Sources of bias in transgenic studies of Stroke Pathophysiology

Matriculation number: 0346901

ABSTRACT

Background and purpose – Hundreds of clinical trails testing neuroprotective agents have disappointingly failed and recently, several systematic reviews have highlighted important deficits and bias in the quality of preclinical stroke research. The aim of this project is to begin the process of systematic review and meta-analysis on all the data from publications on transgenic animals and focal cerebral ischaemia.

Methods – Publications on transgenic animals and focal cerebral ischaemia were found electronically via PubMed, Embase and Biosis. A 95% confidence limit was calculated from preliminary data and comparisons were made between results collected.

Results- 432 were found electronically but only 157 publications were reviewed due to time restrains. 299 experiments on 5316 animals were analysed to produce preliminary data. The highest quality score recorded was 6/10 which may pose as a potential source of bias and a higher proportion of positive experiments were found within the upper limits of quality scores (4-6).

Conclusions- A present meta-analysis cannot be performed on preliminary results due to the presence of bias on a future meta-analysis when all the data is collected. With only 6 out of 299 experiments which used blinded assessment, there can be improvements in the study designs on transgenic animals. (Word count: 199)

INTRODUCTION

Stroke accounted for about 1 of every 15 deaths in the United States in 2003; of all strokes, 88% are ischaemic and 8-12% of ischaemic strokes result in death within 30 days[1]. The estimated direct and indirect cost of stroke for 2006 is $57.9 billion[1] and the strive to gain a deeper understanding of stroke pathophysiology is reflected in the large number of studies on animal models of stroke that have been conducted in the last 10 years.

The ethics of animal experiments are being actively debated in some countries[2] and the public only accept animal testing on the assumption that it benefits humans[3]. The impressive rate of understanding into the pathophysiology of stroke[4] has prompted the development of many neuroprotective agents which are tested on animals.

However, the discrepancy between efficacy in animal data and the lack of efficacy in clinical data; reinforced time and time again by reports of unsuccessful outcomes in trials of candidate neuroprotectants on acute stroke patients has led to several excellent qualitative reviews and commentaries which addressed this issue. (for examples, see previously published studies [5-10])

Pound et al [11] has recently called for urgent, more systematic evaluation of animal data before entering into clinical trials. Animal studies should not only include evidence of neuroprotective activity but also ideas of the limits of efficacy which can impact on the clinical efficacy of the drug[12].

In response to the growing need for urgent evaluation, we set out to examine studies on focal cerebral ischaemia in transgenic animals and the impact on the estimate of efficacy from aspects of study quality and study design. Studies on models of global, haemorrhagic and cell culture method of stroke were excluded because it is thought that focal models mimic acute human stroke more closely[13].

Transgenic animals created by precise and permanent genetic modifications are used to provide insights into gene regulation, development, pathogenesis and the treatment of disease. The use of transgenic animals to demonstrate stroke models has helped researchers understand the progress, stages and symptoms of the disease better, and also to screen potential therapies or drugs. Mice form the majority of transgenic animals developed for this purpose because they are small, easy to manipulate and maintain[14].

The most widely used techniques are pronuclear microinjection because of its simplicity and applicable to a wide range of species. However, this method is often considered relatively crude due to the number of copies integrated and the lack of control of where the transgene integrates in the genome. The second most common methodology is Embryonic stem cell manipulation which can introduce genes as well as ‘knock out’ genes by homologous recombination[14]. This method is only applicable in mice at present.

Using transgenic animals to demonstrate the pathophysiology of stroke is getting more popular as the technology has advanced with many lines of transgenic mice being developed to suit the needs of the experimental designs proposed by investigators[15].

The main aim of the review are therefore (1) to identify all the transgenic animals that have either overexpression or knock-out of receptors with suspected neuroprotective qualities; (2) to compare and describe the study design and study quality and (3) to describe the main classes of neuroprotective receptors of transgenic animals.

METHODS

Identification of relevant studies

(1) Electronic search of Pubmed, EMBASE and BIOSIS using search terms ((stroke) OR (cerebral ischaemia) AND (transgenic) OR (tg) or (targeted deletion) or (overexpression) or (knock out) or (vector)); (2) Handsearching of abstracts of Journal of Neuroscience; and (3) Requests to authors of publication identified above for other published or unpublished data.

(2) Two investigators (LC, ES) independently extracted publications that described controlled studies of transgenic animals given in models of focal cerebral ischaemia induced by occlusion of the middle or anterior cerebral artery or their branches. Disagreements were resolved in discussion with a third investigator (MM).

(3) End Point considered:
The primary outcome measure was infarct area or volume (determined histologically or by cross sectional imaging).

Methods of review[16]:

Quality assessment- There was no quality threshold for inclusion. Study quality was assessed against our published ten item checklist[16] comprising (i) publication in peer reviewed journal, (ii) statement of control of temperature, (iii) randomization of treatment or control, (iv) blinded induction of ischaemia, (v) blinded assessment of outcome, (vi) avoidance of anaesthetics with marked intrinsic neuroprotective properties, (vii) use of animals with hypertension or diabetes, (viii) sample size calculation, (ix) statement of compliance with regulatory requirements, and (x) statement regarding possible conflicts of interest.

Data extraction – From each source we identified individual comparisons where outcome was measured in a group of transgenic animals with a specific knock out or overexpression and compared with outcome in a control group. Where there are more than one transgenic group compared with one control group, this was recorded. For each comparison and for each of transgenic and control group we extracted data for number per group, mean outcome and its standard deviation. Where an outcome was measured serially, only the last measure was used. Where data was given graphically we contacted authors seeking data; where this was not available we estimated values by measurement from publications. Data was extracted onto a data extraction form which was designed and produced specifically by ES.
We also collected other relevant data including anaesthetic used, time of outcome measurement, and method of induction of ischaemia, as well as the individual component items of the quality checklist above.

Analysis- The results were compiled into a table organised by pathway of receptor, the expression of transgenic animals, author, year of publication, sex, type of animal, No of treatment, the true No of control, anaesthetic, quality score, genotype, gene and study effectiveness. To calculate whether the study effectiveness was neutral or positive, i.e. whether the lower 95% confidence limit of effect size crossed the line of no, the experiment is termed Neutral, otherwise it is termed Positive.

Effect size (%) = 100 (1 – Outcome [Treated] / Outcome [Control])*

Where a single control group served multiple transgenic groups, the size of the control group entered to the analysis was adjusted by division by the number of treatment groups served. The following comparisons/observations were made:
Comparisons between the:

(1) Blinded assessments in experimental procedures and the effectiveness of outcome.
(2) The publication type and the effectiveness of outcome.
(3) Quality score and the effectiveness of outcome.
(4) Anaesthetic and effectiveness of outcome.

And Observations on:

(5) The proportion of KO/expression experiments.

(6) The different pathways of the transgenes and the model of ischaemia used in experimental procedures.
(7) The overall heterogeneity of the experiments.
(8) The genotype of the results.
(9) The random allocation to groups.

RESULTS

Electronic searching identified 432 publications describing the use of transgenic animals in models of focal cerebral ischaemia of which in the limited time given to do this project, 238 publications were reviewed. The results described below are merely preliminary results thus no statistical analysis was undertaken. Of these, 81 papers were excluded because they did not contain outcomes of infarct volume/area of transgenic animals and focal ischaemia; thus the analysis was based on 157 papers that included 299 experiments with the use of 5316 animals. (see Appendix 1)

Results of comparisons:

(1) Blinded assessments in experimental procedures and the effectiveness of outcome (see Table 1)

In total, there were 6 out of 299 experiments that had a blinded assessment of outcome. When the results were dichotomised according to the neutral or positive effect, there were little observed differences between the 2 groups.

Outcome Measure	Neutral or positive	Blinded Assessment of Outcome	Sum Of Number in Treatment Group	Sum Of True No of Control	Total Animals	No of Comparisons
Infarct Volume	Neutral	TRUE	20	20	40	2
Infarct Volume	Neutral	FALSE	957	818.68	1775.68	106
Infarct Volume	Positive	TRUE	37	39	76	4
Infarct Volume	Positive	FALSE	1763	1661.31	3424.31	187

Table 1.

(2) The publication type and the effectiveness of outcome (see Table 2)

There were 3 abstracts out of 299 publications and the number of positive full publication experiments (198) slightly exceeds the number of negative full publication experiments (107). The number between negative and positive abstracts are very similar however it

was difficult to compare the data any further as the number of abstracts formed such a small proportion of the total number of publications.

Outcome Measure	Publication Type	Neutral or positive	Sum Of Number in Treatment Group	Sum Of True No of Control	Total Animals	No of Comparisons
Infarct Volume	Abstract	Neutral	9	9	18	1
Infarct Volume	Full Publication	Neutral	968	829.68	1797.68	107
Infarct Volume	Abstract	Positive	33	49	82	2
Infarct Volume	Full Publication	Positive	1767	1651.31	3418.31	189

Table 2.

(3) Quality score and the effectiveness of outcome (see Table 3)

Out of 299 experiments, the highest quality score is 6 out of 10 which may present a potential important source of bias. When the effectiveness of the quality score were dichotomised into less or equal to 3 and more than 3; 43% was ≤3 and 57% was >3 in the neutral group. 34% was ≤3 and 65% was >3 in the positive group. Thus a higher proportional of positive experiments were found in studies that had a higher quality score of 4-6.

Outcome Measure	Quality Score	Neutral or positive	Sum Of Number in Treatment Group	Sum Of True No of Control	Total Animals	No of Comparisons
Infarct Volume	1	Neutral	21	20	41	3
Infarct Volume	2	Neutral	55	56.33	111.33	9
Infarct Volume	3	Neutral	365	267.6	632.6	34
Infarct Volume	4	Neutral	504	468.75	972.75	59
Infarct Volume	5	Neutral	12	6	18	1
Infarct Volume	6	Neutral	20	20	40	2
Infarct Volume	0	Positive	33	49	82	2
Infarct Volume	1	Positive	7	6	13	1
Infarct Volume	2	Positive	177	153.66	330.66	21
Infarct Volume	3	Positive	385	358.4	743.4	41

Infarct Volume	4	Positive	1044	989.25	2033.25	112
Infarct Volume	5	Positive	133	120	253	12
Infarct Volume	6	Positive	21	24	45	2

Table 3.

(4) Anaesthetic and effectiveness of outcome (see Table 4)

There were more positive results in experiments using Halothane and Isoflurane. Otherwise, there were no observed differences in the effectiveness on the other anaesthetics used.

Outcome Measure	Anaesthetic ID	Neutral or positive	Sum Of Number in Treatment Group	Sum Of True No of Control	Total Animals	No of Comparisons
Infarct Volume	Halothane	Neutral	415.5	382	797.5	46
Infarct Volume	Isoflurane	Neutral	147.5	108.25	255.75	17
Infarct Volume	Chloral Hydrate	Neutral	104	88	192	11
Infarct Volume	Pentobarbital	Neutral	23	22	45	2
Infarct Volume	Ketamine	Neutral	118	67.93	185.93	12
Infarct Volume	Unknown	Neutral	107	107.5	214.5	12
Infarct Volume	Tribromoethanol	Neutral	20	20	40	2
Infarct Volume	Sevoflurane	Neutral	27	28	55	3
Infarct Volume	Enflurane	Neutral	15	15	30	3
Infarct Volume	Halothane	Positive	867	822.5	1689.5	94
Infarct Volume	Isoflurane	Positive	370	355.25	725.25	42
Infarct Volume	Chloral Hydrate	Positive	90	84	174	11
Infarct Volume	Ketamine	Positive	150	116.06	266.06	16
Infarct Volume	Unknown	Positive	176	188.5	364.5	17
Infarct Volume	Tribromoethanol	Positive	88	85	173	6
Infarct Volume	Sevoflurane	Positive	44	37	81	4
Infarct Volume	Avertin	Positive	15	12	27	1

Table 4.

Results of observations:

(5) The proportion of KO/expression experiments

Of the 299 experiments, 235 were carried out on Knock out animals and 60 were overexpressed whilst 4 experiments were on both knock outs and overexpressed animals.

(6) The different pathways of the transgenes and the model of ischaemia on experimental procedures.

21 different pathways in total were studied, of which the majority worked on inflammation. 121 studies were carried out on a permanent model of focal ischaemia, 175 were temporary models and 3 were experiments on both temporary and permanent models.

(7) The overall heterogeneity of the experiments (see Table 5)

There was substantial heterogeneity between studies. (X^2= 7351, df= 298, $p<10^{-99}$).

Outcome Measure	Drug Group	No of Comparisons	Sum Of Total Animals	Heterogeneity Stat
Infarct Volume	Transgenics	299	5315.99	7350.555

Table 5.

(8) The genotype of results

Out of the 299 experiments, the majority were homozygous (176 experiments). Heterozygous experiments made up 51 of the total and 72 experiments used transgenic animals of an unknown genotype.

(9) The random allocation to groups (see Table 6)

Out of 299 experiments, only 8 of them randomly allocated animals to a group.

Outcome Measure	Random Allocation to Group	Neutral or positive	Sum Of Number in Treatment Group	Sum Of True No of Control	Total Animals	No of Comparisons
Infarct Volume	TRUE	Neutral	44	44	88	5
Infarct Volume	FALSE	Neutral	933	794.68	1727.68	103
Infarct Volume	TRUE	Positive	26	26	52	3
Infarct Volume	FALSE	Positive	1774	1674.31	3448.31	188

Table 6.

DISCUSSION

It must be noted that the data on this paper only presents the preliminary results and that no statistical analysis could be carried out on only half the number of papers that have been published on transgenic animals and focal cerebral ischaemia. A meta-analysis on the present data will affect the future meta-analysis on the data from all 432 publications because I will be looking for a certain outcome from the previous meta-analysis posing a bias.

It is common practice to carry out clinical trials as a double-blinded assessment; however, with the present data of 299 experiments, only 6 were blinded. It is highly unlikely that all the other 194 papers which have yet to be reviewed will describe all their experiments as blinded. it can therefore be presumed that less than 50% of the total number of assessments on focal ischaemia and transgenic animals are blinded and that this aspect of study quality needs to be improved to provide a more reliable estimate of efficacy.

No study scored more than 6 of 10 items on our quality checklist, and the lack of methodological rigour suggests that the effectiveness of studies calculated might be due to bias. It is therefore very important to be aware of this point when a meta-analysis is carried out on all the experiments of the 432 papers in the future as it has been previously shown that low study quality in reports of animal studies is associated with higher estimates of efficacy[17].

As outlined in the results, only 8 experiments out of 299 randomly allocated animals to groups. This will no doubt affect the study quality of the outcome and poses a further bias into the effectiveness of the results.

The huge heterogeneity has meant that a global estimate of the data may not be representative of the true efficacy. A meta-analysis cannot be used to clump the data together because the differences between the aspects of study characteristics from each paper are too big. It is thus very difficult to make a fair comparison between studies that are so different in nature and in the future, it will be necessary to systematically go through each set of data and its study qualities to find out which aspects of the study are lacking and can be improved.

The analysis on this paper is entirely observational and only simple calculations on the preliminary results were performed due to the time restrains of this project. A lot of results are compared with the effectiveness of the outcome and experiments are grouped as neutral or positive. It must be noted however that in some KO experiments, a neutral result was perhaps the aim and it will produce a more comprehensive analysis if the present papers are reviewed again to discover whether the investigators were trying to produce a positive or neutral result.

Future directions

More accurate estimates of neuroprotective efficacy in transgenic animals and focal ischaemia will be afforded through systematic review and meta-analysis for individual transgenes[18]. Several papers call for urgent evaluation on the contribution of animal studies to clinical medicine[11,19] and systematic reviews and meta-analyses of the existing animal experiments would be an important step forward in this process.

Systemic review uses a methodical approach to minimize the risk of bias in the selection of studies for inclusion, whereas meta-analysis combines results from individual studies to produce a better estimate of treatment effect[12].

The meta-analysis of all the data on transgenic animals and focal ischaemia will provide important information on the effect of study quality and presence of bias on the efficacy of the outcomes. This will go towards improving the study quality of future experiments thus producing outcomes with higher efficacies which is reliable enough to take into clinical trials.

Further meta-analysis on transgenic animals and other models of ischaemic stroke (haemorrhagic and global) will help to elucidate the determinants of efficacy in animal models of stroke.

Conclusion

Stroke ranks No.3 among all causes of death when considered separately from other cardiovascular diseases, behind diseases of the heart and all forms of cancer [1]. It has been a considerable challenge developing safe and effective treatments in experimental and clinical neuroprotection thus it is very important to take the appropriate steps to improve the study quality of animal experiments by systemic review and analysis. This

may with time, help to abolish the misconception that in the case of neuroprotection research, everything works in animals but nothing works on humans. Pound et al have suggested that all animal data should be systemically reviewed before taken into clinical trail. A future meta-analysis of the data on transgenic animals and focal ischaemia will no doubt help to elucidate the pathways which produce higher efficacy and help towards the design of an effective drug and treatment.

Drugs in the past have been taken forward to clinical trials without evidence of superior efficacy in animal models[17], if greater rigour is adopted in the conduct and reporting of animal data from an early stage such as from data on the pathophysiology of stroke, it is more likely that a certain pathway with a superior efficacy will produce a more effective treatment. Using the approach of systematic review and meta-analysis of all animal data, there is every chance that the transition between treatments from the labs to clinical trials will be improved.

(Word count: 2494)

ACKNOWLEDGEMENTS

I would like to thank Emily Sena (ES) for her continuous support on this project and her enormous help with putting the data together and compiling a coherent results table. Without her help and advice, this project would have been impossible to carry out in the limited amount of time that was available. I would also like to thank Dr Malcolm Macleod (MM) for the invaluable information and advice on the importance and the process of systemic reviews and meta-analysis on existing and future animal data as well as his patient approach to the endless questions which were asked by me throughout this project.

REFERENCES

[1] Heart Disease and Stroke Statistics – 2006 update. A report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. AHA Statistical Update

[2] Macleod MR, Sandercock P. Systemic reviews improve clinical research design- can they help improve animal experimental work? RDS News: Winter 2005

[3] Woodman R. Explanations shift attitudes to animal experiments. *BMJ* 1999;318:1438.

[4] Endres M, Dirnagl U. Ischemia and stroke. Adv Exp Med Biol 2002; 513:455-473

[5] Gladstone DJ, black SE, Hakim AM. Toward wisdom from failure: lessons from neuroprotective storke trails and new therapeutic directions. Stroke 2002; 33:2123-2136

[6] Cheng YD, Al-Khoury, Zivin JA. Neuroprotection for ischemic stroke: two decades of success and failure. Neuro Rx. 2004; 1:36-45

[7] Green AR, Odergren T, Ashwood T. Animal models of stroke: do they have value for discovering neuroprotective agents? Trends Pharmacol Sci Vol. 2003; 24: 402-408

[8] Grotta J. Neuroprotection is unlikely to be effective in humans using current trial designs. Stroke 2002; 33: 306-307

[9] Liebeskind DS, Kasner SE. Neuroprotection for ischaemic stroke : an unattainable goal? CNS Drugs Vol. 2001; 15: 165-174

[10] Del Zoppo GJ. Why do all drugs work in animals but none in stroke patients? 1. Drugs promoting cerebral blood flow. J intern Med 1995; 237: 79-88

[11] Pound P, Ebrahim S, Sandercock P, et al. Where is the evidence that animal research benefits humans? Br Med J 2004; 328:514-517

[12] Macleod MR, O'Collins T. Horky LL, Howells DW, Donnan GA. Systemic review and meta-analysis of the efficacy of melatonin in experimental stroke. *Journal of Pineal Research Vol 38 (1) (pp 35-41), 2005* 2005; 35-41

[13] Hunter AJ, Mackay KB, Rogers DC. To what extent have functional studies of iscahaemia in animals been useful in the assessment of potential neuroprotective agents? Trends Pharmacol Sci 1998; 19:59-66

[14] Technology Information, Forecasting & Assessment Council (TIFAC). Transgenic Animal Model Executive Summary, 2003, TMS170. Online at http://www.tifac.org.in/offer/tlbo/rep/TMS170.htm

[15] Petters RM, Sommer JR. Transgenic animals as models for human disease. Kluwer Academic Publishers. *Transgenic Research 9: 347-351, 2000*

[16] Sena E, Wheble P, Sandercock P, Macleod M. Systematic review and meta-analysis of the efficacy of tirilazad in experimental stroke. *Stroke 2007; 38:388*

[17] Macleod MR, O'Collins T, Horky LL, Howells DW, Donnan GA. Systemic review and metaanalysis of the efficacy of FK 506 in experimental stroke. *Journal of Cerebral Blood Flow and Metabolism 2005, 1-9*

[18] O'Collins VE, Macleod MR, Donnan GA, horky LL, van der Worp BH, Howells DW. 1,026 experimental treatments in acute stroke. *Ann Neurol 2006; 59:467-77*

[19] Dirnagl U, Bench to bedside: The quest for quality in experimental stroke research. *Journal of Cerebral Blood Flow and Metabolism.*

Appendix 1

Study Characteristics Table

Pathway	Expression	Author	Year	Sex	Animal	N (C)	N (Rx)	Mode	Anaesthetic	Quality Score	Genotype	Gene	Effectivenes
Angiogenesis													
	KO												
		Asahi,M[1]	2001	Male	Mouse	7	8	Temporary	Halothane	4	unknown	MMP-9	Positive
		Conway,E[2]	2003	Unknown	Mouse	7	7	Permanent	Ketamine	3	heterozygous	Survivin+/-	Neutral
		Copin,J[3]	2005	Unknown	Mouse	5	5	Temporary	Isoflurane	3	Unknown	MMP9	Positive
		Gidday,J[4]	2005	Unknown	Mouse	9	10	Permanent	Halothane	4	Homozygous	MMP-9 -/-	Neutral
		Koistinaho,J[5]	2005	Male	Mouse	5	5	Permanent	Halothane	4	homozygous	MMP-9	Positive
		Sakai,T[6]	2001	Male	Mouse	10	7	Temporary	Halothane	3	homozygous	pFn-null	Positive
Anti-apoptotic													
	KO												
		Dore,S[7]	2000	Male	Mouse	11	10	Temporary	Halothane	4	homozygous	HO2-/-	Positive
		Goto,[8]	2003	Unknown	Mouse	9	7	Temporary	Halothane	4	homozygous	HO2 -/-	Positive
		Hata,R[9]	1999	Unknown	Mouse	5	7	Temporary	Halothane	4	homozygous	bcl-2	Positive
		Lee,E[10]	2001	Unknown	Mouse	15	15	Temporary	Ketamine	4	homozygous	hsp70.1	Positive
		MacManus,J[11]	2003	Unknown	Mouse	4.75	9.5	Temporary	Isoflurane	4	homozygous	E2F1	Neutral
		Martin-Villalba,A[12]	2001	Unknown	Mouse	3	8	Temporary	Ketamine	2	Unknown	gld	Positive
		Siushansian,R[13]	2001	Male	Mouse	12	13	Permanent	Pentobarbital	4	heterozygous	CX43	Neutral
		Taizen,N[14]	2003	Male	Mouse	13	13	Permanent	Unknown	4	heterozygous	CX43	Neutral
	Overexpression												
		Alkayed,N[15]	2001	Female	Mouse	8	8	Temporary	Halothane	4	unknown	bcl-2	Neutral

Pathway	Expression	Author	Year	Sex	Animal	N (C)	N (Rx)	Mode	Anaesthetic	Quality Score	Genotype	Gene	Effectivenes
		DeVries,C[16]	2001	Male	Mouse	5	6	Temporary	Halothane	4	unknown	Bcl-2	Positive
		Lee,E[17]	2001	Male	Mouse	14	13	Permanent	Halothane	4	heterozygous	HSP70	Neutral
		Plumier,J[18]	1997	Unknown	Mouse	5	5	Permanent	Halothane	4	unknown	Hsp-70	Neutral
		Rajdev,S[19]	2000	Male	Mouse	9	12	Permanent	Isoflurane	4	heterozygous	HSP70 tg	Positive
		Tsuchiya,D[20]	2003	Male	Mouse	8	7	Permanent	Isoflurane	4	unknown	HSP70	Positive
		van der Weerd,L[21]	2005	Male	Mouse	7	8	Permanent	Isoflurane	4	homozygous	hsp70	Positive
		Wiessner,C[22]	2006	Male	Mouse	25	20	Permanent	Isoflurane	3	unknown	Bcl-XL	Positive
Antihypertensive													
	KO												
		Walther,T[23]	2002	Female	Mouse	6	6	Permanent	Halothane	3	unknown	AT1	Positive
		Xia,C[24]	2006	Both	Mouse	6	6	Temporary	Isoflurane	4	unknown	Kinin B2	Neutral
Antioxidant													
	KO												
		Crack,P[25]	2006	Male	Mouse	6	6	Temporary	Ketamine	2	homozygous	Gpx1-/-	Positive
		Fujimura,M[26]	2001	Male	Mouse	3	7	Permanent	Isoflurane	4	homozygous	SOD -/-	Neutral
		Keller,J[27]	2000	Unknown	Mouse	6	12	Temporary	Chloral Hydrate	3	homozygous	GPX -/-	Positive
		Kim,G[28]	2001	Male	Mouse	7.5	7.5	Temporary	Isoflurane	4	heterozygous	SOD2 +/-	Positive
		Murakami,K[29]	1998	Male	Mouse	7	8	Permanent	Isoflurane	3	heterozygous	SOD2-/+	Neutral
	Overexpression												
		Crack,P[30]	2003	Unknown	Mouse	1.33	4	Temporary	Ketamine	2	homozygous	SOD 1	Neutral
		Keller,J[31]	1998	Unknown	Mouse	6	6	Temporary	Chloral Hydrate	4	heterozygous	MnSOD	Positive

Pathway	Expression	Author	Year	Sex	Animal	N (C)	N (Rx)	Mode	Anaesthetic	Quality Score	Genotype	Gene	Effectivenes
		Kilic,E[32]	2004	Unknown	Mouse	4.5	4.5	Temporary	Halothane	4	unknown	SOD1 G93 A	Neutral
		Kim,G[33]	2001	Male	Mouse	10	10	Permanent	Isoflurane	4	heterozygous	SOD-1	Positive
		Kinouchi,H[34]	1991	Male	Mouse	15	15	Permanent	Halothane	4	unknown	SOD1	Positive
		Kokubo,Y[35]	2002	Unknown	Mouse	6	7	Temporary	Isoflurane	5	heterozygous	SOD 1	Positive
		Sheng,H[36]	1998	Male	Mouse	19	21	Temporary	Halothane	4	unknown	EC-SOD	Positive
	Overexpression/KO												
		Crack,P[30]	2003	Unknown	Mouse	1.33	4	Temporary	Ketamine	2	homozygous	SOD-1/Gpx-1	Positive
Anti-poptotic													
	KO												
		Rossenbaum,D[37]	2000	Unknown	Mouse	12	15	Permanent	Ketamine	2	Unknown	lpr (loss of	Positive
Calcium													
	KO												
		Chen,Y[38]	1999	Both	Mouse	6	6	Temporary	Halothane	3	Unknown	A2A receptor	Positive
		Connolly,E[39]	1996	Unknown	Mouse	16	18	Temporary	Ketamine	2	homozygous	ICAM-1	Positive
	Overexpression												
		Grilli,M[40]	2000	Unknown	Mouse	18	17	Permanent	Unknown	3	heterozygous	PS1 variant	Neutral
		Mattson,M[41]	2000	Unknown	Mouse	12	12	Temporary	Chloral Hydrate	3	unknown	PS1	Positive
Excitotoxicity													
	KO												
		Bacich,D[42]	2005	Male	Mouse	10.5	10.5	Temporary	Isoflurane	3	homozygous	Folh1-/-	Positive
		Goto,[43]	2002	Male	Mouse	12	9	Temporary	Halothane	4	homozygous	PARP-1	Neutral
		Morikawa,E[44]	1998	Female	Mouse	28	19	Temporary	Halothane	4	heterozygous	GluR e1	Positive

Pathway	Expression	Author	Year	Sex	Animal	N (C)	N (Rx)	Mode	Anaesthetic	Quality Score	Genotype	Gene	Effectivenes
		Namura,S[45]	2002	Unknown	Mouse	9	10	Temporary	Halothane	3	heterozygous	GLT+/-	Neutral
		Parmentier,S[46]	2002	Male	Mouse	2.5	5	Temporary	Isoflurane	4	homozygous	CB1	Positive
	Overexpression												
		Kang,H[47]	2004	Male	Mouse	15	8	Permanent	Chloral Hydrate	3	Unknown	TERT,	Positive
		Le,D[48]	1997	Unknown	Mouse	10	11	Permanent	Isoflurane	4	heterozygous	AMPA	Positive
Genomic Integrity													
	KO												
		Cozzi,A[49]	2006	Male	Mouse	8	8	Permanent	Isoflurane	3	homozygous	PARG110 -/-	Positive
		Jin,G[50]	2005	Male	Mouse	6	6	Temporary	Halothane	4	homozygous	PAR2	Positive
		Kofler,J[51]	2006	Male	Mouse	10	11	Temporary	Halothane	4	homozygous	PAR2 -/-	Positive
Growth Factor													
	KO												
		Bates,B[52]	2002	Unknown	Mouse	10	9	Temporary	Halothane	4	homozygous	NT-3	Positive
		Kiprianova,I[53]	2004	Unknown	Mouse	10	10	Temporary	Halothane	4	homozygous	FGF-2 -/-	Positive
		Tsai,P[54]	2006	Unknown	Mouse	4	5	Permanent	Unknown	1	homozygous	Epo-/-, EpoR-	Neutral
	Overexpression												
		Aoki,Y[55]	2000	Unknown	Mouse	9	9	Temporary	Halothane	3	unknown	nestin-SHP2-	Positive
		Endres,M[56]	2003	Unknown	Mouse	15	15	Temporary	Halothane	4	heterozygous	Bdnf (nt4-ki)	Positive
		Kilic,E[57]	2006	Unknown	Mouse	8	8	Temporary	Halothane	4	unknown	VEGF 165	Positive
		Saarelainen,T[58]	2000	Male	Mouse	5	6	Temporary	Halothane	4	heterozygous	trkB.T1	Positive
hypocholesterole													
	KO												

Pathway	Expression	Author	Year	Sex	Animal	N (C)	N (Rx)	Mode	Anaesthetic	Quality Score	Genotype	Gene	Effectivenes
		Bart,R[59]	1998	Male	Mouse	13	14	Permanent	Halothane	4	homozygous	APOE	Neutral
		Hatcher,J[60]	2002	Male	Mouse	7	7	Permanent	Halothane	3	homozygous	APOE	Neutral
		Kitagawa,K[61]	2001	Male	Mouse	6	6	Permanent	Halothane	4	homozygous	APOE	Neutral
Inflammation													
	KO												
		Arsenijevic,D[62]	2006	Male	Mouse	6	6	Permanent	Ketamine	2	homozygous	PRAPalpha	Neutral
		Arumugam,T[63]	2004	Unknown	Mouse	5	10	Temporary	Ketamine	3	homozygous	LFA-1	Neutral
		Beamer,C[64] Neutral	2006	Both	Mouse	10	10	Permanent	Tribromoethanol	6	homozygous	SHP -1 me/me	
		Bonventre,J[65]	1997	Unknown	Mouse	12	12	Temporary	Halothane	3	homozygous	cPLA2	Positive
		Boutin,H[66]	2001	Male	Mouse	3	9	Temporary	Halothane	4	homozygous	IL-1a, IL-1b,	Neutral
		Cheung,R[67]	2002	Unknown	Mouse	8	11	Permanent	Chloral Hydrate	4	heterozygous	COX 1	Neutral
		Cho,S[68]	2005	Male	Mouse	13	9	Temporary	Isoflurane	4	homozygous	CD36	Positive
		Clark,W[69]	2000	Male	Mouse	7.5	15	Temporary	Halothane	3	Homozygous	IL-6 -/-	Neutral
		Deplanque,D[70]	2003	Male	Mouse	6	6	Temporary	Chloral Hydrate	4	homozygous	APOE	Positive
		Frenkel,D[71]	2005	Female	Mouse	10	9	Temporary	Isoflurane	5	homozygous	IL10-/-	Positive
		Friedlander,R[72]	2006	Both	Mouse	14	11	Permanent	Halothane	6	unknown	ICE (NSE-	Positive
		Grilli,M[73]	2000	Male	Mouse	36	39	Permanent	Tribromoethanol	5	homozygous	IL-10 -/-	Positive
		Groger,M[74]	2005	Male	Mouse	7	7	Temporary	Halothane	4	homozygous	Bradykinin B2	Neutral
		Hermann,D[75]	2003	Both	Mouse	8	9	Temporary	Halothane	3	homozygous	IL-6 (-/-)	Positive
		Hughes,P[76]	2002	Both	Mouse	22	19	Permanent	Isoflurane	4	homozygous	MCP-/-	Positive

Pathway	Expression	Author	Year	Sex	Animal	N (C)	N (Rx)	Mode	Anaesthetic	Quality Score	Genotype	Gene	Effectivenes
		Iadecola,C[77]	2001	Unknown	Mouse	3	7	Permanent	Halothane	3	heterozygous	COX 2	Positive
		Ikeda-Matsuo,Y[78]	2006	Both	Mouse	10	10	Temporary	Halothane	4	homozygous	mPEGS-1	Positive
		Inoue,H[79]	2003	Unknown	Mouse	10	7	Permanent	Halothane	2	Unknown	PPAR alpha	Positive
		Ishikawa,M[80]	2005	Male	Mouse	3.25	6.5	Temporary	Isoflurane	4	homozygous	CD 40 & L	Positive
		Kawano,T[81]	2006	Male	Mouse	6	6	Temporary	Unknown	4	homozygous	EP1 -/-	Positive
		Khanna,S[82]	2005	Male	Mouse	6	6	Temporary	Halothane	3	Unknown	12-	Positive
		Kitagawa,K[83]	2004	Male	Mouse	6	6	Permanent	Halothane	4	homozygous	5-	Neutral
		Komine-Kobayashi,M[84]	2004	Unknown	Mouse	5	5	Temporary	Isoflurane	4	homozygous	FcyR-/-	Positive
		Le Feuvre,R[85]	2003	Male	Mouse	7	7	Temporary	Unknown	3	unknown	P2X7	Positive
		Massberg,S[86]	2005	Male	Mouse	6	6	Temporary	Unknown	2	homozygous	GP Iib -/-	Positive
		McLennan,N[87]	2004	Unknown	Mouse	9.5	17	Permanent	Halothane	3	homozygous	PrP o/o,	Positive
		Nurmi,A[88]	2004	Male	Mouse	9	12	Permanent	Halothane	4	homozygous	p50-/- of NF-kB	Positive
		Ohtaki,H[89]	2003	Male	Mouse	13	12	Temporary	Sevoflurane	4	homozygous	IL-1	Positive
		Pinsky,D[90]	2002	Unknown	Mouse	17	21	Temporary	Unknown	3	homozygous	Cd39	Positive
		Pinteaux,E[91]	2005	Male	Mouse	9	9	Temporary	Halothane	3	homozygous	IL-1ra	Positive
		Prestigiacomo,C[92]	1999	Unknown	Mouse	14	26	Both	Ketamine	3	homozygous	CD18	Neutral
		Rahpeymai,Y[93]	2006	Male	Mouse	7	9	Permanent	Isoflurane	3	homozygous	C3a Receptor	Positive
		Sapirstein,A[94]	2000	Unknown	Mouse	12	12	Temporary	Unknown	2	homozygous	cPLA2	Positive
		Schielke,G[95]	1998	Male	Mouse	5	5	Temporary	Chloral Hydrate	5	homozygous	ICE	Positive

Pathway	Expression	Author	Year	Sex	Animal	N (C)	N (Rx)	Mode	Anaesthetic	Quality Score	Genotype	Gene	Effectivenes
		Schneider,A[96]	1999	Unknown	Mouse	22	15	Temporary	Halothane	3	Unknown	p50	Neutral
		Schroeter,M[97]	2006	Both	Mouse	5	5	Permanent	Enflurane	3	homozygous	OPN-/-	Neutral
		Soriano,S[98]	1999	Male	Mouse	6	9	Temporary	Isoflurane	4	homozygous	P-E selectin -	Neutral
		Wheeler,R[99]	2003	Male	Mouse	9	7	Temporary	Halothane	4	homozygous	IL-18	Neutral
		Zou,L[100]	2006	Both	Mouse	6	6	Permanent	Ketamine	2	heterozygous	COX +/-	Neutral
	Overexpression												
		Chen,Y[101]	2003	Both	Mouse	8.5	8.5	Temporary	Isoflurane	4	unknown	MCP-1	Positive
		Dore,S[102]	2003	Male	Mouse	6	6	Temporary	Halothane	4	unknown	COX 2	Positive
		Koistinaho,J[103]	2002	Male	Mouse	7	7	Permanent	Halothane	4	homozygous	APP751	Positive
		Zhang,Y[104]	2005	Male	Mouse	12	15	Permanent	Avertin	4	unknown	NFkBa	Positive
Ion transporter													
	KO												
		Chen,Y[105]	2005	Unknown	Mouse	3	4	Temporary	Halothane	4	homozygous	NKCC1 -/-,	Positive
		Luo,J[106]	2005	Unknown	Mouse	4	4	Temporary	Halothane	4	heterozygous	NHE +/-	Positive
NO Donor													
	KO												
		Asahi,M[107]	2005	Male	Mouse	10	10	Permanent	Halothane	4	homozygous	eNOS	Neutral
		Atochin,D[108]	2002	Unknown	Mouse	5	10	Permanent	Isoflurane	3	homozygous	eNOS	Neutral
		Chen,Y[109]	2005	Male	Mouse	11	14	Permanent	Halothane	3	homozygous	eNOS	Neutral
		Cho,S[110]	2005	Male	Mouse	7	11	Temporary	Isoflurane	4	homozygous	iNOS	Positive
		Endres,M[111]	2003	Unknown	Mouse	7	5	Temporary	Isoflurane	4	homozygous	eNOS-/-	Neutral

Pathway	Expression	Author	Year	Sex	Animal	N (C)	N (Rx)	Mode	Anaesthetic	Quality Score	Genotype	Gene	Effectivenes
		Gibson,C[112]	2005	Male	Mouse	6	6	Temporary	Isoflurane	4	Homozygous	NOS2-/-	Positive
		Goyagi,T[113]	2001	Male	Mouse	8	8	Temporary	Halothane	4	homozygous	nNOS	Positive
		Hiroi,Y[114]	2006	Unknown	Mouse	6.5	6	Temporary	Unknown	2	homozygous	eNOS	Neutral
		Huang,P[115]	1997	Both	Mouse	10	10	Permanent	Halothane	3	Unknown	eNOS	Neutral
		Iadecola,C[116]	1997	Unknown	Mouse	8	9	Permanent	Halothane	4	homozygous	iNOS	Positive
		Kilic,E[117]	2004	Male	Mouse	6	6	Temporary	Halothane	4	unknown	eNOS	Positive
		McCarter,J[118]	2005	Male	Mouse	13	13	Temporary	Halothane	4	homozygous	nNOS-/-	Positive
		McCullough,L[119]	2005	Male	Mouse	6	6	Temporary	Halothane	4	homozygous	nNOS	Positive
		Namiranian,K[120]	2005	Male	Mouse	12	13	Temporary	Halothane	4	homozygous	nNOS-/-x HO-	Neutral
		Parmentier,S[121]	2006	Male	Mouse	7	7	Temporary	Isoflurane	4	homozygous	iNOS-/-	Positive
	Overexpression/KO												
		Sampei,K[122]	2000	Both	Mouse	10	33	Permanent	Halothane	4	homozygous	nNOS-/-	Positive
Other													
	KO												
		de Bilbao,F[123]	2004	Male	Mouse	5	6	Permanent	Chloral Hydrate	3	homozygous	UCP2-/-	Positive
		Endres,M[124]	2000	Unknown	Mouse	5	4	Temporary	Halothane	4	heterozygous	DNA -methylt	Neutral
		Holschneider,D[125]	1999	Male	Mouse	9	8	Permanent	Halothane	4	unknown	MAO-B	Neutral
		Olsson,T[126]	2004	Male	Mouse	5	10	Temporary	Halothane	4	homozygous	Cystatin C	Positive
	Overexpression												
		Mattiasson,G[127]	2003	Male	Mouse	9	9	Temporary	Halothane	5	unknown	UCP -2/3	Positive
		Miyashita,K[128]	2006	Unknown	Mouse	9	9	Temporary	Halothane	4	unknown	AM-Tg,	Neutral

Pathway	Expression	Author	Year	Sex	Animal	N (C)	N (Rx)	Mode	Anaesthetic	Quality Score	Genotype	Gene	Effectivenes
Oxygen regulation													
	KO												
		Kitano,H[129]	2004	Unknown	Mouse	6	6	Temporary	Halothane	3	heterozygous	ORP150	Positive
	Overexpression												
		Tamatani,M[130]	2001	Unknown	Mouse	12	12	Permanent	Unknown	2	unknown	ORP150	Positive
Pro-Apoptotic													
	KO												
		Chae,H[131]	2004	Unknown	Mouse	6	7	Temporary	Unknown	1	homozygous	BAX inhibitor-1	Positive
		Iadecola,C[132]	1999	Unknown	Mouse	6	5	Permanent	Halothane	4	Heterozygous	IRF-1 +/-	Positive
		Le,D[133]	2002	Unknown	Mouse	9	7	Temporary	Unknown	3	homozygous	caspase-3	Positive
		Nakase,T[134]	2004	Unknown	Mouse	10	10	Permanent	Pentobarbital	4	heterozygous	Cx43	Neutral
		Plesnila,N[135]	2001	Unknown	Mouse	8	8	Temporary	Unknown	1	homozygous	Bid	Neutral
		Schinzel,A[136]	2005	Male	Mouse	4.5	9	Temporary	Unknown	3	Unknown	CypD	Neutral
	Overexpression												
		Arumugam,T[137]	2006	Male	Mouse	12	7	Temporary	Isoflurane	4	unknown	antisense	Positive
		Kerr,L[138]	2004	Male	Mouse	8	9	Temporary	Unknown	2	Unknown	caspase 3	Positive
Protein Kinase													
	KO												
		Aronowski,J[139]	2000	Both	Mouse	12	10	Temporary	Chloral Hydrate	4	homozygous	v PKC	Neutral
		Brecht,S[140]	2005	Male	Mouse	8	8	Permanent	Chloral Hydrate	4	homozygous	JNK1	Positive
		Chou,W[141]	2004	Both	Mouse	8	8	Permenent	Isoflurane	4	homozygous	PKC delta -	Neutral
		Hermann,D[142]	2005	Female	Mouse	20	20	Permanent	Tribromoethanol	5	unknown	IKK2	Positive

Pathway	Expression	Author	Year	Sex	Animal	N (C)	N (Rx)	Mode	Anaesthetic	Quality Score	Genotype	Gene	Effectivenes
		Im,J[143]	2003	Unknown	Mouse	17	14	Temporary	Ketamine	3	homozygous	jip1 -/-	Positive
		Paul,R[144]	2001	Male	Mouse	3.5	7	Permanent	Halothane	3	heterozygous	Src+/-	Neutral
	Overexpression												
		Herrmann,O[142]	2005	Female	Mouse	6	6	Permanent	Tribromoethanol	5	unknown	IKK2 (DN)	Positive
		Ohba,N[145]	2004	Unknown	Mouse	6	5	Permanent	Halothane	4	heterozygous	Akt	Neutral
Serine Protease													
	KO												
		Junge,C[146]	2003	Male	Mouse	31	23	Temporary	Isoflurane	4	homozygous	PAR1 -/-	Positive
		Scarff,K[147]	2004	Unknown	Mouse	9	6	Permanent	Unknown	2	homozygous	SPI3	Neutral
Sex Steroid													
	KO												
		Dubal,D[148]	2001	Unknown	Mouse	7	13	Permanent	Chloral Hydrate	4	homozygous	Eralpha/beta	Neutral
		Hayashi,S[149]	2005	Male	Mouse	6	5	Temporary	Halothane	4	unknown	gamma PKC	Positive
		McCullough,L[150]	2003	Female	Mouse	11	9	Temporary	Halothane	4	homozygous	Aromatase	Positive
		Sampei,K[151]	2000	Female	Mouse	9	9	Temporary	Halothane	4	unknown	ER alpha	Neutral
Thrombolytic													
	KO												
		Kleinschnitz,C[152]	2006	Unknown	Mouse	18	18	Temporary	Unknown	3	homozygous	FX II -/-	Positive
		Nagai,N[153]	1999	Both	Mouse	2.2	11	Permanent	Ketamine	3	homozygous	tPA-/-	Neutral
		Tsuji,K[154]	2005	Male	Mouse	6	8	Temporary	Halothane	4	unknown	tPA	Positive
	Overexpression												
		Nagai,N[155]	2005	Male	Mouse	7	8	Permanent	Isoflurane	4	unknown	PAI-1	Positive

Pathway	Expression	Author	Year	Sex	Animal	N (C)	N (Rx)	Mode	Anaesthetic	Quality Score	Genotype	Gene	Effectivenes
Water regulation													
	KO												
		Manley,G[156]	2000	Male	Mouse	7	7	Permanent	Isoflurane	4	homozygous	AQP4	Positive
	Overexpression												
		Lo,E[157]	2005	Male	Mouse	14	10	Temporary	Halothane	4	homozygous	ET-1 (GET-1)	Positive

Reference List

1. *Asahi M, Wang X, Mori T, Sumii T, Jung JC, Moskowitz MA et al. Effects of matrix metalloproteinase-9 gene knock-out on the proteolysis of blood-brain barrier and white matter components after cerebral ischemia. J.Neurosci. 2001;21:7724-32.*

2. *Conway EM, Zwerts F, Van E, V, DeVriese A, Nagai N, Luo W et al. Survivin-dependent angiogenesis in ischemic brain: molecular mechanisms of hypoxia-induced up-regulation. Am.J.Pathol. 2003;163:935-46.*

3. *Copin JC, Goodyear MC, Gidday JM, Shah AR, Gascon E, Dayer A et al. Role of matrix metalloproteinases in apoptosis after transient focal cerebral ischemia in rats and mice. Eur.J.Neurosci. 2005;22:1597-608.*

4. *Gidday JM, Gasche YG, Copin JC, Shah AR, Perez RS, Shapiro SD et al. Leukocyte-derived matrix metalloproteinase-9 mediates blood-brain barrier breakdown and is proinflammatory after transient focal cerebral ischemia. Am.J.Physiol Heart Circ.Physiol. 2005;289:H558-H568.*

5. *Koistinaho M, Malm TM, Kettunen MI, Goldsteins G, Starckx S, Kauppinen RA et al. Minocycline protects against permanent cerebral ischemia in wild type but not in matrix metalloprotease-9-deficient mice. J.Cereb.Blood Flow Metab. 2005;25:460-7.*

6. *Sakai T, Johnson KJ, Murozono M, Sakai K, Magnuson MA, Wieloch T et al. Plasma fibronectin supports neuronal survival and reduces brain injury following transient focal cerebral ischemia but is not essential for skin-wound healing and hemostasis. Nat.Med. 2001;7:324-30.*

7. *Dore S, Goto S, Sampei K, Blackshaw S, Hester LD, Ingi T et al. Heme oxygenase-2 acts to prevent neuronal death in brain cultures and following transient cerebral ischemia. Neuroscience. 2000;99:587-92.*

8. *Goto S, Sampei K, Alkayed NJ, Dore S, Koehler RC. Characterization of a new double-filament model of focal cerebral ischemia in heme oxygenase-2-deficient mice. Am.J.Physiol Regul.Integr.Comp Physiol. 2003;285:R222-R230.*

9. *Hata R, Gillardon F, Michaelidis TM, Hossmann KA. Targeted disruption of the bcl-2 gene in mice exacerbates focal ischemic brain injury. Metab Brain Dis. 1999;14:117-24.*

10. *Lee SH, Kim M, Yoon BW, Kim YJ, Ma SJ, Roh JK et al. Targeted hsp70.1 disruption increases infarction volume after focal cerebral ischemia in mice. Stroke. 2001;32:2905-12.*

11. *MacManus JP, Jian M, Preston E, Rasquinha I, Webster J, Zurakowski B. Absence of the transcription factor E2F1 attenuates brain injury and improves behavior after focal ischemia in mice. J.Cereb.Blood Flow Metab. 2003;23:1020-8.*

12. *Martin-Villalba A, Hahne M, Kleber S, Vogel J, Falk W, Schenkel J et al. Therapeutic neutralization of CD95-ligand and TNF attenuates brain damage in stroke. Cell Death Differ. 2001;8:679-86.*

13. *Siushansian R, Bechberger JF, Cechetto DF, Hachinski VC, Naus CC. Connexin43 null mutation increases infarct size after stroke. J.Comp Neurol. 2001;440:387-94.*

14. *Nakase T, Fushiki S, Naus CC. Astrocytic gap junctions composed of connexin 43 reduce apoptotic neuronal damage in cerebral ischemia. Stroke 2003;34:1987-93.*

15. *Alkayed NJ, Goto S, Sugo N, Joh HD, Klaus J, Crain BJ et al. Estrogen and Bcl-2: Gene induction and effect of transgene in experimental stroke. Journal of Neuroscience 2001;21:7543-50.*

16. *DeVries AC, Joh HD, Bernard O, Hattori K, Hurn PD, Traystman RJ et al. Social stress exacerbates stroke outcome by suppressing Bcl-2 expression. Proc.Natl.Acad.Sci.U.S A. 2001;98:11824-8.*

17. *Lee JE, Yenari MA, Sun GH, Xu L, Emond MR, Cheng D et al. Differential neuroprotection from human heat shock protein 70 overexpression in in vitro and in vivo models of ischemia and ischemia-like conditions. Exp.Neurol. 2001;170:129-39.*

18. *Plumier JC, Krueger AM, Currie RW, Kontoyiannis D, Kollias G, Pagoulatos GN. Transgenic mice expressing the human inducible Hsp70 have hippocampal neurons resistant to ischemic injury. Cell Stress Chaperones. 1997;2:162-7.*

19. *Rajdev S, Hara K, Kokubo Y, Mestril R, Dillmann W, Weinstein PR et al. Mice overexpressing rat heat shock protein 70 are protected against cerebral infarction. Ann.Neurol. 2000;47:782-91.*

20. *Tsuchiya D, Hong S, Matsumori Y, Shiina H, Kayama T, Swanson RA et al. Overexpression of rat heat shock protein 70 is associated with reduction of early mitochondrial cytochrome C release and subsequent DNA fragmentation after permanent focal ischemia. J.Cereb.Blood Flow Metab. 2003;23:718-27.*

21. *van der WL, Lythgoe MF, Badin RA, Valentim LM, Akbar MT, de Belleroche JS et al. Neuroprotective effects of HSP70 overexpression after cerebral ischaemia--an MRI study. Exp.Neurol. 2005;195:257-66.*

22. *Wiessner C, Allegrini PR, Rupalla K, Sauer D, Oltersdorf T, McGregor AL et al. Neuron-specific transgene expression of Bcl-XL but not Bcl-2 genes reduced lesion size after permanent middle cerebral artery occlusion in mice. Neurosci.Lett. 1999;268:119-22.*

23. *Walther T, Olah L, Harms C, Maul B, Bader M, Hortnagl H et al. Ischemic injury in experimental stroke depends on angiotensin II. FASEB J. 2002;16:169-76.*

24. *Xia CF, Smith RSJ, Shen B, Yang R, Borlongan CV, Chao L et al. Postischemic brain injury is exacerbated in mice lacking the kinin B2 receptor. Hypertension (Baltimore) 2006;47:752-61.*

25. *Crack PJ, Taylor JM, Ali U, Mansell A, Hertzog PJ. Potential contribution of NF-kappaB in neuronal cell death in the glutathione peroxidase-1 knockout mouse in response to ischemia-reperfusion injury. Stroke. 2006;37:1533-8.*

26. *Fujimura M, Morita-Fujimura Y, Copin J, Yoshimoto T, Chan PH. Reduction of copper, zinc-superoxide dismutase in knockout mice does not affect edema or infarction volumes and the early release of mitochondrial cytochrome c after permanent focal cerebral ischemia. Brain Res. 2001;%19;889:208-13.*

27. *Keller JN, Huang FF, Zhu H, Yu J, Ho YS, Kindy TS. Oxidative stress-associated impairment of proteasome activity during ischemia-reperfusion injury. J.Cereb.Blood Flow Metab. 2000;20:1467-73.*

28. *Kim GW, Kondo T, Noshita N, Chan PH. Manganese superoxide dismutase deficiency exacerbates cerebral infarction after focal cerebral ischemia/reperfusion in mice: implications for the production and role of superoxide radicals. Stroke. 2002;33:809-15.*

29. *Murakami K, Kondo T, Kawase M, Li Y, Sato S, Chen SF et al. Mitochondrial susceptibility to oxidative stress exacerbates cerebral infarction that follows permanent focal cerebral ischemia in mutant mice with manganese superoxide dismutase deficiency. J.Neurosci. 1998;18:205-13.*

30. *Crack PJ, Taylor JM, De Haan JB, Kola I, Hertzog P, Iannello RC. Glutathione peroxidase-1 contributes to the neuroprotection seen in the superoxide dismutase-1 transgenic mouse in response to ischemia/reperfusion injury. J.Cereb.Blood Flow Metab. 2003;23:19-22.*

31. *Keller JN, Kindy MS, Holtsberg FW, St Clair DK, Yen HC, Germeyer A et al. Mitochondrial manganese superoxide dismutase prevents neural apoptosis and reduces ischemic brain injury: suppression of peroxynitrite production, lipid peroxidation, and mitochondrial dysfunction. J.Neurosci. 1998;18:687-97.*

32. *Kilic E, Weishaupt JH, Kilic U, Rohde G, Yulug B, Peters K et al. The superoxide dismutase1 (SOD1) G93A mutation does not promote neuronal injury after focal brain ischemia and optic nerve transection in mice. Neuroscience. 2004;128:359-64.*

33. *Kim GW, Lewen A, Copin J, Watson BD, Chan PH. The cytosolic antioxidant, copper/zinc superoxide dismutase, attenuates blood-brain barrier disruption and oxidative cellular injury after photothrombotic cortical ischemia in mice. Neuroscience. 2001;105:1007-18.*

34. *Kinouchi H, Epstein CJ, Mizui T, Carlson E, Chen SF, Chan PH. Attenuation of focal cerebral ischemic injury in transgenic mice overexpressing CuZn superoxide dismutase. Proc.Natl.Acad.Sci.U.S.A. 1991;88:11158-62.*

35. *Kokubo Y, Matson GB, Derugin N, Hill T, Mancuso A, Chan PH et al. Transgenic mice expressing human copper-zinc superoxide dismutase exhibit attenuated apparent diffusion coefficient reduction during reperfusion following focal cerebral ischemia. Brain Res. 2002;947:1-8.*

36. *Sheng H, Bart RD, Oury TD, Pearlstein RD, Crapo JD, Warner DS. Mice overexpressing extracellular superoxide dismutase have increased resistance to focal cerebral ischemia. Neuroscience. 1999;88:185-91.*

37. *Rosenbaum DM, Gupta G, D'Amore J, Singh M, Weidenheim K, Zhang H et al. Fas (CD95/APO-1) plays a role in the pathophysiology of focal cerebral ischemia. Journal of Neuroscience Research.Vol.61(6)()(pp 686-692), 2000.Date of Publication: 15 SEP 2000. 2000;686-92.*

38. *Chen JF, Huang Z, Ma J, Zhu J, Moratalla R, Standaert D et al. A(2A) adenosine receptor deficiency attenuates brain injury induced by transient focal ischemia in mice. J.Neurosci. 1999;19:9192-200.*

39. *Connolly ES, Jr., Winfree CJ, Springer TA, Naka Y, Liao H, Yan SD et al. Cerebral protection in homozygous null ICAM-1 mice after middle cerebral artery occlusion. Role of neutrophil adhesion in the pathogenesis of stroke. J.Clin.Invest. 1996;97:209-16.*

40. *Grilli M, Diodato E, Lozza G, Brusa R, Casarini M, Uberti D et al. Presenilin-1 regulates the neuronal threshold to excitotoxicity both physiologically and pathologically. Proc.Natl.Acad.Sci.U.S.A. 2000;97:12822-7.*

41. *Mattson MP, Zhu H, Yu J, Kindy MS. Presenilin-1 mutation increases neuronal vulnerability to focal ischemia in vivo and to hypoxia and glucose deprivation in cell culture: Involvement of perturbed calcium homeostasis. Journal of Neuroscience.Vol.20(4)()(pp 1358-1364), 2000.Date of Publication: 15 FEB 2000. 2000;1358-64.*

42. *Bacich DJ, Wozniak KM, Lu XC, O'Keefe DS, Callizot N, Heston WD et al. Mice lacking glutamate carboxypeptidase II are protected from peripheral neuropathy and ischemic brain injury. J.Neurochem. 2005;95:314-23.*

43. *Goto S, Xue R, Sugo N, Sawada M, Blizzard KK, Poitras MF et al. Poly(ADP-ribose) polymerase impairs early and long-term experimental stroke recovery. Stroke. 2002;33:1101-6.*

44. *Morikawa E, Mori H, Kiyama Y, Mishina M, Asano T, Kirino T. Attenuation of focal ischemic brain injury in mice deficient in the epsilon1 (NR2A) subunit of NMDA receptor. J.Neurosci. 1998;18:9727-32.*

45. *Namura S, Maeno H, Takami S, Jiang XF, Kamichi S, Wada K et al. Inhibition of glial glutamate transporter GLT-1 augments brain edema after transient focal cerebral ischemia in mice. Neurosci.Lett. 2002;324:117-20.*

46. *Parmentier-Batteur S, Jin K, Mao XO, Xie L, Greenberg DA. Increased severity of stroke in CB1 cannabinoid receptor knock-out mice. J.Neurosci. 2002;22:9771-5.*

47. *Kang HJ, Choi YS, Hong SB, Kim KW, Woo RS, Won SJ et al. Ectopic expression of the catalytic subunit of telomerase protects against brain injury resulting from ischemia and NMDA-induced neurotoxicity. J.Neurosci. 2004;24:1280-7.*

48. *Le D, Das S, Wang YF, Yoshizawa T, Sasaki YF, Takasu M et al. Enhanced neuronal death from focal ischemia in AMPA-receptor transgenic mice. Brain Res.Mol.Brain Res. 1997;52:235-41.*

49. *Cozzi A, Cipriani G, Fossati S, Faraco G, Formentini L, Min W et al. Poly(ADP-ribose) accumulation and enhancement of postischemic brain damage in 110-kDa poly(ADP-ribose) glycohydrolase null mice. J.Cereb.Blood Flow Metab. 2006;26:684-95.*

50. *Jin G, Hayashi T, Kawagoe J, Takizawa T, Nagata T, Nagano I et al. Deficiency of PAR-2 gene increases acute focal ischemic brain injury. J.Cereb.Blood Flow Metab. 2005;25:302-13.*

51. *Kofler J, Otsuka T, Zhang Z, Noppens R, Grafe MR, Koh DW et al. Differential effect of PARP-2 deletion on brain injury after focal and global cerebral ischemia. J.Cereb.Blood Flow Metab. 2006;26:135-41.*

52. *Bates B, Hirt L, Thomas SS, Akbarian S, Le D, Amin-Hanjani S et al. Neurotrophin-3 promotes cell death induced in cerebral ischemia, oxygen-glucose deprivation, and oxidative stress: Possible involvement of oxygen free radicals. Neurobiology of Disease.Vol.9(1)()(pp 24-37), 2002. 2002;24-37.*

53. *Kiprianova I, Schindowski K, Bohlen und HO, Krause S, Dono R, Schwaninger M et al. Enlarged infarct volume and loss of BDNF mRNA induction following brain ischemia in mice lacking FGF-2. Exp.Neurol. 2004;189:252-60.*

54. *Tsai PT, Ohab JJ, Kertesz N, Groszer M, Matter C, Gao J et al. A critical role of erythropoietin receptor in neurogenesis and post-stroke recovery. J.Neurosci. 2006;26:1269-74.*

55. *Aoki Y, Huang Z, Thomas SS, Bhide PG, Huang I, Moskowitz MA et al. Increased susceptibility to ischemia-induced brain damage in transgenic mice overexpressing a dominant negative form of SHP2. FASEB J. 2000;14:1965-73.*

56. *Endres M, Fan G, Hirt L, Jaenisch R. Stroke damage in mice after knocking the neutrophin-4 gene into the brain-derived neurotrophic factor locus. J.Cereb.Blood Flow Metab. 2003;23:150-3.*

57. *Kilic E, Kilic U, Wang Y, Bassetti CL, Marti HH, Hermann DM. The phosphatidylinositol-3 kinase/Akt pathway mediates VEGF's neuroprotective activity and induces blood brain barrier permeability after focal cerebral ischemia. FASEB J. 2006;20:1185-7.*

58. *Saarelainen T, Lukkarinen JA, Koponen S, Grohn OH, Jolkkonen J, Koponen E et al. Transgenic mice overexpressing truncated trkB neurotrophin receptors in neurons show increased susceptibility to cortical injury after focal cerebral ischemia. Mol.Cell Neurosci. 2000;16:87-96.*

59. *Bart RD, Sheng H, Laskowitz DT, Pearlstein RD, Warner DS. Regional CBF in apolipoprotein E-deficient and wild type mice during focal cerebral ischemia. Neuroreport. 1998;9:2615-20.*

60. *Hatcher JP, Virley D, Hadingham SJ, Roberts J, Hunter AJ, Parsons AA. The behavioural effect of middle cerebral artery occlusion on apolipoprotein-E deficient mice. Behav.Brain Res. 2002;131:139-49.*

61. *Kitagawa K, Matsumoto M, Kuwabara K, Ohtsuki T, Hori M. Delayed, but marked, expression of apolipoprotein E is involved in tissue clearance after cerebral infarction. J.Cereb.Blood Flow Metab. 2001;21:1199-207.*

62. *Arsenijevic D, de Bilbao F, Plamondon J, Paradis E, Vallet P, Richard D et al. Increased infarct size and lack of hyperphagic response after focal cerebral ischemia in peroxisome proliferator-activated receptor beta-deficient mice. J.Cereb.Blood Flow Metab. 2006;26:433-45.*

63. *Arumugam TV, Salter JW, Chidlow JH, Ballantyne CM, Kevil CG, Granger DN. Contributions of LFA-1 and Mac-1 to brain injury and microvascular dysfunction induced by transient middle cerebral artery occlusion. American Journal of Physiology - Heart & Circulatory Physiology.Vol.287(6 56-6)()(pp H2555-H2560), 2004. 2004;H2555-H2560.*

64. *Beamer CA, Brooks DM, Lurie DI. Motheaten (me/me) mice deficient in SHP-1 are less susceptible to focal cerebral ischemia. J.Neurosci.Res. 2006;83:1220-30.*

65. *Bonventre JV, Huang Z, Taheri MR, O'Leary E, Li E, Moskowitz MA et al. Reduced fertility and postischaemic brain injury in mice deficient in cytosolic phospholipase A2. Nature. 1997;390:622-5.*

66. *Boutin H, LeFeuvre RA, Horai R, Asano M, Iwakura Y, Rothwell NJ. Role of IL-1alpha and IL-1beta in ischemic brain damage. J.Neurosci. 2001;21:5528-34.*

67. *Cheung RT, Pei Z, Feng ZH, Zou LY. Cyclooxygenase-1 gene knockout does not alter middle cerebral artery occlusion in a mouse stroke model. Neurosci.Lett. 2002;330:57-60.*

68. *Cho S, Park EM, Febbraio M, Anrather J, Park L, Racchumi G et al. The class B scavenger receptor CD36 mediates free radical production and tissue injury in cerebral ischemia. J.Neurosci. 2005;25:2504-12.*

69. *Clark WM, Rinker LG, Lessov NS, Hazel K, Hill JK, Stenzel-Poore M et al. Lack of interleukin-6 expression is not protective against focal central nervous system ischemia. Stroke. 2000;31:1715-20.*

70. *Deplanque D, Gele P, Petrault O, Six I, Furman C, Bouly M et al. Peroxisome proliferator-activated receptor-alpha activation as a mechanism of preventive neuroprotection induced by chronic fenofibrate treatment. J.Neurosci. 2003;23:6264-71.*

71. *Frenkel D, Huang Z, Maron R, Koldzic DN, Moskowitz MA, Weiner HL. Neuroprotection by IL-10-producing MOG CD4+ T cells following ischemic stroke. J.Neurol.Sci. 2005;233:125-32.*

72. *Friedlander RM, Gagliardini V, Hara H, Fink KB, Li W, Macdonald G et al. Expression of a dominant negative mutant of interleukin-1 beta converting enzyme in transgenic mice prevents neuronal cell death induced by trophic factor withdrawal and ischemic brain injury. J.Exp.Med. 1997;185:933-40.*

73. *Grilli M, Barbieri I, Basudev H, Brusa R, Casati C, Lozza G et al. Interleukin-10 modulates neuronal threshold of vulnerability to ischaemic damage. Eur.J.Neurosci. 2000;12:2265-72.*

74. *Groger M, Lebesgue D, Pruneau D, Relton J, Kim SW, Nussberger J et al. Release of bradykinin and expression of kinin B2 receptors in the brain: role for cell death and brain edema formation after focal cerebral ischemia in mice. J.Cereb.Blood Flow Metab. 2005;25:978-89.*

75. *Herrmann O, Tarabin V, Suzuki S, Attigah N, Coserea I, Schneider A et al. Regulation of body temperature and neuroprotection by endogenous interleukin-6 in cerebral ischemia. Journal of Cerebral Blood Flow & Metabolism 2003;23:406-15.*

76. *Hughes PM, Allegrini PR, Rudin M, Perry VH, Mir AK, Wiessner C. Monocyte chemoattractant protein-1 deficiency is protective in a murine stroke model. J.Cereb.Blood Flow Metab. 2002;22:308-17.*

77. *Iadecola C, Niwa K, Nogawa S, Zhao X, Nagayama M, Araki E et al. Reduced susceptibility to ischemic brain injury and N-methyl-D-aspartate-mediated neurotoxicity in cyclooxygenase-2-deficient mice. Proc.Natl.Acad.Sci.U.S.A. 2001;98:1294-9.*

78. *Ikeda-Matsuo Y, Ota A, Fukada T, Uematsu S, Akira S, Sasaki Y. Microsomal prostaglandin E synthase-1 is a critical factor of stroke-reperfusion injury. Proceedings of the National Academy of Sciences of the United States of America.Vol.103(31)()(pp 11790-11795), 2006.Date of Publication: 01 AUG 2006. 2006;11790-5.*

79. *Inoue H, Jiang XF, Katayama T, Osada S, Umesono K, Namura S. Brain protection by resveratrol and fenofibrate against stroke requires peroxisome proliferator-activated receptor alpha in mice. Neurosci.Lett. 2003;352:203-6.*

80. *Ishikawa M, Vowinkel T, Stokes KY, Arumugam TV, Yilmaz G, Nanda A et al. CD40/CD40 ligand signaling in mouse cerebral microvasculature after focal ischemia/reperfusion. Circulation. 2005;111:1690-6.*

81. *Kawano T, Anrather J, Zhou P, Park L, Wang G, Frys KA et al. Prostaglandin E2 EP1 receptors: downstream effectors of COX-2 neurotoxicity. Nat.Med. 2006;12:225-9.*

82. *Khanna S, Roy S, Slivka A, Craft TK, Chaki S, Rink C et al. Neuroprotective properties of the natural vitamin E alpha-tocotrienol. Stroke. 2005;36:2258-64.*

83. *Kitagawa K, Matsumoto M, Hori M. Cerebral ischemia in 5-lipoxygenase knockout mice. Brain Res. 2004;1004:198-202.*

84. *Komine-Kobayashi M, Chou N, Mochizuki H, Nakao A, Mizuno Y, Urabe T. Dual role of Fcgamma receptor in transient focal cerebral ischemia in mice. Stroke. 2004;35:958-63.*

85. *Le Feuvre RA, Brough D, Touzani O, Rothwell NJ. Role of P2X7 receptors in ischemic and excitotoxic brain injury in vivo. J.Cereb.Blood Flow Metab. 2003;23:381-4.*

86. *Massberg S, Schurzinger K, Lorenz M, Konrad I, Schulz C, Plesnila N et al. Platelet adhesion via glycoprotein IIb integrin is critical for atheroprogression and focal cerebral ischemia: An in vivo study in mice lacking glycoprotein IIb. Circulation.Vol.112(8)()(pp 1180-1188), 2005.Date of Publication: 23 AUG 2005. 2005;1180-8.*

87. *McLennan NF, Brennan PM, McNeill A, Davies I, Fotheringham A, Rennison KA et al. Prion protein accumulation and neuroprotection in hypoxic brain damage. Am.J.Pathol. 2004;165:227-35.*

88. *Nurmi A, Lindsberg PJ, Koistinaho M, Zhang W, Juettler E, Karjalainen-Lindsberg ML et al. Nuclear factor-kappaB contributes to infarction after permanent focal ischemia. Stroke. 2004;35:987-91.*

89. *Ohtaki H, Funahashi H, Dohi K, Oguro T, Horai R, Asano M et al. Suppression of oxidative neuronal damage after transient middle cerebral artery occlusion in mice lacking interleukin-1. Neurosci.Res. 2003;45:313-24.*

90. *Pinsky DJ, Broekman MJ, Peschon JJ, Stocking KL, Fujita T, Ramasamy R et al. Elucidation of the thromboregulatory role of CD39/ectoapyrase in the ischemic brain. J.Clin.Invest. 2002;109:1031-40.*

91. *Pinteaux E, Rothwell NJ, Boutin H. Neuroprotective actions of endogenous interleukin-1 receptor antagonist (IL-1ra) are mediated by glia. Glia. 2006;53:551-6.*

92. *Prestigiacomo CJ, Kim SC, Connolly ES, Jr., Liao H, Yan SF, Pinsky DJ. CD18-mediated neutrophil recruitment contributes to the pathogenesis of reperfused but not nonreperfused stroke. Stroke. 1999;30:1110-7.*

93. *Rahpeymai Y, Hietala MA, Wilhelmsson U, Fotheringham A, Davies I, Nilsson A-K et al. Complement: A novel factor in basal and ischemia-induced neurogenesis. EMBO Journal.Vol.25(6)()(pp 1364-1374), 2006.Date of Publication: 22 MAR 2006. 2006;1364-74.*

94. *Sapirstein A,.Bonventre JV. Phospholipases A2 in ischemic and toxic brain injury. Neurochem.Res. 2000;25:745-53.*

95. *Schielke GP, Yang G-Y, Shivers BD, Betz AL. Reduced ischemic brain injury in interleukin-1beta converting enzyme- deficient mice. Journal of Cerebral Blood Flow & Metabolism.Vol.18(2)()(pp 180-185), 1998. 1998;180-5.*

96. *Schneider A, Martin-Villalba A, Weih F, Vogel J, Wirth T, Schwaninger M. NF-kappaB is activated and promotes cell death in focal, cerebral ischemia. Nature Medicine.Vol.5(5)()(pp 554-559), 1999. 1999;554-9.*

97. *Schroeter M, Zickler P, Denhardt DT, Hartung HP, Jander S. Increased thalamic neurodegeneration following ischaemic cortical stroke in osteopontin-deficient mice. Brain. 2006;129:1426-37.*

98. *Soriano SG, Wang YF, Wagner DD, Frenette PS. P- and E-selectin-deficient mice are susceptible to cerebral ischemia-reperfusion injury. Brain Res. 1999;835:360-4.*

99. *Wheeler RD, Boutin H, Touzani O, Luheshi GN, Takeda K, Rothwell NJ. No role for interleukin-18 in acute murine stroke-induced brain injury. Journal of Cerebral Blood Flow & Metabolism.Vol.23(5)()(pp 531-535), 2003.Date of Publication: 01 MAY 2003. 2003;531-5.*

100. *Liang YZ, Cheung RTF, Liu S, Li G, Huang L. Melatonin reduces infarction volume in a photothrombotic stroke model in the wild-type but not cyclooxygenase-1-gene knockout mice. Journal of Pineal Research.Vol.41(2)()(pp 150-156), 2006. 2006;150-6.*

101. *Chen Y, Hallenbeck JM, Ruetzler C, Bol D, Thomas K, Berman NE et al. Overexpression of monocyte chemoattractant protein 1 in the brain exacerbates ischemic brain injury and is associated with recruitment of inflammatory cells. J.Cereb.Blood Flow Metab. 2003;23:748-55.*

102. *Dore S, Otsuka T, Mito T, Sugo N, Hand T, Wu L et al. Neuronal overexpression of cyclooxygenase-2 increases cerebral infarction. Ann.Neurol. 2003;54:155-62.*

103. *Koistinaho M, Kettunen MI, Goldsteins G, Keinanen R, Salminen A, Ort M et al. Beta-amyloid precursor protein transgenic mice that harbor diffuse A beta deposits but do not form plaques show increased ischemic vulnerability: role of inflammation. Proc.Natl.Acad.Sci.U.S.A. 2002;99:1610-5.*

104. *Zhang W, Potrovita I, Tarabin V, Herrmann O, Beer V, Weih F et al. Neuronal activation of NF-kappaB contributes to cell death in cerebral ischemia. J.Cereb.Blood Flow Metab. 2005;25:30-40.*

105. *Chen H, Luo J, Kintner DB, Shull GE, Sun D. Na(+)-dependent chloride transporter (NKCC1)-null mice exhibit less gray and white matter damage after focal cerebral ischemia. J.Cereb.Blood Flow Metab. 2005;25:54-66.*

106. *Luo J, Chen H, Kintner DB, Shull GE, Sun D. Decreased neuronal death in Na+/H+ exchanger isoform 1-null mice after in vitro and in vivo ischemia. J.Neurosci. 2005;25:11256-68.*

107. *Asahi M, Huang Z, Thomas S, Yoshimura S, Sumii T, Mori T et al. Protective effects of statins involving both eNOS and tPA in focal cerebral ischemia. J.Cereb.Blood Flow Metab. 2005;25:722-9.*

108. *Atochin DN, Clark J, Demchenko IT, Moskowitz MA, Huang PL. Rapid cerebral ischemic preconditioning in mice deficient in endothelial and neuronal nitric oxide synthases. Stroke. 2003;34:1299-303.*

109. *Chen J, Zacharek A, Zhang C, Jiang H, Li Y, Roberts C et al. Endothelial nitric oxide synthase regulates brain-derived neurotrophic factor expression and neurogenesis after stroke in mice. J.Neurosci. 2005;25:2366-75.*

110. *Cho S, Park EM, Zhou P, Frys K, Ross ME, Iadecola C. Obligatory role of inducible nitric oxide synthase in ischemic preconditioning. J.Cereb.Blood Flow Metab. 2005;25:493-501.*

111. *Endres M, Gertz K, Lindauer U, Katchanov J, Schultze J, Schrock H et al. Mechanisms of stroke protection by physical activity. Ann.Neurol. 2003;54:582-90.*

112. *Gibson CL, Constantin D, Prior MJ, Bath PM, Murphy SP. Progesterone suppresses the inflammatory response and nitric oxide synthase-2 expression following cerebral ischemia. Exp.Neurol. 2005;193:522-30.*

113. *Goyagi T, Goto S, Bhardwaj A, Dawson VL, Hurn PD, Kirsch JR. Neuroprotective effect of sigma(1)-receptor ligand 4-phenyl-1-(4-phenylbutyl) piperidine (PPBP) is linked to reduced neuronal nitric oxide production. Stroke. 2001;32:1613-20.*

114. *Hiroi Y, Kim H-H, Ying H, Furuya F, Huang Z, Simoncini T et al. Rapid nongenomic actions of thyroid hormone. Proceedings of the National Academy of Sciences of the United States of America.Vol.103(38)()(pp 14104-14109), 2006.Date of Publication: 19 SEP 2006. 2006;14104-9.*

115. *Huang Z, Chen K, Huang PL, Finklestein SP, Moskowitz MA. bFGF ameliorates focal ischemic injury by blood flow-independent mechanisms in eNOS mutant mice. Am.J.Physiol. 1997;272:H1401-H1405.*

116. *Iadecola C, Zhang F, Casey R, Nagayama M, Ross ME. Delayed reduction of ischemic brain injury and neurological deficits in mice lacking the inducible nitric oxide synthase gene. J.Neurosci. 1997;17:9157-64.*

117. *Kilic E, Kilic U, Matter CM, Luscher TF, Bassetti CL, Hermann DM. Aggravation of focal cerebral ischemia by tissue plasminogen activator is reversed by 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor but does not depend on endothelial NO synthase. Stroke. 2005;36:332-6.*

118. *McCullough LD, Zeng Z, Blizzard KK, Debchoudhury I, Hurn PD. Ischemic nitric oxide and poly (ADP-ribose) polymerase-1 in cerebral ischemia: Male toxicity, female protection. Journal of Cerebral Blood Flow & Metabolism.Vol.25(4)()(pp 502-512), 2005. 2005;502-12.*

119. *McCullough LD, Zeng Z, Li H, Landree LE, McFadden J, Ronnett GV. Pharmacological inhibition of AMP-activated protein kinase provides neuroprotection in stroke. J.Biol.Chem. 2005;280:20493-502.*

120. *Namiranian K, Koehler RC, Sapirstein A, Dore S. Stroke outcomes in mice lacking the genes for neuronal heme oxygenase-2 and nitric oxide synthase. Curr.Neurovasc.Res. 2005;2:23-7.*

121. *Park EM, Cho S, Frys KA, Glickstein SB, Zhou P, Anrather J et al. Inducible nitric oxide synthase contributes to gender differences in ischemic brain injury. J.Cereb.Blood Flow Metab. 2006;26:392-401.*

122. *Sampei K, Mandir AS, Asano Y, Wong PC, Traystman RJ, Dawson VL et al. Stroke outcome in double-mutant antioxidant transgenic mice. Stroke. 2000;31:2685-91.*

123. *de Bilbao F, Arsenijevic D, Vallet P, Hjelle OP, Ottersen OP, Bouras C et al. Resistance to cerebral ischemic injury in UCP2 knockout mice: evidence for a role of UCP2 as a regulator of mitochondrial glutathione levels. J.Neurochem. 2004;89:1283-92.*

124. *Endres M, Meisel A, Biniszkiewicz D, Namura S, Prass K, Ruscher K et al. DNA methyltransferase contributes to delayed ischemic brain injury. J.Neurosci. 2000;20:3175-81.*

125. *Holschneider DP, Scremin OU, Huynh L, Chen K, Shih JC. Lack of protection from ischemic injury of monoamine oxidase B-deficient mice following middle cerebral artery occlusion. Neurosci.Lett. 1999;259:161-4.*

126. *Olsson T, Nygren J, Hakansson K, Lundblad C, Grubb A, Smith ML et al. Gene deletion of cystatin C aggravates brain damage following focal ischemia but mitigates the neuronal injury after global ischemia in the mouse. Neuroscience. 2004;128:65-71.*

127. *Mattiasson G, Shamloo M, Gido G, Mathi K, Tomasevic G, Yi S et al. Uncoupling protein-2 prevents neuronal death and diminishes brain dysfunction after stroke and brain trauma. Nat.Med. 2003;9:1062-8.*

128. *Miyashita K, Itoh H, Arai H, Suganami T, Sawada N, Fukunaga Y et al. The neuroprotective and vasculo-neuro-regenerative roles of adrenomedullin in ischemic brain and its therapeutic potential. Endocrinology. 2006;147:1642-53.*

129. *Kitano H, Nishimura H, Tachibana H, Yoshikawa H, Matsuyama T. ORP150 ameliorates ischemia/reperfusion injury from middle cerebral artery occlusion in mouse brain. Brain Res. 2004;1015:122-8.*

130. *Tamatani M, Matsuyama T, Yamaguchi A, Mitsuda N, Tsukamoto Y, Taniguchi M et al. ORP150 protects against hypoxia/ischemia-induced neuronal death. Nature Medicine.Vol.7(3)()(pp 317-323), 2001. 2001;317-23.*

131. *Chae HJ, Kim HR, Xu C, Bailly-Maitre B, Krajewska M, Krajewski S et al. BI-1 regulates an apoptosis pathway linked to endoplasmic reticulum stress. Mol.Cell. 2004;15:355-66.*

132. *Iadecola C, Salkowski CA, Zhang F, Aber T, Nagayama M, Vogel SN et al. The transcription factor interferon regulatory factor 1 is expressed after cerebral ischemia and contributes to ischemic brain injury. J.Exp.Med. 1999;189:719-27.*

133. *Le DA, Wu Y, Huang Z, Matsushita K, Plesnila N, Augustinack JC et al. Caspase activation and neuroprotection in caspase-3- deficient mice after in vivo cerebral ischemia and in vitro oxygen glucose deprivation. Proc.Natl.Acad.Sci.U.S.A. 2002;99:15188-93.*

134. *Nakase T, Sohl G, Theis M, Willecke K, Naus CC. Increased apoptosis and inflammation after focal brain ischemia in mice lacking connexin43 in astrocytes. Am.J.Pathol. 2004;164:2067-75.*

135. *Plesnila N, Zinkel S, Le DA, Amin-Hanjani S, Wu Y, Qiu J et al. BID mediates neuronal cell death after oxygen/ glucose deprivation and focal cerebral ischemia. Proc.Natl.Acad.Sci.U.S.A. 2001;98:15318-23.*

136. *Schinzel AC, Takeuchi O, Huang Z, Fisher JK, Zhou Z, Rubens J et al. Cyclophilin D is a component of mitochondrial permeability transition and mediates neuronal cell death after focal cerebral ischemia. Proc.Natl.Acad.Sci.U.S.A. 2005;102:12005-10.*

137. *Arumugam TV, Chan SL, Jo DG, Yilmaz G, Tang SC, Cheng A et al. Gamma secretase-mediated Notch signaling worsens brain damage and functional outcome in ischemic stroke. Nat.Med. 2006;12:621-3.*

138. *Kerr LE, McGregor AL, Amet LE, Asada T, Spratt C, Allsopp TE et al. Mice overexpressing human caspase 3 appear phenotypically normal but exhibit increased apoptosis and larger lesion volumes in response to transient focal cerebral ischaemia. Cell Death Differ. 2004;11:1102-11.*

139. *Aronowski J, Grotta JC, Strong R, Waxham MN. Interplay between the gamma isoform of PKC and calcineurin in regulation of vulnerability to focal cerebral ischemia. J.Cereb.Blood Flow Metab. 2000;20:343-9.*

140. *Brecht S, Kirchhof R, Chromik A, Willesen M, Nicolaus T, Raivich G et al. Specific pathophysiological functions of JNK isoforms in the brain. Eur.J.Neurosci. 2005;21:363-77.*

141. *Chou WH, Choi DS, Zhang H, Mu D, McMahon T, Kharazia VN et al. Neutrophil protein kinase Cdelta as a mediator of stroke-reperfusion injury. J.Clin.Invest. 2004;114:49-56.*

142. *Herrmann O, Baumann B, de Lorenzi R, Muhammad S, Zhang W, Kleesiek J et al. IKK mediates ischemia-induced neuronal death. Nat.Med. 2005;11:1322-9.*

143. *Im JY, Lee KW, Kim MH, Lee SH, Ha HY, Cho IH et al. Repression of phospho-JNK and infarct volume in ischemic brain of JIP1-deficient mice. J.Neurosci.Res. 2003;74:326-32.*

144. *Paul R, Zhang ZG, Eliceiri BP, Jiang Q, Boccia AD, Zhang RL et al. Src deficiency or blockade of Src activity in mice provides cerebral protection following stroke. Nat.Med. 2001;7:222-7.*

145. *Ohba N, Kiryu-Seo S, Maeda M, Muraoka M, Ishii M, Kiyama H. Transgenic mouse overexpressing the Akt reduced the volume of infarct area after middle cerebral artery occlusion. Neurosci.Lett. 2004;359:159-62.*

146. *Junge CE, Sugawara T, Mannaioni G, Alagarsamy S, Conn PJ, Brat DJ et al. The contribution of protease-activated receptor 1 to neuronal damage caused by transient focal cerebral ischemia. Proc.Natl.Acad.Sci.U.S.A. 2003;100:13019-24.*

147. *Scarff KL, Ung KS, Nandurkar H, Crack PJ, Bird CH, Bird PI. Targeted disruption of SPI3/Serpinb6 does not result in developmental or growth defects, leukocyte dysfunction, or susceptibility to stroke. Mol.Cell Biol. 2004;24:4075-82.*

148. *Dubal DB, Zhu H, Yu J, Rau SW, Shughrue PJ, Merchenthaler I et al. Estrogen receptor alpha, not beta, is a critical link in estradiol-mediated protection against brain injury. Proc.Natl.Acad.Sci.U.S.A. 2001;98:1952-7.*

149. *Hayashi S, Ueyama T, Kajimoto T, Yagi K, Kohmura E, Saito N. Involvement of gamma protein kinase C in estrogen-induced neuroprotection against focal brain ischemia through G protein-coupled estrogen receptor. J.Neurochem. 2005;93:883-91.*

150. *McCullough LD, Blizzard K, Simpson ER, Oz OK, Hurn PD. Aromatase cytochrome P450 and extragonadal estrogen play a role in ischemic neuroprotection. J.Neurosci. 2003;23:8701-5.*

151. *Sampei K, Goto S, Alkayed NJ, Crain BJ, Korach KS, Traystman RJ et al. Stroke in estrogen receptor-alpha-deficient mice. Stroke. 2000;31:738-43.*

152. *Kleinschnitz C, Stoll G, Bendszus M, Schuh K, Pauer HU, Burfeind P et al. Targeting coagulation factor XII provides protection from pathological thrombosis in cerebral ischemia without interfering with hemostasis. J.Exp.Med. 2006;%20;203:513-8.*

153. *Nagai N, De Mol M, Lijnen HR, Carmeliet P, Collen D. Role of plasminogen system components in focal cerebral ischemic infarction: a gene targeting and gene transfer study in mice. Circulation. 1999;99:2440-4.*

154. *Tsuji K, Aoki T, Tejima E, Arai K, Lee SR, Atochin DN et al. Tissue plasminogen activator promotes matrix metalloproteinase-9 upregulation after focal cerebral ischemia. Stroke. 2005;36:1954-9.*

155. *Nagai N, Suzuki Y, Van Hoef B, Lijnen HR, Collen D. Effects of plasminogen activator inhibitor-1 on ischemic brain injury in permanent and thrombotic middle cerebral artery occlusion models in mice. J.Thromb.Haemost. 2005;3:1379-84.*

156. *Manley GT, Fujimura M, Ma T, Noshita N, Filiz F, Bollen AW et al. Aquaporin-4 deletion in mice reduces brain edema after acute water intoxication and ischemic stroke. Nat.Med. 2000;6:159-63.*

157. *Lo AC, Chen AY, Hung VK, Yaw LP, Fung MK, Ho MC et al. Endothelin-1 overexpression leads to further water accumulation and brain edema after middle cerebral artery occlusion via aquaporin 4 expression in astrocytic end-feet. J.Cereb.Blood Flow Metab. 2005;25:998-1011.*

Personal Review and Self-Assessment

Project Objectives:

Systemic analysis of preclinical efficacy data for candidate stroke drugs suggests an important confounding effect of bias caused by aspects of study design. Using a similar approach, I will seek to determine whether these same factors confound data from transgenic studies investigating stroke pathophysiology by collecting all the publications found on transgenic animals and focal cerebral ischaemia. Aspects of the study design from the 432 papers found were entered into a database and these data are to be systemically reviewed and meta-analysed to find evidence of bias in the reported efficacy of the outcomes.

Personal objective: Having not taken an BSc and with no experience of any literature research, I hoped to gained the skills of carrying out a literature review. I also hope to improve my communication skills with my peers and learn to work in a small group where I can express my opinions. I hoped to learn how to keep calm when problems arose and work under pressure. A literature review engages different computer skills which I would not have used before and it will be good to gain the ability to use programmes such as Microsoft Access for future studies.

Project Review

As it has become clear that with the failure of many clinical trials on neuroprotective agents despite being developed from studies which yielded high efficacy data, evaluation on the study quality and sources of bias is needed on the existing and future animal data before a certain drug is taken into trial. This project addressed this issue by looking at the potential sources of bias in the pathophysiology of stroke which contributes to the study and development of potential neuroprotective drugs.

1504 references were found initially from electronic searching and it was extremely time consuming and mind-numbing to sieve through each abstract to find the 432 publications which were relevant to transgenic animals and focal ischaemia.

432 is a huge number of papers to read in 14 weeks and I spent as much time as I could trying to get through the large amount of papers. In the time that was given with the addition of the 2 weeks of Christmas holidays, I managed to go through 238 papers of which 157 qualified for analysis. Reviewing papers and entering the relevant data was

also time consuming and become quite dull at times but the thought that once all the data has been collected will hopefully help to improve the study quality of future animal experiments gave me incentive to keep going.

For the 4 weeks of the course which were dedicated to doing this SSC project, I also went through a leg operation which meant I was housebound for a month. However, with the project being a systemic review, it was possible to read the papers from home. I found this period the most difficult because when problems arose, being at home made it harder for them to be solved. Due to the vast numbers of papers that needed to be reviewed, I had to do most of them during the Christmas Holidays and found it very difficult not being able to walk and doing papers thus not having much of a holiday. Despite working throughout the holidays, I could not get through 432 papers and it has meant that a meta-analysis could not be carried out which was disappointing. However, I am going to go back to Dr Macleod's office in June and finish the rest of the papers, carry out a meta-analysis without the presence of bias and hopefully find results that are significant enough to get a publication.

Project Review - Doing this project has been an extremely valuable learning experience because I did not undertake a BSc thus did not have the opportunity to carry out any literature reviews. I learnt how to find appropriate papers online which will be extremely valuable to me in the future and the experience of working in an office on a specific project with the aim of producing a publication is very different from the other aspects of the medical degree.

Working with my supervisor Dr Macleod and his pHD student Emily helped me to improve my communication skills as I learnt to be less afraid of asking questions and I enjoyed the experience of working in a group with a smaller number of people as I found it less intimidating to express my own opinions.

Due to the vast numbers of papers which needed to be reviewed, I found a strict discipline, especially during the holidays, whilst carrying out this project which will be useful to my studies in the future. I've learned various computing skills which I would not have otherwise and because I needed to manage my time with the rest of the course, gaining the ability to be well-organised was vital. I have also gained an additional interest in this area of stroke research and understand more about how papers are systematically reviewed and meta-analysed.

Estimation of grade

a) **performance:** A/B
b) **written report:** B
c) **overall grade:** A/B

Although I did put in a lot of time and effort into this project, due to the vast numbers of papers that needed to be reviewed, I was not able to carry out a meta-analysis. This meant I could only use simple statistics to describe my results which was disappointing and I hope to finish reviewing the rest of the papers in the summer.