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ORIGINAL RESEARCH ARTICLE

Isoflurane preconditioning induced genomic changes in mouse cortex

Umeshkumar Athiraman[1,2,*] and Tusar Giri[1]

[1]Department of Anesthesiology, Washington University, St. Louis, MO, USA and [2]Department of Neurological Surgery, Washington University, St. Louis, MO, USA

*Corresponding author. E-mail: uathira@wustl.edu

Abstract

Background: Altered patterns of genetic expression induced by isoflurane preconditioning in mouse brain have not yet been investigated. The aim of our pilot study is to examine the temporal sequence of changes in the transcriptome of mouse brain cortex produced by isoflurane preconditioning.

Methods: Twelve-wk-old wild-type (C57BL/6J) male mice were randomly assigned for the experiments. Mice were exposed to isoflurane 2% in air for 1 h and brains were harvested at the following time points—immediately (0 h), and at 6, 12, 24, 36, 48, and 72 h after isoflurane exposure. A separate cohort of mice were exposed to three doses of isoflurane on days 1, 2, and 3 and brains were harvested after the third exposure. The NanoString mouse neuropathology panel was used to analyse isoflurane-induced gene expression in the cortex. The neuropathology panel included 760 genes covering pathways involved in neurodegeneration and other nervous system diseases, and 10 internal reference genes for data normalisation.

Results: Genes involving several pathways were upregulated and downregulated by isoflurane preconditioning. Interestingly, a biphasic response was noted, meaning, an early expression of genes (until 6 h), followed by a transient pause (until 24 h), and a second wave of genomic response beginning at 36 h of isoflurane exposure was noted.

Conclusions: Isoflurane preconditioning induces significant alterations in the genes involved in neurodegeneration and other nervous system disorders in a temporal sequence. These data could aid in the identification of molecular mechanisms behind isoflurane preconditioning-induced neuroprotection in various central nervous system diseases.

Keywords: genomics; isoflurane preconditioning; mouse cortex; nervous system disorders; neurodegeneration

Conditioning is a strategy where a sub-lethal noxious stimulus presented to any tissue in the body invokes endogenous protective mechanisms so that the organ develops tolerance towards a subsequent similar or stronger insult.[1,2] It is not only powerful, but also remarkably pleiotropic, as conditioning strategies have highly protective effects on all major cell types of the central nervous system including neurones, glia, and vascular cells.[1,3–5] The adaptive responses induced by conditioning involve molecular sensors and transducers, transcription factors, genes, and effectors that ultimately produce a protective phenotype.[1,2] Conditioning can be applied before or after the insult, and referred to as preconditioning or postconditioning, respectively. Several distinct conditioning agents have been shown to provide protection in a variety of cerebral disorders through conditioning-based mechanisms.[6–11] Specifically, volatile anaesthetics (such as isoflurane, sevoflurane, and desflurane) have been shown to induce strong neuroprotection against several nervous system disorders.[12–16] These volatile anaesthetics are FDA approved, have an excellent safety profile in humans, and are used in millions of patients on a regular basis. Therefore, understanding the molecular foundations of volatile anaesthetic conditioning will identify new molecular targets for drug development and heighten the translational potential of anaesthetic conditioning-based therapeutics.

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Intriguingly, several preclinical studies in various brain injury models have demonstrated the neuroprotective effects of anaesthetic conditioning in mature adult brains, but have shown opposite effects in developing and ageing brains.[17] Although several studies have begun to elucidate the molecular mechanisms of the preconditioning response, comprehensive mechanisms underlying anaesthetic preconditioning-induced neuroprotection have not yet been explored. In this study, we examine the impact of a volatile anaesthetic, isoflurane, on genomic expression in mature adult mouse cortex with the aim of identifying the genes involved in isoflurane preconditioning-induced neuroprotection. To achieve this, we utilised a mouse neuropathology panel from NanoString, Seattle, Washington, 98109, which includes 760 genes covering the pathways involved in neurodegeneration and other nervous system diseases.

Methods

All experiments in the study were approved by the Washington University in Saint Louis animal care and use committee. Twelve-wk-old C57BL/6J adult male mice (20–30 g) were obtained from the Jackson Laboratory (Bar Harbor, ME, USA). Mice were housed in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) accredited facility in a 12-hr dark–light cycle with controlled temperature and humidity. Five mice were placed in each cage with unlimited access to food and water. A total of 45 mice were used in the experiments with n=5 in each group. Animals were randomly allocated to each experimental group. The experimental groups were a control group (mice exposed to room air for 1 h), and an isoflurane group (mice were exposed to isoflurane 2% with room air for 1 h). After isoflurane exposure, cortical tissues were harvested at different time points, immediately (0 h), and at 6, 12, 24, 36, 48, and 72 h later. Immediately after the tissue extraction, the sample was placed on dry ice and stored at −80°C until used for analysis. A further group of mice was exposed to three doses of isoflurane 2%, 24 h apart to examine the impact of repetitive exposure of isoflurane on genomic expression. The cortical tissue in this group was harvested immediately after the last dose of isoflurane exposure. The cerebral cortex was chosen for the genomic analysis as it plays a crucial role in almost all the higher functions of brain.

Isoflurane preconditioning

A medium sized anaesthetic induction chamber (Smiths Medical, Dublin, OH, USA) was used to administer isoflurane. Isoflurane preconditioning was accomplished by exposing spontaneously breathing mice to isoflurane 2% with air for 1 h. The control groups were placed in the same chamber and exposed to air, but not the isoflurane, for 1 h. A homeothermic blanket was utilised (HTP-1500 heat therapy pump, Kent Scientific Corporation, CT, Torrington, USA, accuracy; plus or minus 2°F) to maintain normal temperature throughout the anaesthetic exposure. Isoflurane and carbon dioxide concentrations in the induction chamber were measured using an anaesthetic gas analyser (Capnomac Ultima, Datex Ohmeda, Louisville, Kentucky, USA). The dose of isoflurane 2% was chosen for this study, as it has been used safely in several experimental studies examining the impact of isoflurane conditioning-induced neuroprotection.[7,13,14]

RNA extraction

Total RNA was prepared from cerebral cortex using the RNeasy mini kit (cat# 74104, Qiagen Inc., Germantown, Maryland), following the manufacturer's instruction. In brief, the brain tissue was homogenised in RLT buffer containing 2-mercaptoethanol (1:100 dilution, Sigma Aldrich Inc., Missouri, United States), using a Bullet blender (model, Storm 24, Next Advance Inc., Troy, NY) and an RNase-free bead lysis kit (Navy 1.5 ml RINO). After centrifugation at 12 000×g at +4°C for 5 min, the supernatant was transferred to a QIA shredder (Qiagen Inc.) column and centrifuged at maximum speed for 3 min at room temperature (RT). The flow-through was collected and RNA was precipitated with the addition of an equal volume of ethanol 70% and mixed with gentle pipetting. The mixture was transferred to an RNeasy Mini spin column and centrifuged at <9000×g for 15 s, RT. On-column DNase digestion was done using an RNase-free DNase set (cat# 79254, Qiagen Inc.), following the manufacturer's instructions. Following RNA clean-up steps, the RNA was eluted in RNeasy-free water. The RNA concentration for each sample was measured using a NanoDrop 2000, and all RNA samples were diluted to a final concentration of 20 ng μl^{-1} in RNeasy-free water and stored immediately at −80°C for future NanoString analysis.

NanoString nCounter® mouse neuropathology gene expression analysis and statistics

The nCounter® Neuropathology panel was selected in particular to examine the effects of isoflurane on the specific gene sets involving neurodegenerative diseases. This specific panel provides comprehensive assessment of neurodegenerative pathways and processes, and also in profiling the abundance of important neural cell types. nCounter® gene expression analysis from the mouse Neuropathology panel was performed with nSolver 4.0 and Advanced Analysis package 2.0 (NanoString Technologies) according to the recommended procedures (NanoString User Manual C0019-08 and 1003-03) and as previously described.[18] Heatmaps for raw data show the level of raw gene expression for each sample in the data set. Raw counts were normalised to the geometric mean of the selected housekeeping genes followed by log2 transformation. For normalised data heatmaps, samples are plotted by z-score and clustered by sample. All statistical analyses were performed on log2 transformed normalised counts. Differential gene expression analysis was performed for control *vs* different isoflurane treatment groups (0, 12, 24, 36, 48, 72, repetitive 72 h). Adjustment of the significance threshold was done using a Benjamini-Yekutieli correction to maintain or control the false discovery rate (FDR) below the 0.05 threshold.[19] It is important to note that when multiple hypotheses are tested, FDR is a very useful measure of accuracy compared with false positive rate (FPR) as FDR considers all the hypotheses being tested rather than measuring each metric independently. Global significance scores were calculated as the square root of the mean squared t-statistic for the genes in a gene set, with t-statistics coming from the linear regression underlying the differential expression analysis. The directed global significance score is calculated as the square root of the mean signed squared t-statistic for the genes in a gene set, with t-statistics coming from the linear regression underlying the differential expression analysis. Heatmaps of global significance scores used unsupervised hierarchical clustering of genes and samples that were scaled by gene or score.

Results

Isoflurane administration affected a range of genes involved in neurodegeneration and other nervous system disorders. Out of 760 genes in the neuropathology panel, 76 unique transcripts were differentially expressed (compared with control) after a brief single exposure to isoflurane 2%. Out of those 76 genes, 62 were upregulated and 14 were downregulated. During the early phase (0, 6 h), isoflurane exposure caused the differential expression of 33 genes (five genes overlapped between 0 h and 6 h groups, so 28 unique genes were expressed), and during the late phase (36, 48, 72 h), 73 genes were differentially expressed (25 genes overlapped between 36 h, 48 h, and 72 h groups, so 48 unique genes expressed). Out of the 76 unique genes expressed, only eight genes overlapped between the early and late phase. There were six unique genes that appeared in the repetitive isoflurane exposure group compared with the other single time isoflurane exposure groups.

Heatmaps of the raw and normalised data generate a series of high-level plots that describe the data overall and are useful for identifying anomalous data, covariates, or both. Figure 1a is generated from raw data and allows identification of samples and gene sets with low signal. The blue bar labelled below threshold on the left indicates probes the counts of which have fallen below threshold in all samples. The heatmap (Fig. 1a) shows that the data set with low raw counts (<25) are minimal, indicating that the overall data are highly reliable and reproducible.

Differential expression of genes and volcano plot

The genes which appeared to be significant by adjusted *P*-value after correcting for FDR are listed in the Supplementary material. Each figure represents genes differentially expressed by isoflurane at different time points compared with the control group. All the genes and the corresponding pathways were summarised into six themes as following: (1) compartmentalisation and structural integrity; (2) metabolism; (3) neuroinflammation; (4) neurone–glia interaction; (5) neuroplasticity, development, and ageing; and (6) neurotransmission. A significant expression of genes during the early phase (0 and 6 h), with a transient pause (12 and 24 h), followed by a second peak of gene expression (36, 48, and 74 h) was noticed. The complete list of genes differentially expressed by isoflurane exposure compared with control at various time points is given in the Supplementary material.

Global significance scores

The undirected global significance score (Fig. 2) shows that genes are extensively differentially expressed at 0, 48, 72, and repeat 72 h groups and a limited gene expression was noted at 6, 12, 24, and 36 h after isoflurane preconditioning. The direct global significance score indicates that the majority of gene sets were downregulated during the initial few hours of isoflurane preconditioning (0, 6 h).

Discussion

Examining the time-based impact of isoflurane-induced genomic changes reveals several interesting findings. (1) Isoflurane exposure applied for a brief period (2% for 1 h) induced significant gene expression changes in the mouse cortex that lasted for at least 72 h. (2) A temporal sequence of isoflurane-induced genomic changes was noted. An immediate early change in expression of genes (0, 6 h), followed by a quiescent phase (12, 24 h), and an eventual late change in expression of genes (36, 48, 72 h) was observed. (3) The genes differentially expressed after exposure to isoflurane during the early phase (0, 6 h), were largely distinct from the genes differentially expressed during the late phase (36, 48, 72 h), indicating the possibility that the noted genomic modifications were secondary changes induced by isoflurane rather than the primary effect of the anaesthetic itself. 4) Repetitive exposure to isoflurane (isoflurane 2% for 1 h, three doses, 24 h apart) caused the differential expression of a distinct set of genes compared with a single time isoflurane exposure group, at the examined 72-h time point.

A single brief exposure of isoflurane (2% for 1 h) resulted in the altered appearance of transcripts that persisted for at least 3 days. These genes were involved in a wide array of processes including compartmentalisation and structural integrity, metabolism, neuroinflammation, neurone–glia interaction, neuroplasticity, development and ageing, and neurotransmission. During the early phase (mainly at 0 h), though isoflurane altered expression of genes in all the above-mentioned themes, it was more evident with the neuroplasticity of the brain during development and ageing (upregulated—*Ddit3*, *Pik3r1*, *Col4a2*, *Col4a1*, *Ncl*, *Tnfrsf12a*, *Gata2*, *Efna1*, *AcvrI1*, *bid*; downregulated—*Bdnf*, *Egr1*). Specifically, biological roles of these genes are connected with the pathways including growth factors, angiogenesis, chromatin modification, and apoptosis. During the late phase (mainly at 48 h), the effect of isoflurane was more evident on compartmentalisation and structural integrity (upregulated—*Lrp1*, *Mtor*, *Cacna1b*, *Grin1*, *Dlg4*, *Fus*, *Htt*, *Grik2*, *Dnm2*, *Rims1*, *Hdac6*, *Cacna1c*, *Unc13a*; downregulated—*clu*), metabolism (upregulated—*Atp1312*, *Smpd4*, *Mtor*, *Park2*, *Gga1*, *Atrn*, *Taz*, *Pdgfrb*, *Tcerg1*, *Akt1s1*, *Dnm2*, *Trpm2*, *Atxn2*, *Htra2*, *Hdac6*, *Tcirg1*, *Man2b1*; downregulated—*Atf4*), neuroplasticity, development and ageing (upregulated—*Cacna1b*, *Flt1*, *Inpp4a*, *Notch3*, *Cspg4*, *Col4a2*, *Pdgfrb*, *Atxn7*, *Egfl7*, *Nelfa*, *Hdac7*, *Htra2*, *Ring1*, *Hdac6*, *Cacna1c*, *Col4a1*, *Sirt7*, *Cacna1d*; downregulated—*Cdkn1a*, *Tnfrsf12a*, *Atf4*, *Clu*), and neurotransmission (upregulated—*Cacna1b*, *Grin1*, *Dlg4*, *Flt1*, *Ryr2*, *Tnr*, *Pdgfrb*, *Grik2*, *Dnm2*, *Rims1*, *Trpm2*, *Ryr1*, *Cacna1c*, *Tcirg1*, *Cacna1d*, *Unc13a*; downregulated—*Atf4*) pathways. Interestingly, isoflurane exposure significantly altered the expression of genes at a later time point compared with the early phase and these genes are involved in several pathways controlling biological processes such as tissue integrity, neuronal connectivity and cytoskeleton, axon and dendrite structure, lipid and carbohydrate metabolism, autophagy, oxidative stress, unfolded protein response, transcription and mRNA splicing, transmitter synthesis, storage, reuptake and release, vesicular trafficking, growth factors, angiogenesis, chromatin modification and apoptosis (nanostring.com/neuroscience/neuropathology/).

The above-mentioned findings may have wider implications for translational science research, as the majority of experimental studies are conducted using isoflurane anaesthesia and the ability of isoflurane to affect several pathways after a brief exposure may potentially influence the results of the study. Supporting this notion, numerous studies have implicated a protective role of isoflurane preconditioning in several *in vivo* and *in vitro* brain injury models, including ischaemic stroke, spinal cord injury, hypoxic ischaemic brain injury, and oxygen glucose deprivation injury models.[12–17] Interestingly, Gaidhani and colleagues,[20] using a common

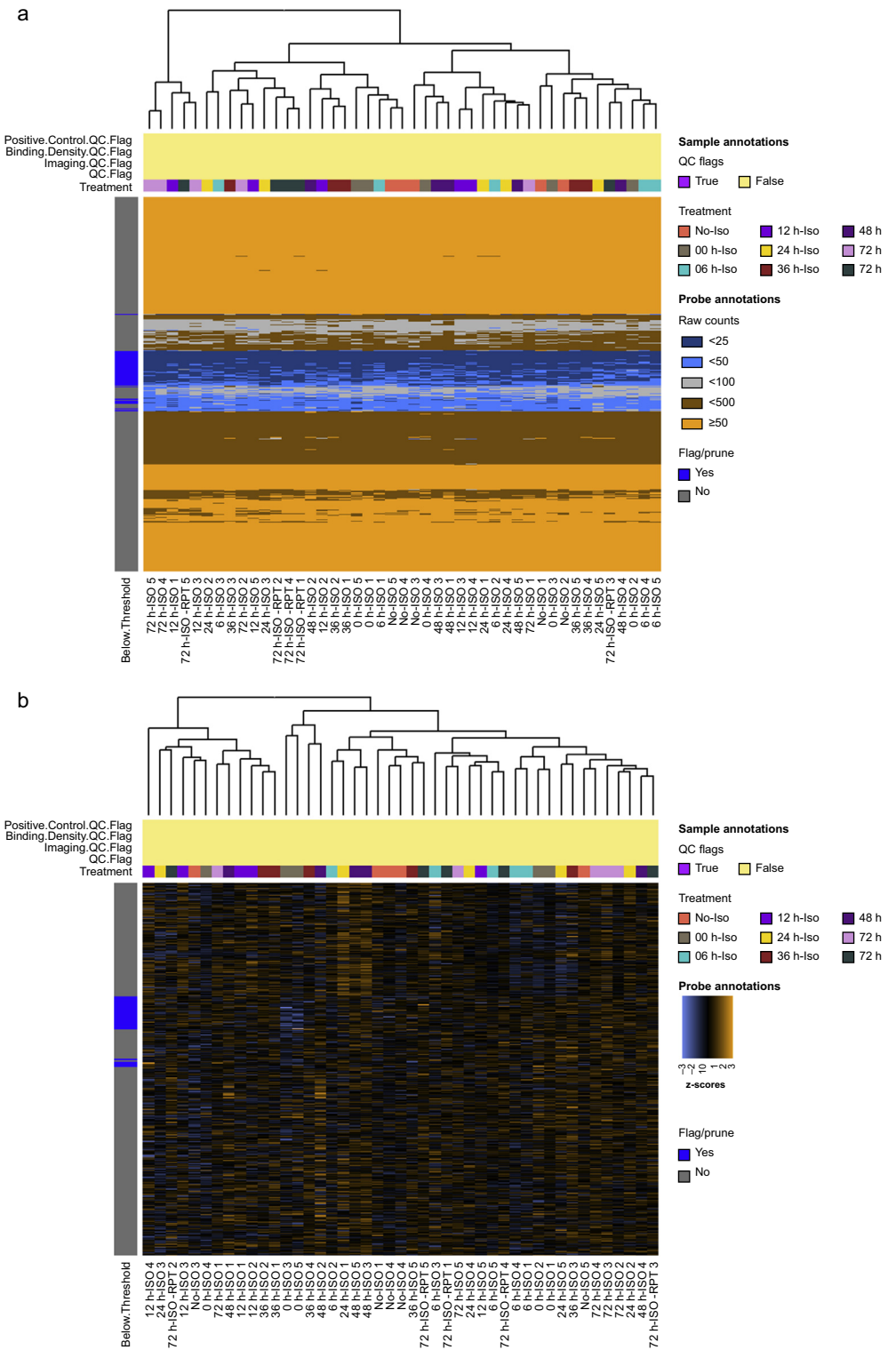

Fig 1. Heatmap of raw and normalised data. (a) The plot provides an overview of robustness of the raw expression levels across samples and gene sets. Each row of the heatmap is a single probe, and each column is a single sample. Coloured horizontal bars along the top of the plot identify the quality control (QC) flag status and covariate categorisation. The dark blue bar (counts less than background, 25) indicates low signal data and was trimmed out of the analysis. Datasets that entirely lack higher level expressions (e.g. counts >100) may indicate experimental failure or low input. (b) Heatmap of the normalised data, scaled to give all genes equal variance, generated via unsupervised clustering. Orange indicates high expression; blue indicates low expression. This plot provides a high-level exploratory view of the data. Iso, isoflurane.

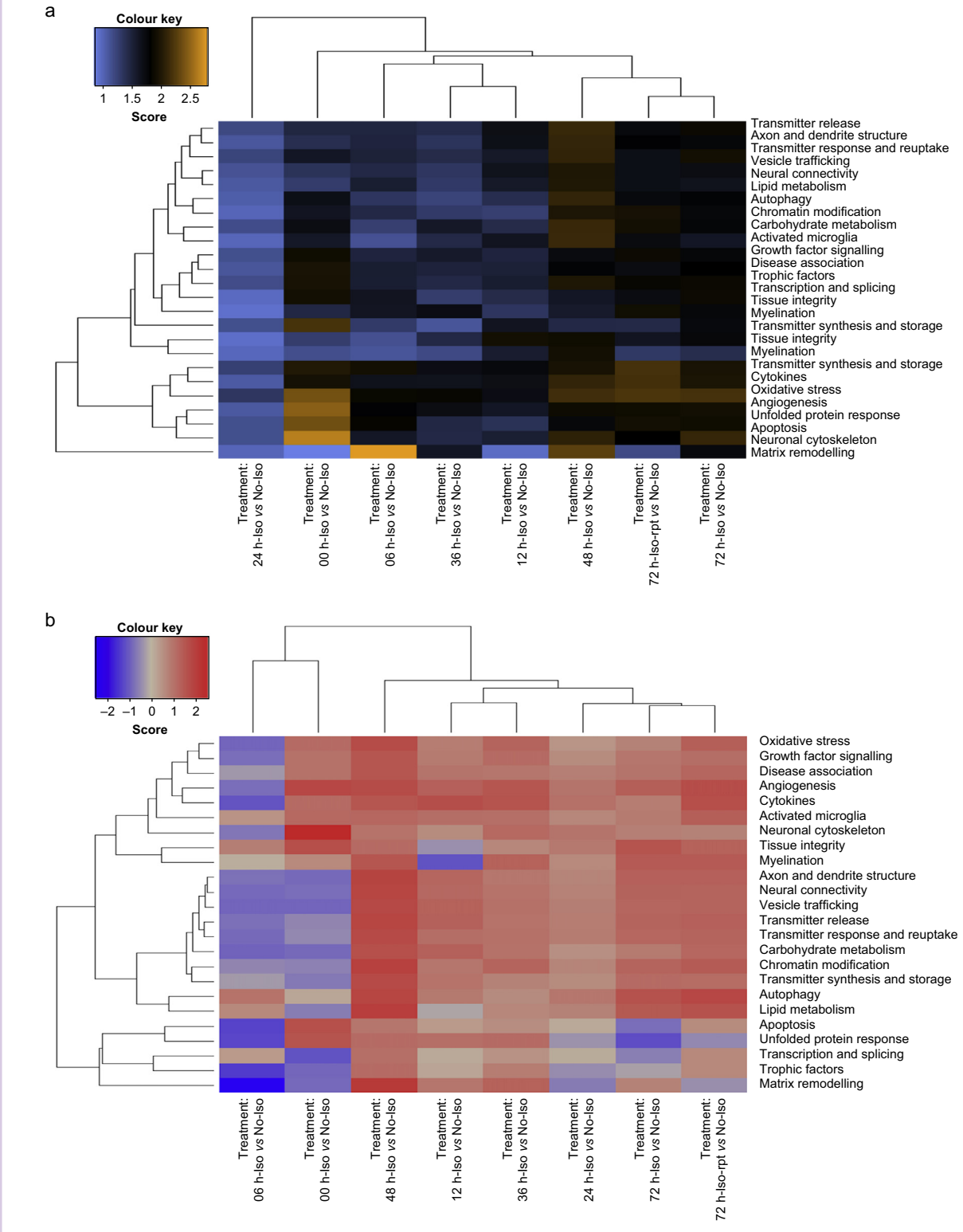

Fig 2. Global significance score. (a) Heatmap displaying each sample's global significance scores. Global significance statistics measure the extent of differential expression of a gene set's genes with a covariate, ignoring whether each gene within the set is upregulated or downregulated. Orange denotes gene sets the genes of which exhibit extensive differential expression with the covariate, blue denotes gene sets with less differential expression. (b) Heatmap displaying each sample's directed global significance scores. Directed global significance statistics measure the extent to which a gene set's genes are upregulated or downregulated with the variable. Red denotes gene sets the genes of which exhibit extensive overexpression with the covariate, blue denotes gene sets with extensive underexpression. Iso, isoflurane.

ischaemic stroke model (transient middle cerebral artery occlusion in rats) showed that isoflurane-induced neuroprotection was directly proportional to the duration of anaesthesia, suggesting that isoflurane anaesthesia for experimental procedures should be restricted to 20–30 min to avoid the confounding results during novel drug testing. Whether this duration applies to other animal/brain injury models and the molecular changes induced by isoflurane exposure (<1 h) remains to be investigated.

Another intriguing finding in our study is the biphasic regulation of gene expression by isoflurane preconditioning—an early change in expression of genes (0, 6 h) followed by a late change in expression (36, 48, and 72 h). This late regulation of gene expression by isoflurane conditioning has several possible explanations. (1) Residual effect of isoflurane: it is possible that residual isoflurane in the brain affects the expression of genes at later time points. The mismatch found between the early and late phase regulation of genes and the fact that isoflurane is minimally metabolised (0.2%) and the majority of isoflurane (95%) is eliminated within a few minutes from lung alveoli, does not support this hypothesis.[21] (2) Impact of trifluoroacetic acid: another possibility is that the metabolite of isoflurane, trifluoroacetic acid (a potential immunogen) could mediate late regulation of genes.[22] Currently, studies specifically examining the impact of trifluoroacetic acid on gene regulation are lacking. (3) Isoflurane's secondary effect: a highly likely hypothesis is that isoflurane-induced translation of proteins from early phase genes stimulates the expression of late phase genes. The fact that a nearly distinct set of genes is differentially expressed in the late phase compared with the early phase suggests that this is not a direct effect of isoflurane, rather a secondary effect mediated by the transcriptional changes induced by isoflurane conditioning.

Interestingly, studies have shown that the protection offered by anaesthetic preconditioning occurs in two phases: (1) early phase—which starts immediately after the anaesthetic conditioning, lasting for minutes to a few hours and this protection depends on the multiple intracellular phosphorylation signalling cascades, and (2) the late phase—which starts after 12–24 h, and has the potential to provide a sustained protection lasting somewhere between 3 and 14 days, leading to improved long-term neurological outcomes. This delayed protection depends on altered protein expression induced by the early transcriptional changes caused by anaesthetic preconditioning.[23–27] Thus far, no experimental studies have evaluated the impact of isoflurane-induced genomic changes in a temporal sequence, and the results from our current study support the above notion indicating that isoflurane preconditioning-induced neuroprotection may occur in two phases: (1) immediate protection by the early expressing genes, where a significant upregulation of genes by isoflurane conditioning at 0 h was noticed. The observed changes in gene expression were transient as only 50% of genes were differentially expressed at 6 h compared with 0 h and the majority of genes differentially expressed at 6 h were downregulated, and (2) the delayed protection by the late phase gene expression changes at 36–72 h after isoflurane conditioning. A dormant period of ~24 h was noticed before the next set of differentially expressed genes emerged at 36 h followed by a more robust differential gene expression at 48 and 72 h after isoflurane conditioning. This dormant period may correlate with the transcriptional changes required for the delayed protection induced by isoflurane conditioning.

We also examined the effect of repetitive exposure of isoflurane on transcriptional changes, as there is conflicting evidence regarding repeated isoflurane exposure, with some studies showing a neuroprotective effect and others noting a detrimental effect.[28–31] Interestingly, we noted that although several genes were overlapping between the single and repeated isoflurane exposure groups measured at 72 h, a few genes were differentially expressed in only one of the groups. Notably six genes (*Naglu*, *Pla2g4e*, *Bche*, *Tor1a*, *Akt1*, and *Erlec1*) were exclusively differentially expressed in the repetitive isoflurane exposure group compared with all other groups examined and most of these genes are implicated in neuroprotection.[32–36]

Strengths and limitations

Our study has several strengths. (1) We have examined genomic expression in a large set of neuropathology genes in young adult mice. (2) To the best of our knowledge, this is the first study which has examined the isoflurane-induced gene expression in temporal sequence. (3) The impact of repetitive exposure of isoflurane has been evaluated. (4) We have presented the genes which were significant by FDR-adjusted *P*-values. This is important to avoid high FPRs when comparing hundreds of genes. Our study also has several limitations. (1) Only cortical tissue was studied in the current study, and the genomic expression in other areas such as hippocampus needs to be evaluated in the future. (2) We did not include female mice, or older mice in the study. (3) Haemodynamic and physiological variables were not measured to avoid any interference during the experiments which may induce other gene expressions. (4) The impact of isoflurane on protein expression was not examined in this study. Though mRNA is required for the production of protein, isoflurane may affect translation and other aspects of protein expression. (5) Long-term effects of isoflurane have not been studied. (6) The impact of other commonly used volatile and intravenous anaesthetics such as sevoflurane, desflurane, propofol, and anaesthetic adjuvants such as opioids, benzodiazepines, and neuromuscular blockers, on gene and protein expression were not studied. For example, a recent study examining the impact of a clinically relevant dose of sevoflurane and propofol in human fetal brain cells showed that both anaesthetics extensively altered transcriptomic profiling across different brain cells.[37] Hence it is important to examine the effect of other commonly used anaesthetics on genomic and proteomic expressions in neurodegenerative disorders which could eventually help in the identification of the molecular mechanisms underlying anaesthetic conditioning-induced neuroprotection. (7) Finally, it is important to note that this is an exploratory analysis and the sample size utilised in the current study may limit our power to detect small effect sizes. Our data will aid power calculations for future studies in this area.

Conclusions

Our preliminary study shows that isoflurane administration, as used for preconditioning, alters expression of RNA transcripts that are involved in neurodegeneration and other central nervous system disorders in two phases. Further studies are warranted to examine the corresponding molecular pathways mediated by these genes that could provide insights into isoflurane conditioning-induced neuroprotection in several central nervous system disorders.

Author's contributions

Conceptualisation, formal analysis, NanoString; investigation, writing—original draft preparation, supervision, project administration, funding acquisition: UA.

Methodology, software, NanoString; validation, writing—review and editing: UA, TG.

Read and agreed to the published version of the manuscript: both authors.

Declaration of interest

The authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bjao.2024.100268.

References

1. Gidday JM. Cerebral preconditioning and ischaemic tolerance. *Nat Rev Neurosci* 2006; **7**: 437–48
2. Dirnagl U, Becker K, Meisel A. Preconditioning and tolerance against cerebral ischaemia: from experimental strategies to clinical use. *Lancet Neurol* 2009; **8**: 398–412
3. Wacker BK, Park TS, Gidday JM. Hypoxic preconditioning-induced cerebral ischemic tolerance: role of microvascular sphingosine kinase 2. *Stroke* 2009; **40**: 3342–8
4. Stowe AM, Altay T, Freie AB, Gidday JM. Repetitive hypoxia extends endogenous neurovascular protection for stroke. *Ann Neurol* 2011; **69**: 975–85
5. Trendelenburg G, Dirnagl U. Neuroprotective role of astrocytes in cerebral ischemia: focus on ischemic preconditioning. *Glia* 2005; **50**: 307–20
6. Masada T, Hua Y, Xi G, Ennis SR, Keep RF. Attenuation of ischemic brain edema and cerebrovascular injury after ischemic preconditioning in the rat. *J Cereb Blood Flow Metab* 2001; **21**: 22–33
7. Liu M, Jayaraman K, Norris AJ, et al. Isoflurane conditioning-induced delayed cerebral ischemia protection in subarachnoid hemorrhage-role of inducible nitric oxide synthase. *J Am Heart Assoc* 2023; **12**, e029975
8. Bastide M, Gele P, Petrault O, et al. Delayed cerebrovascular protective effect of lipopolysaccharide in parallel to brain ischemic tolerance. *J Cereb Blood Flow Metab* 2003; **23**: 399–405
9. Hua Y, Keep RF, Hoff JT, Xi G. Thrombin preconditioning attenuates brain edema induced by erythrocytes and iron. *J Cereb Blood Flow Metab* 2003; **23**: 1448–54
10. Digicaylioglu M, Lipton SA. Erythropoietin-mediated neuroprotection involves cross-talk between jak2 and nf-kappab signalling cascades. *Nature* 2001; **412**: 641–7
11. Li YX, Ding SJ, Xiao L, Guo W, Zhan Q. Desferoxamine preconditioning protects against cerebral ischemia in rats by inducing expressions of hypoxia inducible factor 1 alpha and erythropoietin. *Neurosci Bull* 2008; **24**: 89–95
12. Jayaraman K, Liu M, Zipfel GJ, Athiraman U. Sevoflurane and desflurane exposures following aneurysmal subarachnoid hemorrhage confer multifaceted protection against delayed cerebral ischemia. *Biomedicines* 2021; **9**: 820
13. Archer DP, Walker AM, McCann SK, Moser JJ, Appireddy RM. Anesthetic neuroprotection in experimental stroke in rodents: a systematic review and meta-analysis. *Anesthesiology* 2017; **126**: 653–65
14. Archer DP, McCann SK, Walker AM, et al. Neuroprotection by anaesthetics in rodent models of traumatic brain injury: a systematic review and network meta-analysis. *Br J Anaesth* 2018; **121**: 1272–81
15. Sang H, Cao L, Qiu P, Xiong L, Wang R, Yan G. Isoflurane produces delayed preconditioning against spinal cord ischemic injury via release of free radicals in rabbits. *Anesthesiology* 2006; **105**: 953–60
16. Gigante PR, Appelboom G, Hwang BY, et al. Isoflurane preconditioning affords functional neuroprotection in a murine model of intracerebral hemorrhage. *Acta Neurochir Suppl* 2011; **111**: 141–4
17. Neag MA, Mitre AO, Catinean A, Mitre CI. An overview on the mechanisms of neuroprotection and neurotoxicity of isoflurane and sevoflurane in experimental studies. *Brain Res Bull* 2020; **165**: 281–9
18. Danaher P, Warren S, Dennis L, et al. Gene expression markers of tumor infiltrating leukocytes. *J Immunother Cancer* 2017; **5**: 18
19. Benjamini Y, Yekutieli D. The control of the false discovery rate in multiple testing under dependency. *Ann Stat* 2001; **29**: 1165–88
20. Gaidhani N, Sun F, Schreihofer D, Uteshev VV. Duration of isoflurane-based surgical anesthesia determines severity of brain injury and neurological deficits after a transient focal ischemia in young adult rats. *Brain Res Bull* 2017; **134**: 168–76
21. Davidkova T, Kikuchi H, Fujii K, et al. Biotransformation of isoflurane: urinary and serum fluoride ion and organic fluorine. *Anesthesiology* 1988; **69**: 218–22
22. Kharasch ED, Hankins DC, Cox K. Clinical isoflurane metabolism by cytochrome P450 2E1. *Anesthesiology* 1999; **90**: 766–71
23. Tomai F, Crea F, Chiariello L, Gioffre PA. Ischemic preconditioning in humans: models, mediators, and clinical relevance. *Circulation* 1999; **100**: 559–63
24. Nandagopal K, Dawson TM, Dawson VL. Critical role for nitric oxide signaling in cardiac and neuronal ischemic preconditioning and tolerance. *J Pharmacol Exp Ther* 2001; **297**: 474–8
25. Uecker M, Da Silva R, Grampp T, Pasch T, Schaub MC, Zaugg M. Translocation of protein kinase C isoforms to subcellular targets in ischemic and anesthetic preconditioning. *Anesthesiology* 2003; **99**: 138–47
26. Dirnagl U, Simon RP, Hallenbeck JM. Ischemic tolerance and endogenous neuroprotection. *Trends Neurosci* 2003; **26**: 248–54
27. Lucchinetti E, Aguirre J, Feng J, et al. Molecular evidence of late preconditioning after sevoflurane inhalation in healthy volunteers. *Anesth Analg* 2007; **105**: 629–40

28. Xiong L, Zheng Y, Wu M, et al. Preconditioning with isoflurane produces dose-dependent neuroprotection via activation of adenosine triphosphate-regulated potassium channels after focal cerebral ischemia in rats. *Anesth Analg* 2003; **96**: 233–7
29. Gjendal K, Ottesen JL, Olsson IAS, Sørensen DB. Effect of repeated exposure to isoflurane on nest building and burrowing in mice. *J Am Assoc Lab Anim Sci* 2020; **59**: 30–6
30. Bajwa NM, Lee JB, Halavi S, Hartman RE, Obenaus A. Repeated isoflurane in adult male mice leads to acute and persistent motor decrements with long-term modifications in corpus callosum microstructural integrity. *J Neurosci Res* 2019; **97**: 332–45
31. Long Ii RP, Aroniadou-Anderjaska V, Prager EM, Pidoplichko VI, Figueiredo TH, Braga MF. Repeated isoflurane exposures impair long-term potentiation and increase basal GABAergic activity in the basolateral amygdala. *Neural Plast* 2016; **2016**: 8524560
32. Di Natale P, Di Domenico C, Gargiulo N, et al. Treatment of the mouse model of mucopolysaccharidosis type IIIB with lentiviral-NAGLU vector. *Biochem J* 2005; **388**(Pt 2): 639–46
33. Pérez-González M, Mendioroz M, Badesso S, et al. PLA2G4E, a candidate gene for resilience in Alzheimer's disease and a new target for dementia treatment. *Prog Neurobiol* 2020; **191**, 101818
34. Jasiecki J, Targońska M, Wasąg B. The role of butyrylcholinesterase and iron in the regulation of cholinergic network and cognitive dysfunction in Alzheimer's disease pathogenesis. *Int J Mol Sci* 2021; **22**: 2033
35. Cao S, Gelwix CC, Caldwell KA, Caldwell GA. Torsin-mediated protection from cellular stress in the dopaminergic neurons of *Caenorhabditis elegans*. *J Neurosci* 2005; **25**: 3801–12
36. Kim H, Park J, Kang H, et al. Activation of the Akt1-CREB pathway promotes RNF146 expression to inhibit PARP1-mediated neuronal death. *Sci Signal* 2020; **13**, eaax7119
37. Chang E, Wang Y, Zhu R, et al. General anesthetic action profile on the human prefrontal cortex cells through comprehensive single-cell RNA-seq analysis. *iScience* 2023; **26**, 106534

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