism in mice but also a higher level of ischemic stress and protein damage in mouse brains shortly after ischemia.

Ischemia triggered endogenous neuroprotective responses in both rats and mice. Among the neuroprotective genes, the expression of Npas4 in mice was first stimulated but declined with time in mice, while in rats it had a much higher expression at 6 h after pMCAO. Npas4 has been identified as a critical neuroprotective gene that regulates neuronal

Table 3 Comparison of pMCAO regulated pathways (stroke-related) between mice and rats after 2 h or 6 h ischemia

pMCAO regulated pathways (stroke-related)							
2 h							
Mouse				Rat			
Pathway description	Gene count	False discovery rate	Matching genes in the network (labels)	Pathway description	Gene count	False discovery rate	Matching genes in the network (labels)
MAPK Signaling pathway	6	2.74E−05	Fos,Gadd45g,Hspa1a,Hspa1b,Il1a,Jun				
Estrogen Signaling pathway	5	1.25E−05	Fkbp5,Fos,Hspa1a,Hspa1b,Jun				
Toll-like receptor signaling pathway	3	0.00396	Ccl3,Fos,Jun				
Oxytocin signaling pathway	3	0.0114	Camkk1,Fos,Jun				
Protein processing in endoplasmic reticulum	3	0.0122	Dnajb1,Hspa1a,Hspa1b				
B cell receptor signaling pathway	2	0.0324	Fos,Jun				
6 h							
Mouse				Rat			
Pathway description	Gene count	False discovery rate	Matching genes in the network (labels)	Pathway description	Gene count	False discovery rate	Matching genes in the network (labels)
MAPK Signaling pathway	18	3.05E−07	Bdnf,Cacna1a,Cd14,Ddit3,Dusp16,Fas,Fos,Gadd45b,Gadd45g,Hspa1a,Hspa1b,Hspb1,Il1a,Jun,Mapkapk2,Nlk,Pdgfa,Pdgfb	MAPK signaling pathway	16	2.59E-06	Atf4,Ddit3,Dusp1,Dusp6,Fos,Gadd45b,Gadd45g,Hspa8,Hspb1,Il1a,Jun,Jund,Map3k14,Nfkb1,Nr4a1,Srf
MicroRNAs in cancer	11	0.000111	Cd44,Cdk6,Cdkn1a,Cyp1b1,Gls,Mcl1,Pdgfa,Pdgfb,Ptgs2,Stat3,Vim	Transcriptional misregulation in cancer	10	0.000177	Cd86,Ddit3,Dusp6,Eya1,Mlf1,Nfkb1,Nr4a3,Plat,Rel,Smad1
Oxytocin signaling pathway	8	0.0458	Cdkn1a,Fos,Jun,Kcnj3,Prkab2,Ptgs2,Rcan1,Rgs2	TNF signaling pathway	9	0.000101	Atf4,Cxcl1,Fos,Jun,Junb,Map3k14,Nfkb1,Nfkbia,Ptgs2
Protein processing in endoplasmic reticulum	8	0.0482	Ddit3,Dnaja1,Dnajb1,Hspa1a,Hspa1b,Hsph1,Sar1a,Sec61a1	FoxO signaling pathway	9	0.00028	Gadd45b,Gadd45g,Homer1,Plk2,Plk3,Prkaa1,Prkab2,Rbl2,Stat3
Estrogen signaling pathway	6	0.0482	Fos,Hbegf,Hspa1a,Hspa1b,Jun,Kcnj3	Toll-like receptor signaling pathway	8	0.000177	Ccl3,Ccl4,Cd86,Fos,Jun,Nfkb1,Nfkbia,Spp1
p53 signaling pathway	5	0.0484	Cdk6,Cdkn1a,Fas,Gadd45b,Gadd45g	NF-kappa B signaling pathway	7	0.000819	Ccl4,Gadd45b,Lyn,Map3k14,Nfkb1,Nfkbia,Ptgs2
				Oxytocin signaling pathway	7	0.0114	Fos,Jun,Prkaa1,Prkab2,Ptgs2,Rcan1,Rgs2
				Protein processing in endoplasmic reticulum	7	0.0174	Atf4,Ddit3,Hspa5,Hspa8,Hsph1,Ngly1,Ppp1r15a

Table 3 (continued)

6 h							
Mouse				Rat			
Pathway description	Gene count	False discovery rate	Matching genes in the network (labels)	Pathway description	Gene count	False discovery rate	Matching genes in the network (labels)
				Chemokine signaling pathway	7	0.02	Ccl3,Ccl4,Cxcl1,Lyn,Nfkb1,Nfkbia,Stat3
				B cell receptor signaling pathway	6	0.00194	Fcgr2b,Fos,Jun,Lyn,Nfkb1,Nfkbia
				Estrogen signaling pathway	5	0.0218	Atf4,Fos,Hbegf,Hspa8,Jun
				T cell receptor signaling pathway	5	0.0381	Fos,Jun,Map3k14,Nfkb1,Nfkbia
				HIF-1 signaling pathway	5	0.0405	Hmox1,Nfkb1,Serpine1,Stat3,Timp1

Significantly regulated pathways with FDR <0.05 are listed

survival genes such as BDNF (Pruunsild et al. 2011), promotes angiogenesis (Esser et al. 2017) and protects neurons from ischemic cell death (Choy et al. 2016). The significant stimulation of Npas4 in rats may suggest an emphasis on Npas4-dependent survival signaling. The decline of Npas4 seen in mice may imply a reduced neuroprotective capability with time or a faster ischemic progression than rats. Interestingly, the release of endogenous tissue plasminogen activator Plat was seen in both species after ischemia with a higher expression in rats, suggesting an intrinsic ability to dissolve blood clots.

As expected after ischemia, cell death mediator expression was altered. Apoptotic genes Bag3 and Fas were significantly increased in mice, which may render them more vulnerable to ischemic brain injury than rats.

Several ion channels were significantly downregulated in mice after focal ischemia, including voltage-dependent calcium channels, kainate glutamate receptors, serotonin receptors, voltage-gated potassium channels and inwardly-rectifying potassium channels. Since ischemia disrupts channel activities and ionic homeostasis, leading to excitotoxicity and ischemic cell death, the downregulation of ion channels and excitatory neurotransmitter receptors might act to counter the ionic imbalance in ischemia and reduce the effects of excitotoxicity.

In addition to the different expressions seen in the previously reported stroke-related genes, rat and mouse stroke models were also very different when comparing the most regulated genes. Among the top 10 up- and downregulated genes by 2 h pMCAO, mice mostly upregulated heat shock proteins, stress response genes and inflammatory mediators, while in rats, only two genes were significantly upregulated. Cyr61 gene was significantly increased in both species, which could be a universal neuroprotective gene that might have a clinical interest. After 6 h pMCAO, the continuous high elevation of heat shock proteins in mice, and the significant increase of Npas4 in rats may suggest their dependency on different neuroprotective pathways. Additionally, mice and rats also have different sets of most downregulated genes composed of mostly microRNAs (miRNAs). miRNAs are short non-coding RNA molecules involved in the post-transcriptional regulation of gene expression. In recent years, miRNAs have emerged as regulators of ischemic injury. They can regulate several different mRNAs simultaneously, making them good candidates for therapeutic targets (Weiss et al. 2012). Previous reports have shown that miR382, 323, 543, 495, let7f-1, 181b-1, 29c, and let7c-1 were differentially regulated following ischemia (Yang et al. 2015). Among these miRNAs, the downregulation of miR323, let7f-1, 495, and miR181b-1 was shown to be neuroprotective against ischemia(Peng et al. 2013; Selvamani et al. 2012; Welten et al. 2014; Yang et al. 2015), and the downregulation of miR-29c increased

Table 4 Validation of data from microarray analysis by RT-qPCR

Gene expression (fold change) by RT-qPCR				
Genes	2 h MCAO		6 h MCAO	
	Rat	Mouse	Rat	Mouse
Plat	2.28	1.06	6.34	1.67
Timp1	4.05	1.32	24.99	6.52
Dusp1	2.38	1.58	6.56	1.47
MMP12	1.6	1.23	12.35	100.38
Gene expression (fold change) by Microarray				
Genes	2 h MCAO		6 h MCAO	
	Rat	Mouse	Rat	Mouse
Plat	1.91	0	3.46	0
Timp1	1.71	0	4.97	0
Dusp1	0	0	2.78	0
MMP12	0	0	0	1.937

Change in gene expression by ischemia was calculated as the average fold change in sham samples subtracted from the average fold change in ischemic animals

Plat plasminogen activator, tissue, *Timp1* tissue inhibitor metalloproteinase I, *Dusp1* dual specificity phosphatase 1, *MMP12* matrix metallopeptidase 12

the risk of ischemic neuronal death (Pandi et al. 2013). Apart from previously reported miRNAs, we also observed a significant downregulation of miR667, 485, 136, 434 379, 154 after 2 h pMCAO and miR128-2, 154, 374b, 876, and 128-1 after 6 h pMCAO in mice. In rats, we found a significant downregulation of miR27b, 543, 154, 421, 300, 374 after 2 h pMCAO, and miR-let7c-1 after 6 h pMCAO. Our findings may suggest the involvement of these miRNAs in ischemia.

Functional pathways were also different in mice and rats after pMCAO. At 2 h, pathways such as MAPK signaling pathway and toll-like receptor signaling were activated in mice but not in rats. This further supports that mice may be more sensitive to ischemia than rats at early hours. After 6 h, MAPK signaling was mostly regulated by ischemia in both rats and mice. Studies have suggested a role of MAPK signaling in regulating inflammatory cytokines and cell apoptosis in ischemia (Sun and Nan 2016). Thus, MAPK signaling pathways have the potential to serve as therapeutic targets against ischemic injury. Furthermore, 6 h pMCAO in rats stimulated TNF, FoxO, HIF-1, NF-kappa B, toll-like receptor and chemokine signaling pathways involved in inflammation, oxidative stress responses, immune responses and apoptosis, which were not differentially regulated in mice. The regulation of TNF and chemokine signaling pathways in rats after stroke confirms the previous research that inflammatory cytokines are involved in ischemic damage and are potential targets in future stroke therapy (Lambertsen et al. 2012).

Future studies should seek to match as much as possible the degree and duration of ischemia in the sampled brain regions in order to determine whether the inter-species differences in gene expression reported here are due largely to biological inter-species differences or due to altered regional physiological responses, such as different degrees of ischemia or collateral flow. Since the pMCAO model produces phenotypically similar acute strokes in rats and mice, experiments might be expanded to determine whether similar differences between the species are found during the recovery phase or in response to treatment. Experiments should also seek to compare the gene responses of mice and rats to those documented in humans. Moreover, similar analyses might be performed in a temporary MCAO model in mice and rats to evaluate these species' reaction to reperfusion. Future experiments could be conducted to address the impact of neuroprotective agents or other therapies on these gene expression patterns and resultant pathways. Such experiments may help elucidate previously unrecognized mechanisms of action of such therapies. Future behavioral studies may be conducted in the two species exposed to the ischemic insult to further understand the phenotypical projection of the discovered fundamental genetic responses. Lastly, experiments may expand to non-human primates, which bear genetic, anatomical, and behavioral similarities to humans, to correlate the gene response of primates with those of rats and mice. In addition, we chose only males for the study so that the differences in gene expression would be related to inter-species differences only and not obscured

by gender differences. The gender difference in different animal models is another important topic of stroke translational research for further investigation.

Conclusion

Mice and rats respond to ischemia by regulating different groups of genes and pathways. These findings may be due to genetic differences in stroke response or to fundamental differences in depth of ischemia between species. This may have implications for the interpretation of stroke studies and decision-making in translational stroke research.

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Data Availability All data in this paper are available to scientific communities upon reasonable request to the corresponding author.

Declarations

Conflict of interest Dr. Michael Tymianski (M.T.) is a Canada Research Chair (Tier 1) in Translational Stroke Research, and the CEO of NoNO Inc., a biotechnology company developing nerinetide (also termed NA-1 or Tat-NR2B9c) for clinical use. Q.J.W., X.J.S., L.T. and D.M. have no competing interests.

Ethical Approval All procedures performed involving animals were approved by the University Health Network animal care committee, conformed to Canadian Council of Animal Care guidelines, and ARRIVE guidelines (Kilkenny et al. 2010).

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