

UC Davis

UC Davis Previously Published Works

Title

Metabolomic Signatures of Brainstem in Mice following Acute and Subchronic Hydrogen Sulfide Exposure

Permalink

<https://escholarship.org/uc/item/1pv6n7dg>

Journal

Metabolites, 14(1)

ISSN

2218-1989

Authors

Kim, Dong-Suk

Santana Maldonado, Cristina M

Giulivi, Cecilia

et al.

Publication Date

2024-01-14

DOI

10.3390/metabo14010053



Copyright Information

This work is made available under the terms of a Creative Commons Attribution License, available at <https://creativecommons.org/licenses/by/4.0/>

Peer reviewed

Article

Metabolomic Signatures of Brainstem in Mice following Acute and Chronic Hydrogen Sulfide Exposure

Dong-Suk Kim ¹, Cristina M. Santana Maldonado ^{1,2}, Cecilia Giulivi ¹  and Wilson Kiiza Rumbeiha ^{1,*} 

¹ Department of Molecular Biosciences, School of Veterinary Medicine, UC Davis, Davis, CA 95616, USA; dskkim@ucdavis.edu (D.-S.K.); csantanamaldonado@mriglobal.org (C.M.S.M.); cgiulivi@ucdavis.edu (C.G.)

² MRI Global, Kansas City, MO 64110, USA

* Correspondence: wkrumbeiha@ucdavis.edu; Tel.: +1-530-752-6285 or +1-517-944-2022

Abstract: Hydrogen sulfide (H₂S) is an environmental toxicant of significant health concern. The brain is a major target in acute H₂S poisoning. This study was conducted to test the hypothesis that acute and subchronic ambient H₂S exposures alter the brain metabolome. Male 7–8-week-old C57BL/6J mice were exposed by whole-body inhalation to 1000 ppm H₂S for 45 min and euthanized at 5 min or 72 h for acute exposure. For subchronic study, mice were exposed to 5 ppm H₂S 2 h/day, 5 days/week for 5 weeks. Control mice were exposed to room air. The brainstem was removed for metabolomic analysis. Enrichment analysis showed that the metabolomic profiles in acute and subchronic H₂S exposures matched with those of cerebral spinal fluid from patients with seizures or Alzheimer's disease. Acute H₂S exposure decreased excitatory neurotransmitters, aspartate, and glutamate, while the inhibitory neurotransmitter, serotonin, was increased. Branched-chain amino acids and glucose were increased by acute H₂S exposure. Subchronic H₂S exposure within OSHA guidelines surprisingly decreased serotonin concentration. In subchronic H₂S exposure, glucose was decreased, while polyunsaturated fatty acids, inosine, and hypoxanthine were increased. Collectively, these results provide important mechanistic clues for acute and subchronic ambient H₂S poisoning and show that H₂S alters brainstem metabolome.

Keywords: hydrogen sulfide; metabolomics; brainstem; brain; metabolism; biomarkers; neurotransmitters; branched-chain amino acids; polyunsaturated fatty acids



Citation: Kim, D.-S.; Santana Maldonado, C.M.; Giulivi, C.; Rumbeiha, W.K. Metabolomic Signatures of Brainstem in Mice following Acute and Chronic Hydrogen Sulfide Exposure. *Metabolites* **2024**, *14*, 53. <https://doi.org/10.3390/metabo14010053>

Academic Editor: Guorong Wu

Received: 21 December 2023

Revised: 5 January 2024

Accepted: 11 January 2024

Published: 14 January 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

The brain is a highly complex organ that regulates many body functions. To perform these roles, it relies on complex metabolic processes which are energy dependent [1,2]. Consequently, the brain is susceptible to toxic environmental chemicals that target mitochondria and impair energy metabolism. An example of such chemicals is hydrogen sulfide (H₂S), a potent gas produced endogenously and in the environment. Endogenously produced H₂S plays key roles in brain signaling and is essential for normal brain physiologic functions. However, excessive amounts of H₂S in the brain, either from aberrant endogenous metabolic pathways (genetic defects) or environmental exposures, leads to neuronal dysfunction, including neurodegeneration [3–10]. Ethylmalonic encephalopathy (OMIM 602473), a life-limiting disease, is caused by a genetic defect in H₂S metabolism in mitochondria, resulting in chronic excessive H₂S exposure in the brain [11].

Studies from our laboratory showed that acute exogenous H₂S exposure induces transcriptomic and proteomic changes in the brainstem, thalamus, and other brain regions [3,5–9,12,13]. A wealth of literature shows that H₂S impairs mitochondrial function, specifically targeting cytochrome c oxidase (Complex IV) in the electron transport chain, thus inhibiting ATP production [14–17]. Chemical hypoxia and/or energy deficits are linked to a loss of central respiratory drive, convulsions, and death following acute H₂S exposures [18,19]. In cases without a fatal outcome, it is likely that deficits in brain metabolism

may cause immediate, intermediate, delayed, and long-term neurotoxicity following acute or chronic H₂S exposure. It is possible that some of these H₂S-induced toxicological changes may predispose exposed individuals to other neurological conditions, including neurodegenerative conditions via gene–environment interactions. To our knowledge, despite the well-known impact of H₂S on cellular metabolism, there are no metabolomic studies on the brainstem upon acute and chronic H₂S exposures. This is a knowledge gap curtailing our understanding of toxic mechanisms of H₂S-induced neurotoxicity.

We postulated that metabolic signatures of the brainstem could be observed in an inhalation whole-mouse model because human exposure to H₂S gas is typically via inhalation. For this project, we focused on the brainstem because this region has been previously shown to undergo neurodegeneration following acute H₂S exposure and also because it controls vital autonomic functions such as breathing [3]. Impaired breathing is widely implicated as a cause of death following acute H₂S poisoning [18]. It was also previously reported that H₂S preferentially accumulates in the brainstem region [20]. Moreover, the exact underlying mechanisms by which H₂S suppresses breathing, causing death, remain to be elucidated. Therefore, understanding the metabolomic changes in the brainstem following H₂S exposure will advance our knowledge of the mechanisms underlying H₂S-induced neurotoxicity, neuropathology, and death.

2. Materials and Methods

2.1. Animals

All animal studies were approved by the Institutional Animal Care and Use Committee (IACUC) of Iowa State University (ISU) and the University of California at Davis (UC Davis). IACUC-18-136, February 2016, and IACUC-21819, October 2020, were approved by ISU and UC Davis, respectively. Care and use of animals were performed in accordance with the IACUC, and animals were treated humanely and handled with care. We also followed the ARRIVE guidelines in the design and execution of this study [21].

Briefly, 7–8-week-old male C57BL/6J mice were purchased from Jackson Laboratories (Sacramento, CA and Bar Harbor, ME, USA). Only male mice were used because previous studies in our laboratory found male mice to be more sensitive to acute H₂S poisoning than females [9]. Mice were randomly assigned to different groups and housed in Laboratory Animal Resources at the College of Veterinary Medicine, ISU, and in the Teaching and Research Animal Care Services facility (TRACS) at the School of Veterinary Medicine, UC Davis, with a 12:12 h light and dark cycle. Room temperature and relative humidity were maintained at 22 °C and 50 ± 10%, respectively. Protein rodent maintenance diet (Teklad HSD Inc., Madison, WI, USA) and drinking water were provided ad libitum. Mice were acclimated for 1 week before the start of the experiments.

2.2. Gas Exposure

We conducted two separate experiments to mimic the two real-world scenarios of H₂S exposure. In the first scenario, we conducted acute H₂S exposure to mimic situations involving large scale industrial accidents or nefarious acts (Figure 1A). In the second scenario, we conducted subchronic exposure to low level ambient H₂S to mimic occupational settings (Figure 1B). According to the OSHA guidelines, workers can be exposed to up to 10 ppm H₂S for 8 h a day (TWA), 5 days a week without negatively impacting their health [22]. For this reason, we chose to use H₂S at a concentration of 5 ppm.

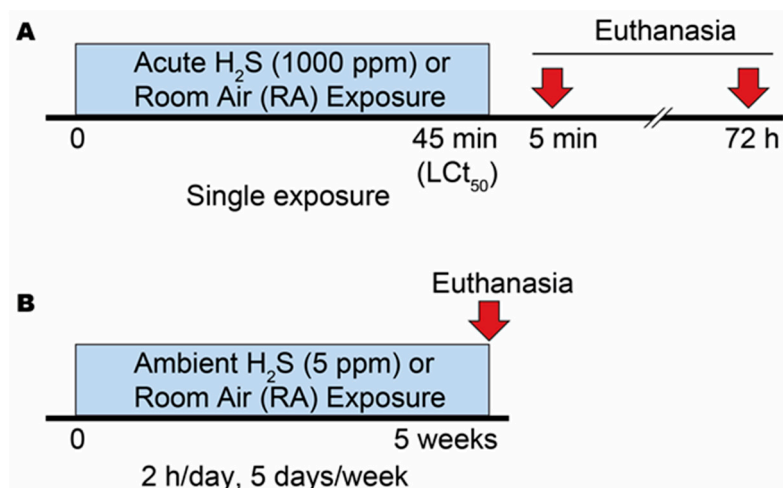


Figure 1. A summary of experimental exposure paradigms. **(A)** Exposure paradigm of single acute exposure to 1000 ppm H₂S. This exposure model induces 50% mortality (LC₅₀). Control mice were exposed to room air. **(B)** Exposure paradigm of subchronic ambient exposure to 5 ppm H₂S. In this model, mice were exposed to 5 ppm H₂S for 2 h/day, 5 days/week for 5 weeks. Control mice were exposed to room air.

2.2.1. Acute Exposure Experiment

For the acute study, we followed our previously published LC₅₀ model [3,5–9,12,13] (Figure 1A). Briefly, two cohorts of male mice ($n = 10$) were exposed to 1000 ppm of H₂S for 45 min using a whole-body inhalation exposure chamber, as reported previously [3,5–9,12,13]. This level and length of exposure results in 50% mortality (lethal concentration time 50 [LC₅₀]) during exposure, and mice typically exhibit dyspnea, convulsions, loss of consciousness, and death in that order [9]. For the metabolomic analysis, we used mice that survived the LC₅₀ exposure. One cohort of surviving mice was sacrificed 5 min post exposure in order to determine the metabolic profile of mice immediately after exposure while the mice were still manifesting clinical effects of acute exposure. The other cohort was sacrificed 72 h post H₂S exposure in order to determine metabolic changes at the time when neurodegenerative changes are typically initially observed in this model histologically [3,5,9]. For the controls, we used two cohorts of mice exposed once to normal room air (RA) for the same duration as H₂S-exposed mice. As was the case for H₂S-exposed mice, one cohort of control mice ($n = 5$) was euthanized at 5 min post RA exposure while the other was euthanized at 72 post RA exposure. All mice that received RA survived. Both H₂S gas and RA were introduced to the exposure chamber from pressurized gas cylinders. The concentration of H₂S in the exposure chamber was monitored in real time using a H₂S sensor (RKI Eagle, RKI Instrument, Union City, CA, USA). Following exposure, mice were euthanized by decapitation and the brainstem (medulla and pons) was immediately further micro-dissected and the pontine reticular nucleus and the gigantocellular reticular nucleus isolated on ice. These tissue samples were immediately stored at -80°C until metabolomic analysis.

2.2.2. Ambient Exposure Experiment

In this experiment, male mice ($n = 5$) were exposed to 5 ppm H₂S, 2 h/day, 5 days/week for 5 weeks, while the control group of mice ($n = 4$) were exposed to normal room air (RA). This dosage was chosen based on our prior pilot studies and also because 5 ppm is the midpoint concentration of the OSHA guideline of a time weighted average of 10 ppm of H₂S in an 8 h work day, 5 days a week for the construction and shipyard industries [23]. Both H₂S gas and RA were delivered to the exposure chambers from pressurized gas cylinders. None of the mice in this subchronic experiment died. The experimental design is shown in Figure 1B.

2.3. Metabolomic Analysis

The micro-dissected brainstem tissue samples were stored at -80°C before submission to the West Coast Metabolomics Center, UC Davis, for untargeted metabolomics analysis. These metabolomic analyses consisted of three assays: (1) primary metabolism by GC-TOF MS, (2) biogenic amines (hydrophilic compounds) by HILIC-MS/MS, and (3) lipidomics by RPLC-MS/MS. Control samples from mice exposed to RA and samples from H_2S -exposed mice were processed identically. Detailed procedures of metabolomic analysis is described in the supplementary data [24–27].

2.4. Data Analysis

Raw data peak heights were normalized to the sum of internal standards for primary amine and lipidomics data analyses. Data of primary metabolites were normalized by a vector normalization method by calculating the sum of all peak heights of all identified metabolites. Data were further processed to identify outliers by using a boxplot with $1.5\times$ interquartile range (IQR). Outliers were removed for the definitive analysis. The dataset of metabolomic analysis is publicly available in dryad, an open data publishing platform (<https://doi.org/10.5061/dryad.tdz08kq5n>) (accessed on 21 December 2023).

2.5. Enrichment Analysis

Quantitative metabolite set enrichment analysis (MSEA) was performed using MetaboAnalyst 5.0 (<https://www.metaboanalyst.ca>) (accessed on 21 December 2023). Metabolites that were significantly altered compared to the RA groups were further analyzed by quantitative MSEA.

2.6. Heatmap

Metabolites that were significantly altered compared to the RA groups were summarized and visualized in a heatmap. Concentration changes in metabolites were visualized as a log 2-fold change. Visualization was processed using Python version 3.0 (<https://www.python.org>) accessed on 21 December 2023.

2.7. Statistical Analysis

Data are presented as mean and standard deviation of the mean. ANOVA with post hoc Tukey HSD test was performed for metabolites of acute H_2S exposure using the statsmodels module in Python (<https://www.python.org>, version 3.0) accessed on 21 December 2023. Unpaired Student's *t*-test was performed for the metabolites of the ambient H_2S exposure study using Excel software of Microsoft 365. *p*-values ≤ 0.1 were considered statistically significant for this explorative analysis. Statistical analysis of the metabolite set enrichment data was performed in MetaboAnalyst 5.0 (<https://www.metaboanalyst.ca>) accessed on 21 December 2023.

3. Results

3.1. Exposure to Acute and Ambient Hydrogen Sulfide

Mice exposed acutely to H_2S for 45 min manifested seizures, dyspnea, ataxia, and knockdown during exposure, as previously reported [3,5–7,9,12,13]. Mice that survived after acute H_2S exposure and were euthanized at 5 min post H_2S exposure exhibited weak, ataxic, and labored breathing. Mice euthanized at 72 h post H_2S exposure were active and moved about normally. However, mice acutely exposed to H_2S exhibited a reduction in body weight [3]. In contrast, mice from the 5 ppm exposure group did not exhibit any abnormal clinical signs or behavior, despite a reduction in body weight 30 days post ambient H_2S exposure.

3.2. Metabolomic Changes in the Brainstem following Acute and Ambient Hydrogen Sulfide

H_2S induced significant metabolomic changes in the brainstem. Metabolomic changes in brainstems from mice given a single high dose of acute exposure were different from

those of mice repeatedly administered low levels of H₂S over a month. The results of the changes from the metabolomic analyses included primary metabolites, biogenic amine metabolites, and lipidomic metabolites (summarized in heatmaps for acute and ambient H₂S exposures; Figures 2 and 3). The Venn diagram shows the number of significantly altered identified metabolites which were increased more than 50% or decreased 33% compared to the room air control group (Figure 4). The Venn diagram represents the number of significantly altered metabolites in common or which were unique at 5 min and at 72 h post acute H₂S exposure and following subchronic ambient H₂S exposures. The significantly altered metabolites are listed in Tables 1 and 2 for 5 min and 72 h post acute H₂S exposure and following subchronic ambient H₂S exposure, respectively.

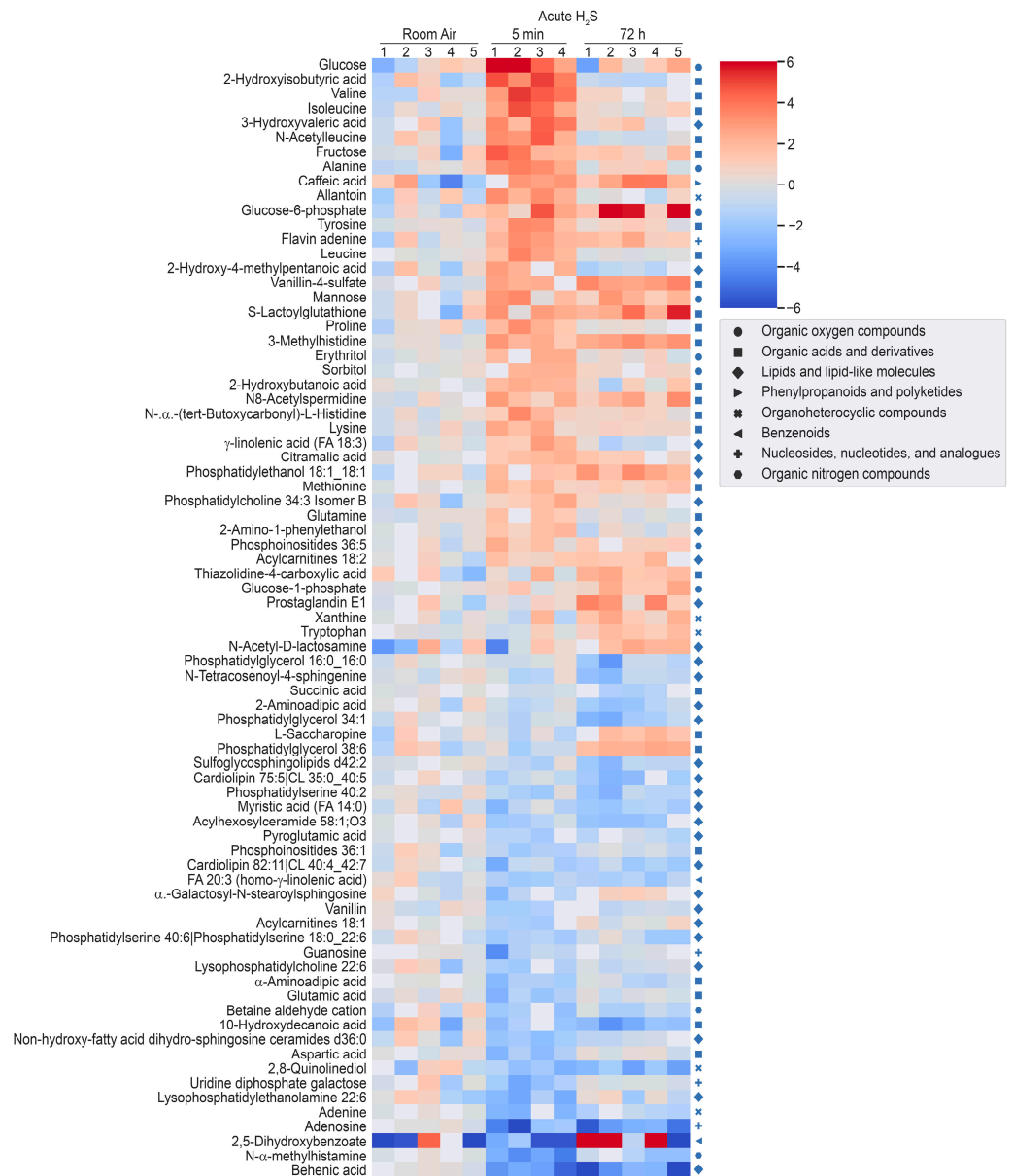


Figure 2. Heatmap analysis of significantly altered metabolites following a single acute H₂S or RA exposure. Concentrations of significantly altered metabolites from primary metabolites, biogenic amines, and lipidomic metabolites are visualized in a heatmap by fold change (log₂ scale). Each metabolite was compared to the average concentration of RA control group. Classification of individual metabolites is shown on the right side of the heatmap.

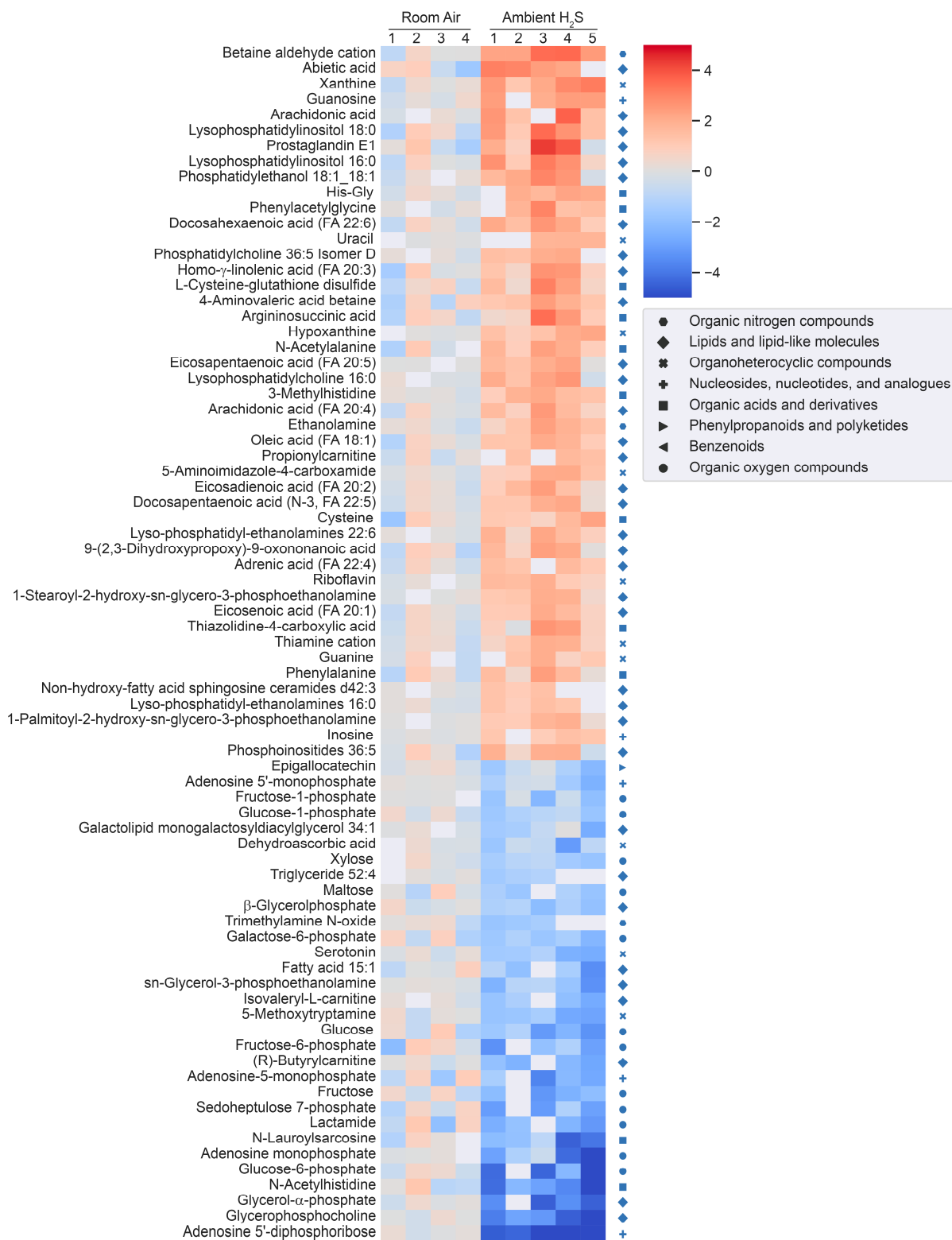


Figure 3. Heatmap analysis of significantly altered metabolites following subchronic ambient H₂S or RA exposure. Concentrations of significantly altered primary organic metabolites, hydrophilic compounds including biogenic amines, and lipidomic metabolites are visualized in a heatmap by fold change (log₂ scale). Each metabolite was compared to the average concentration of RA control group. Classification of individual metabolites is shown on the right side of the heatmap.

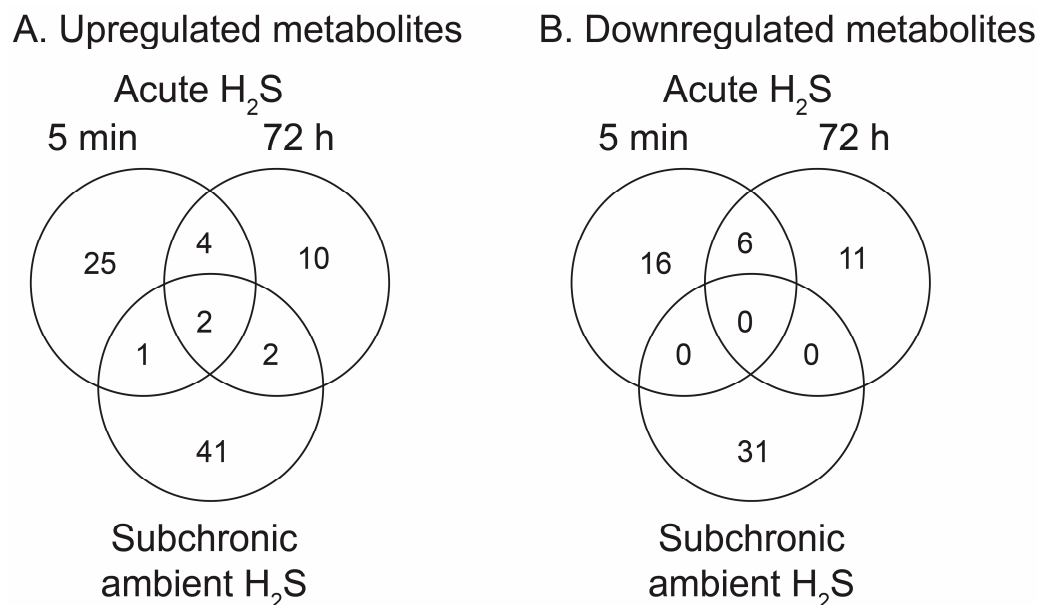


Figure 4. Venn diagrams summarizing numbers of significantly altered metabolites following acute and ambient H₂S exposure. Immediate (5 min) and early (72 h) responses to acute H₂S and response to ambient H₂S exposure in brainstem are shown. (A) Increased metabolites compared to RA control group. (B) Decreased metabolites compared to RA control group.

Table 1. Significantly altered metabolites at 5 min post acute H₂S exposure compared to RA control group. Common metabolite name, mass-to-charge ratio, fold change (log₂ scale), and statistical significance are listed. ANOVA with post hoc Tukey HSD test was performed for statistical significance. Asterisks indicate significant differences compared to RA group. *p* * < 0.1, ** < 0.05, *** < 0.01, and **** < 0.001.

| Metabolites | <i>m/z</i> | 5 min | | 72 h | |
|----------------------------------|------------|-------------|--------------|-------------|--------------|
| | | Fold Change | Significance | Fold Change | Significance |
| 10-hydroxydecanoic acid | 187.135 | 1.77 | **** | 0.31 | |
| 2,5-dihydroxybenzoate | 329.025 | 1.52 | **** | 0.21 | |
| 2,8-quinolinediol | 162.057 | 1.46 | *** | 0.32 | |
| 2-amino-1-phenylethanol | 120.08 | 1.41 | **** | −0.2 | |
| 2-aminoadipic acid | 162.076 | 1.36 | *** | 0.48 | |
| 2-hydroxy-4-methylpentanoic acid | 131.071 | 1.35 | **** | 0.23 | |
| 2-hydroxybutanoic acid | 131 | 1.22 | * | 1.18 | ** |
| 2-hydroxyisobutyric acid | 103.041 | 1.15 | *** | 0.02 | |
| 3-hydroxyvaleric acid | 117.056 | 1.15 | | 2.88 | * |
| 3-methylhistidine | 170.091 | 1.11 | *** | 0.25 | |
| Acylcarnitines 18:1 | 426.358 | 0.9 | **** | 0.31 | *** |
| Acylcarnitines 18:2 | 424.344 | 0.87 | *** | 0.19 | |
| Acylhexosylceramide 58:1; O3 | 1048.91 | 1.07 | *** | −0.04 | |
| Adenine | 136.062 | 1.1 | | 1.33 | * |
| Adenosine | 236 | 1.07 | *** | 0.68 | ** |
| Alanine | 116 | 1.01 | *** | −0.44 | |
| Allantoin | 157.038 | 1 | *** | 1.29 | **** |

Table 1. Cont.

| Metabolites | m/z | 5 min | | 72 h | |
|--|-----------------------|-------------|--------------|-------------|--------------|
| | | Fold Change | Significance | Fold Change | Significance |
| α -galactosyl-N-stearoylsphingosine | 728.598 | 1.79 | **** | −0.43 | |
| α -aminoadipic acid | 260 | 0.97 | * | 0.81 | |
| Aspartic acid | 232 | 0.96 | | 1.49 | ** |
| Behenic acid | 117 | 0.93 | *** | 0.08 | |
| Betaine aldehyde cation | 120.1019_ 102.0893 | 0.93 | *** | 1.19 | **** |
| Caffeic acid | 181.057 | 0.91 | **** | 0.13 | |
| Cardiolipin 75:5 Cardiolipin 35:0_40:5 | 1496.04 | 0.79 | *** | 0.29 | |
| Cardiolipin 82:11 Cardiolipin 40:4_42:7 | 1582.09 | 0.78 | ** | −0.24 | |
| Citramalic acid | 247 | 0.83 | ** | 0.26 | |
| Erythritol | 217 | 0.78 | * | 0.76 | ** |
| Flavin adenine | 246 | 0.66 | ** | 0.2 | |
| Fructose | 307 | 0.63 | **** | −0.06 | |
| γ -Linolenic acid (FA 18:3) | 277.216 | 0.69 | * | 1.06 | *** |
| Glucose | 319 | 0.62 | *** | −0.02 | |
| Glucose-1-phosphate | 217 | 0.59 | * | 0.45 | |
| Glucose-6-phosphate | 387 | 0.47 | * | 0.65 | ** |
| Glutamic acid | 246 | 0.27 | | 0.8 | * |
| Glutamine | 156 | 0.26 | | 0.7 | ** |
| Guanosine | 324 | 0.12 | | 1.07 | ** |
| Homo- γ -linolenic acid (FA 20:3) | 305.249 | 0.68 | *** | 0.48 | ** |
| Isoleucine | 158 | 0.09 | | 0.71 | * |
| Leucine | 158 | −0.06 | | 0.87 | * |
| L-Saccharopine | 277.139 | −0.23 | *** | −0.58 | **** |
| Lysine | 317 | −0.24 | | −0.62 | ** |
| Lysophosphatidylcholine 22:6 | 568.339 | −0.07 | | −0.61 | ** |
| Lysophosphatidylethanolamine 22:6 | 526.288 | −0.18 | | −0.58 | ** |
| Mannose | 205 | −0.24 | | −0.8 | *** |
| Methionine | 150.056 | −0.26 | | 0.71 | ** |
| Myristic acid (FA 14:0) | 227.202 | 0.75 | *** | 0.47 | ** |
| N-(Octadecanoyl)-sphinganine (Cer-NDS d36:0) | 566.547 | 0.86 | * | 0.88 | * |
| N- α -(Tert-Butoxycarbonyl)-L-histidine | 110.069 | −0.27 | | 0.93 | **** |
| N ⁸ -Acetylspermidine | 188.175 | −0.28 | | −0.6 | ** |
| N-Acetyl-D-lactosamine | 406.13 | −0.35 | | −0.9 | *** |
| N-Acetyl-leucine | 172.096 | −0.4 | | −0.66 | ** |
| N- α -Methylhistamine | 126.101 | −0.49 | | −0.68 | * |

Table 1. Cont.

| Metabolites | m/z | 5 min | | 72 h | |
|--|-----------------------|-------------|--------------|-------------|--------------|
| | | Fold Change | Significance | Fold Change | Significance |
| N-tetracosenoyl-4-sphinganine | 648.6272_ 630.6146 | −0.5 | * | −0.89 | ** |
| Phosphatidylcholine 34:3 Isomer B | 756.5538_ 778.5289 | −0.58 | *** | −0.39 | *** |
| Phosphatidylglycerol 16:0_16:0 | 721.502 | −0.6 | ** | −0.6 | ** |
| Phosphatidylserine 40:2 | 844.603 | −0.69 | * | −0.19 | |
| Phosphatidylserine 40:6 PS 18:0_22:6 | 836.547 | −0.74 | *** | −0.29 | * |
| Phosphoinositides 34:1 | 835.536 | −0.61 | ** | −0.59 | ** |
| Phosphoinositides 36:1 | 863.56 | −0.65 | ** | 0.38 | * |
| Phosphoinositides 36:5 | 855.5 | −0.66 | *** | −0.3 | |
| Phosphoinositides 38:6 | 881.512 | −0.66 | *** | 0.07 | |
| Phostatidylethanol 18:1_18:1 | 727.527 | −0.6 | * | −0.19 | |
| Proline | 142 | −0.66 | * | −0.37 | |
| Prostaglandin E1 | 353.232 | −0.67 | ** | −0.16 | |
| Pyroglutamic acid | 130.05 | −0.78 | **** | −0.11 | |
| S-Lactoylglutathione | 380.111 | −0.82 | | −1.13 | * |
| Sorbitol | 217 | −0.83 | ** | −0.3 | |
| Succinic acid | 117.019 | −0.83 | **** | −0.02 | |
| Sulfoglycosphingolipids d42:2 | 890.639 | −0.8 | * | −0.41 | |
| Thiazolidine-4-carboxylic acid | 134.025 | −0.84 | * | −1.05 | ** |
| Tryptophan | 202 | −0.87 | ** | −0.08 | |
| Tyrosine | 218 | −1.06 | * | −0.05 | |
| Uridine diphosphate galactose | 565.044 | −1.18 | **** | −0.49 | **** |
| Valine | 144 | −1.42 | **** | −1.58 | **** |
| Vanillin | 151.04 | −1.53 | | 4.94 | * |
| Vanillin-4-sulfate | 230.996 | −1.54 | **** | −0.64 | *** |
| Xanthine | 153.037 | −1.6 | **** | −1.71 | **** |

Table 2. Significantly altered metabolites following subchronic ambient H₂S exposure compared to RA control group. Common metabolite name, mass-to-charge ratio, fold change (log₂ scale), and statistical significance are listed. Unpaired Student's *t*-test was performed for statistical significance. Asterisks indicate significant differences compared to RA group. *p* * < 0.1, ** < 0.05, *** < 0.01, and **** < 0.001.

| Metabolites | m/z | Fold Change | Significance |
|--|----------|-------------|--------------|
| (R)-Butyrylcarnitine | 232.1547 | −1.18 | *** |
| 1-Palmitoyl-2-hydroxy-sn-glycero-3-phosphoethanolamine | 454.2927 | 0.61 | ** |
| 1-Stearoyl-2-hydroxy-sn-glycero-3-phosphoethanolamine | 482.3181 | 0.68 | ** |
| 3-Methylhistidine | 170.0913 | 0.77 | *** |

Table 2. Cont.

| Metabolites | m/z | Fold Change | Significance |
|--|--|-------------|--------------|
| 4-Aminovaleric acid betaine | 160.133 | 0.8 | ** |
| 5-Aminoimidazole-4-carboxamide | 110.0322 | 0.72 | ** |
| 5-Methoxytryptamine | 174 | −1.03 | *** |
| 9-(2,3-Dihydroxypropoxy)-9-oxononanoic acid | 261.1345 | 0.7 | ** |
| Abietic acid | 301.216 | 1.34 | *** |
| Adenosine 5'-diphosphoribose | 560.0761_582.0571 | −3.46 | **** |
| Adenosine 5'-monophosphate | 370.0487 | −0.61 | ** |
| Adenosine monophosphate | 695.1267 | −1.48 | ** |
| Adenosine-5-monophosphate | 315 | −1.2 | * |
| Adrenic acid (FA 22:4) | 331.2637 | 0.69 | *** |
| Arachidonic acid | 303.2328 | 1.13 | * |
| Arachidonic acid (FA 20:4) | 303.2333 | 0.77 | ** |
| Argininosuccinic acid | 291.127 | 0.8 | * |
| β-Glycerolphosphate | 243 | −0.76 | *** |
| Betaine aldehyde cation | 120.1019_102.0893 | 1.4 | *** |
| Cysteine | 220 | 0.71 | ** |
| Dehydroascorbic acid | 173 | −0.65 | ** |
| Docosahexaenoic acid (FA 22:6) | 327.2328 | 0.96 | *** |
| Docosapentaenoic acid (n-3, FA 22:5) | 329.2473 | 0.71 | ** |
| Eicosadienoic acid (FA 20:2) | 307.2637 | 0.72 | ** |
| Eicosapentaenoic acid (FA 20:5) | 301.2167 | 0.79 | ** |
| Eicosenoic acid (FA 20:1) | 309.2798 | 0.68 | ** |
| Epigallocatechin | 307.0835 | −0.59 | ** |
| Ethanolamine | 62.0596 | 0.76 | ** |
| Fatty acid 15:1 | 239.2009 | −0.92 | ** |
| Fructose | 307 | −1.21 | ** |
| Fructose-1-phosphate | 387 | −0.62 | ** |
| Fructose-6-phosphate | 315 | −1.13 | * |
| Galactolipid monogalactosyldiacylglycerol 34:1 | 774.605 | −0.64 | ** |
| Galactose-6-phosphate | 387 | −0.82 | * |
| Glucose | 319 | −1.11 | ** |
| Glucose-1-phosphate | 217 | −0.63 | ** |
| Glucose-6-phosphate | 387 | −1.9 | *** |
| Glycerol-alpha-phosphate | 357 | −1.97 | **** |
| Glycerophosphocholine | 515.2116_772.3096_ 280.0915_258.1106_ 296.0648 | −2.04 | **** |
| Guanine | 152.0564 | 0.64 | * |

Table 2. Cont.

| Metabolites | <i>m/z</i> | Fold Change | Significance |
|--|------------|-------------|--------------|
| Guanosine | 324 | 1.16 | **** |
| His-Gly | 213.0949 | 1.01 | **** |
| Homo- γ -linolenic acid (FA 20:3) | 305.2487 | 0.86 | ** |
| Hypoxanthine | 265 | 0.8 | *** |
| Inosine | 230 | 0.6 | **** |
| Isovaleryl-L-carnitine | 246.17 | −0.98 | *** |
| Lactamide | 90.0544 | −1.25 | * |
| L-Cysteine-glutathione disulfide | 427.0924 | 0.81 | * |
| Lysophosphatidylcholine 16:0 | 540.3289 | 0.78 | ** |
| Lyso-phosphatidyl-ethanolamines 16:0 | 452.2776 | 0.62 | **** |
| Lyso-phosphatidyl-ethanolamines 22:6 | 526.2883 | 0.7 | ** |
| Lysophosphatidylinositol 16:0 | 571.2842 | 1.03 | ** |
| Lysophosphatidylinositol 18:0 | 599.3185 | 1.11 | ** |
| Maltose | 361 | −0.76 | * |
| N-Acetylglycine | 130.0515 | 0.79 | * |
| N-Acetylhistidine | 198.0888 | −1.96 | ** |
| N-Lauroylsarcosine | 272.2221 | −1.35 | * |
| Non-hydroxy-fatty acid sphingosine ceramides d42:3 | 704.6138 | 0.62 | *** |
| Oleic acid (FA 18:1) | 281.2491 | 0.75 | *** |
| Phenylacetyl-glycine | 192.0664 | 0.97 | ** |
| Phenylalanine | 164.0721 | 0.62 | * |
| Phosphatidylcholine 36:5 Isomer D | 780.555 | 0.88 | *** |
| Phosphoinositides 36:5 | 855.5001 | 0.6 | * |
| Phosphatidylethanol 18:1_18:1 | 727.5266 | 1.01 | ** |
| Propionylcarnitine | 218.1366 | 0.75 | *** |
| Prostaglandin E1 | 353.2316 | 1.07 | * |
| Riboflavin | 377.1473 | 0.69 | *** |
| Sedoheptulose 7-phosphate | 387 | −1.24 | ** |
| Serotonin | 174 | −0.91 | **** |
| sn-glycerol-3-phosphoethanolamine | 238.0433 | −0.96 | *** |
| Thiamine cation | 265.1075 | 0.65 | ** |
| Thiazolidine-4-carboxylic acid | 134.0252 | 0.66 | * |
| Triglyceride 52:4 | 872.7704 | −0.67 | *** |
| Trimethylamine N-oxide | 76.0748 | −0.8 | ** |
| Uracil | 241 | 0.89 | **** |
| Xanthine | 353 | 1.23 | *** |
| Xylose | 103 | −0.66 | * |

In the Venn diagram, immediate response reflects metabolic changes in brainstems of mice acutely exposed to H₂S and euthanized 5 min post acute exposure, while early response reflects changes at 72 h post acute exposure. The results show that both acute and subchronic H₂S exposure significantly altered brainstem metabolites. Several metabolites including a phosphatidylinositol (PI 36:5), prostaglandin E1, thiazolidine-4-carboxylic acid, 3-methylhistidine, and phosphatidylethanol 18:1_18:1 (PEtOH 18:1_18:1) were increased both at 5 min post acute H₂S and subchronic ambient H₂S, while no common metabolites were decreased by both acute and subchronic ambient H₂S exposures relative to the RA group (Figure 4B). 3-Methylhistidine and PEtOH 18:1_18:1 were increased at all three euthanasia time points. Metabolites which increased in common due to acute H₂S exposure both at 5 min and 72 h include caffeic acid, N⁸-acetylspermidine, vanillin-4-sulfate, flavin adenine, 3-methylhistidine, and PEtOH 18:1_18:1, while those which decreased due to acute H₂S exposure were N- α -methylhistamine, 2,8-quinolinediol, vanillin, a glycerophospholipid (Cardiolipin 40:4_42:7), adenosine, and behenic acid. Metabolites presented in the heatmap analyses are further listed in Tables 1 and 2 with mass-to-charge ratio and fold change (log₂ scale).

3.3. Enrichment Analysis

We performed enrichment analysis using Metaboanalyst 5.0 to investigate whether H₂S-induced metabolic profiles cause any biological dysregulation and whether H₂S-induced metabolic dysregulations share any similarity with any existing pathological signature databases in Metaboanalyst 5.0. While the database did not have brainstem as a tissue, we chose the human cerebral spinal fluid (CSF) dataset as this most closely matched our experimental design. The results showed several matches with human neuropathological conditions which are summarized in Figures 5–7. Briefly, the metabolomic profiles following acute H₂S poisoning matched with several neurological disorders including seizures, epilepsy, Alzheimer’s disease, and Glut-1 deficiency syndrome, among others (Figures 5 and 6). The metabolomic profiles of subchronic ambient H₂S exposure matched with several pathological conditions including Alzheimer’s disease, dementia, Parkinson’s disease, seizures, Glut-1 deficiency, and anoxia, among others (Figure 7).

3.4. H₂S Dysregulated Brainstem Neurotransmitters

We analyzed neurotransmitters following both acute and subchronic ambient H₂S exposures in the brainstem region. Interestingly, excitatory neurotransmitters, namely, aspartate and glutamate, were decreased, while serotonin, an inhibitory neurotransmitter, was increased at 5 min after single acute H₂S exposure (Figure 8A). Adenosine, which acts both as a central excitatory and as an inhibitory neurotransmitter in the brain, was decreased following both acute and subchronic H₂S exposures. Subchronic ambient H₂S exposure decreased serotonin concentration in the brainstem (Figure 8B). Tyrosine, a precursor of catecholamines (excitatory), was also increased at 5 min post acute H₂S exposure, while tryptophan, a precursor of serotonin, was increased at 72 h post acute H₂S exposure. Tryptophan was also increased due to subchronic ambient H₂S exposure (Figure 9B).

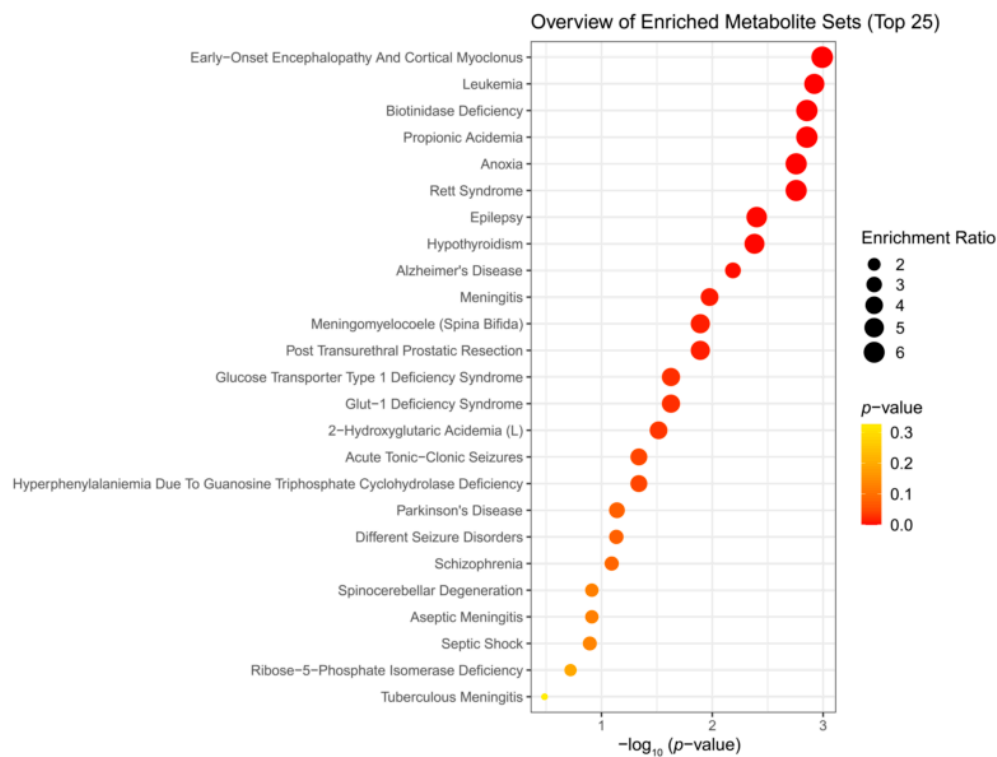


Figure 5. Disease-based enrichment analysis of significantly altered metabolites of immediate response to H₂S exposure (5 min post single acute H₂S exposure) for primary metabolites, biogenic amines, and lipidomic metabolites.

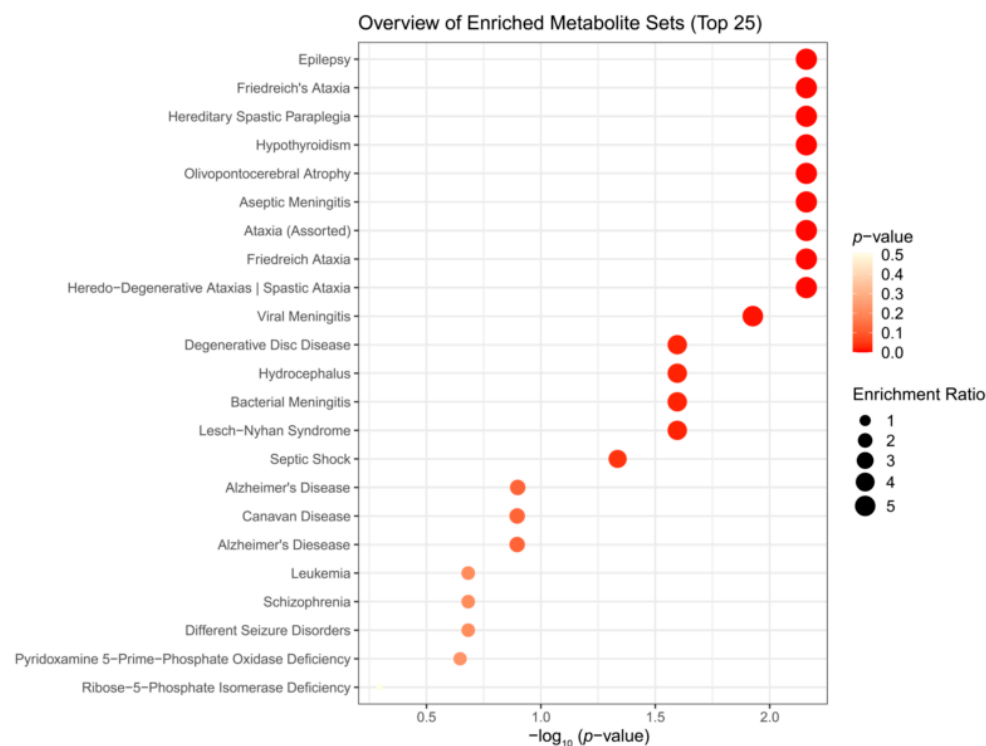


Figure 6. Disease-based enrichment analysis of significantly altered metabolites of early response 72 h post single acute H₂S exposure for primary metabolites, biogenic amines, and lipidomic metabolites.

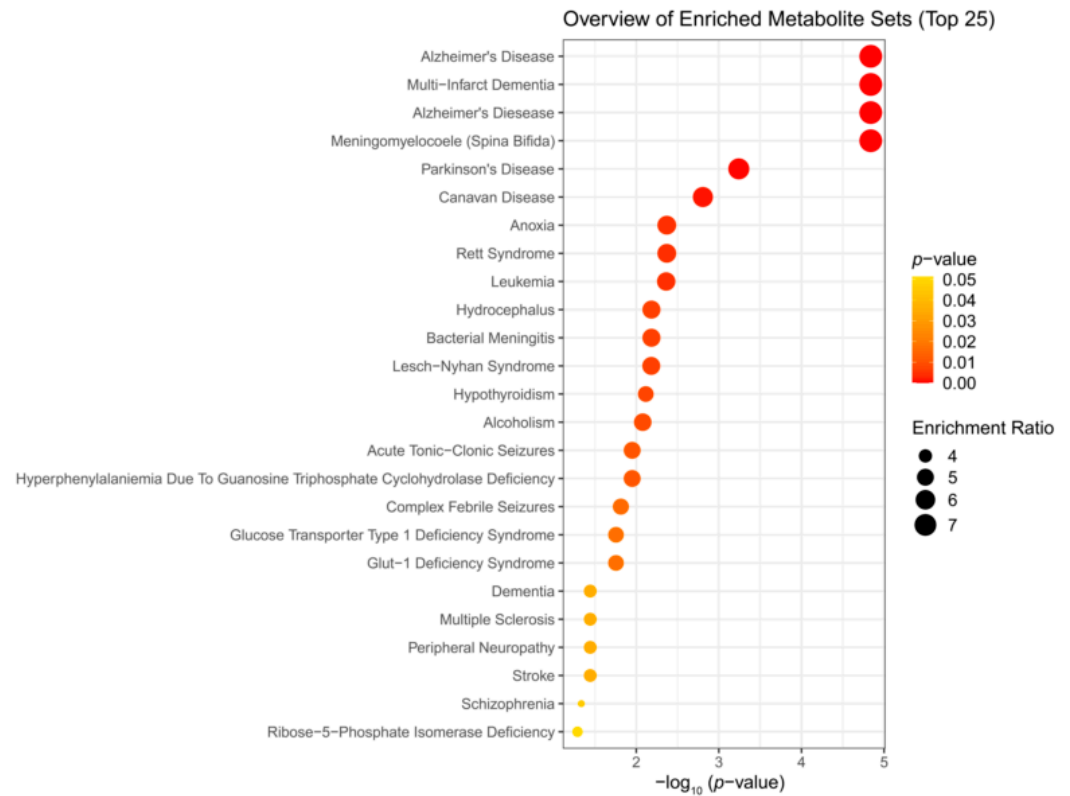


Figure 7. Disease-based enrichment analysis of significantly altered metabolites of subchronic response to repeated H_2S exposure for 5 weeks for primary metabolites, biogenic amines, and lipidomic metabolites.

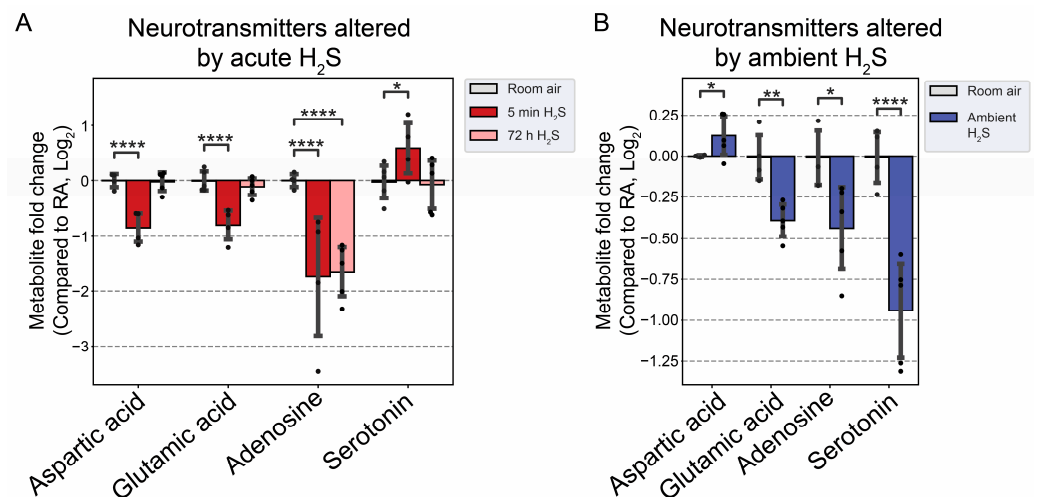


Figure 8. Neurotransmitter changes following H_2S exposure. (A) Fold change in neurotransmitters following acute 1000 ppm H_2S exposure. (B) Fold change in neurotransmitters following subchronic ambient H_2S exposure. H_2S groups were compared to RA control group. Values are presented as mean \pm standard deviation. ANOVA with post hoc Tukey HSD test was performed for acute H_2S exposure groups, while unpaired Student's *t*-test was performed for subchronic ambient H_2S exposure groups to test for statistical significance. Asterisks indicate significant differences compared to RA control group. *p* * < 0.1, ** < 0.05, and **** < 0.001.

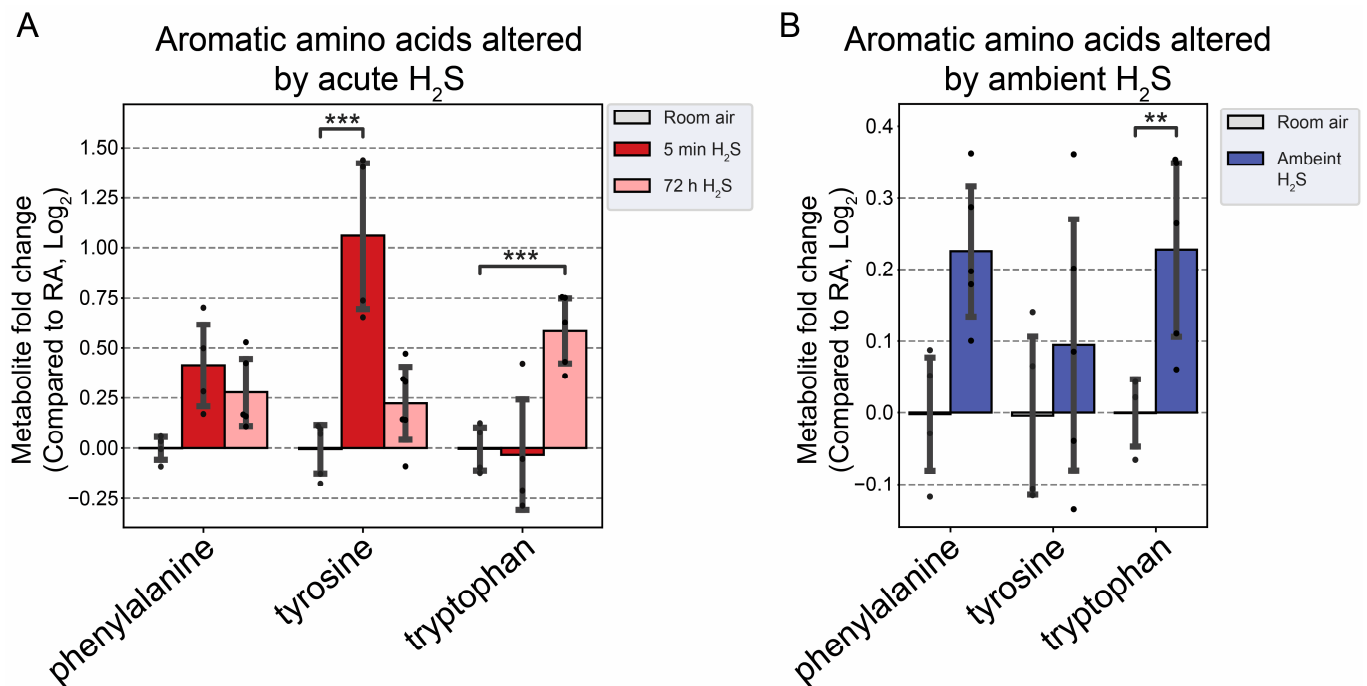


Figure 9. Altered aromatic amino acids following H₂S exposure. (A) Fold change in aromatic amino acids following acute 1000 ppm H₂S exposure. (B) Fold change in aromatic amino acids following subchronic ambient H₂S exposure. H₂S groups were compared to RA control group. Values are presented as mean \pm standard deviation. ANOVA with post hoc Tukey HSD test was performed for acute H₂S exposure groups, while unpaired Student's *t*-test was performed for subchronic ambient H₂S exposure groups to test for statistical significance. Asterisks indicate statistically significant differences compared to RA control group. *p* ** < 0.05 and *** < 0.01.

3.5. H₂S Dysregulated Energy Homeostasis

We analyzed to determine changes in lipid metabolism in the brainstem following acute and subchronic H₂S exposures. Immediately following acute H₂S exposure, BCAAs were significantly increased more than two-fold (Figure 10A). Interestingly, subchronic ambient H₂S also significantly increased BCAAs, albeit less than acute exposure (Figure 10B).

Behenic acid concentration, a very long-chain fatty acid, was significantly decreased more than two-fold following acute H₂S exposure (Figure 11A). Following subchronic ambient H₂S exposure, major fatty acids in the brainstem including oleic acid, arachidonic acid (AA), and docosahexaenoic acid (DHA) were significantly increased compared to control (Figure 11B).

Cellular respiration was also dysregulated following both acute and subchronic ambient H₂S exposures. Glucose concentration was significantly elevated at 5 min post acute H₂S exposure, while it decreased following ambient H₂S exposure (Figure 12). Elevated glucose concentration can affect multiple biological pathways. The polyol pathway converts glucose to sorbitol and then to fructose. Sorbitol and fructose concentrations were also significantly increased at 5 min post acute H₂S exposure. Lactate is formed via glycolysis and was also increased following acute H₂S exposure, but it was decreased following subchronic ambient H₂S exposure. However, ribulose 5-phosphate and UDP-GlcNAc, intermediates of the pentose phosphate pathway (PPP) and hexosamine biosynthetic pathways (HBP), respectively, were not significantly altered. Succinate, fumarate, and malate which are intermediates of the Krebs cycle were also increased at 5 min post acute H₂S exposure.

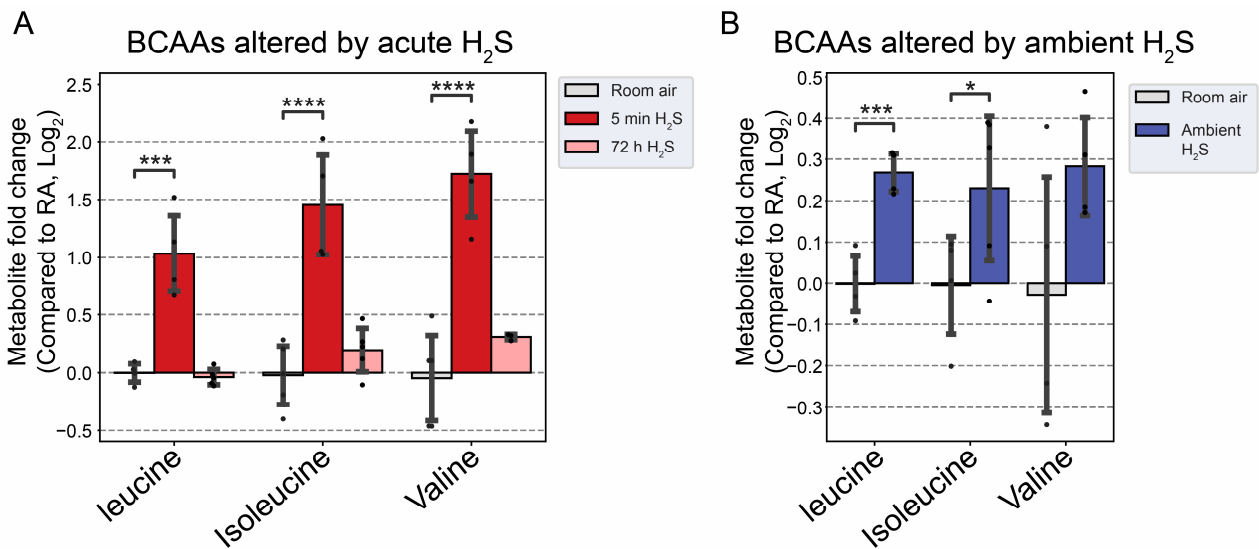


Figure 10. Fold change in branched-chain amino acids following H₂S exposure. (A) Fold change in BCAAs following acute H₂S exposure. (B) Fold change in BCAAs following subchronic ambient H₂S exposure. H₂S groups were compared to RA control group. Values are presented as mean ± standard deviation. ANOVA with post hoc Tukey HSD test was performed for acute H₂S exposure groups, while unpaired Student's *t*-test was performed for subchronic ambient H₂S exposure groups for statistical significance. Asterisks indicate significant differences compared to RA control group. *p* * < 0.1, *** < 0.01, and **** < 0.001.

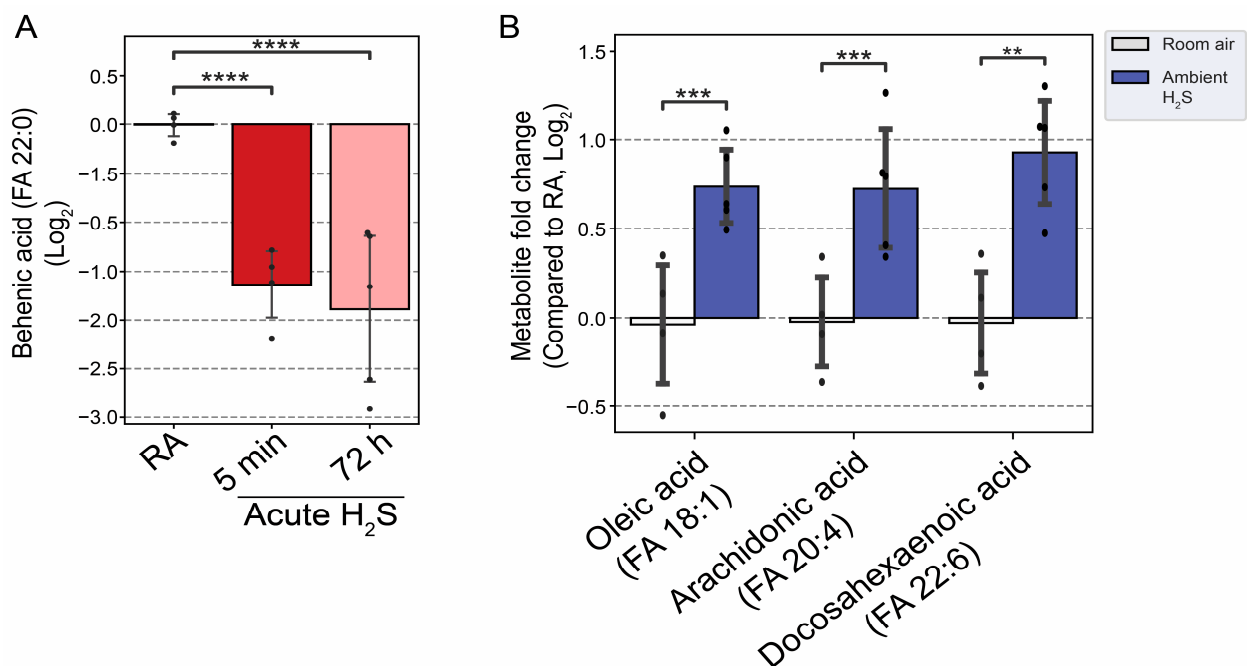


Figure 11. Altered fatty acids following H₂S exposure. (A) Decreased behenic acid, a very long-chain fatty acid (VLCFA), following acute H₂S exposure. (B) Major unsaturated fatty acids in brain were increased following subchronic ambient H₂S exposure. H₂S groups were compared to RA control group. Values are presented as mean ± standard deviation. ANOVA with post hoc Tukey HSD test was performed for acute H₂S exposure groups, while unpaired Student's *t*-test was performed for subchronic ambient H₂S exposure groups for statistical significance. Asterisks indicate significant differences compared to RA control group. *p* ** < 0.05, *** < 0.01, and **** < 0.001.

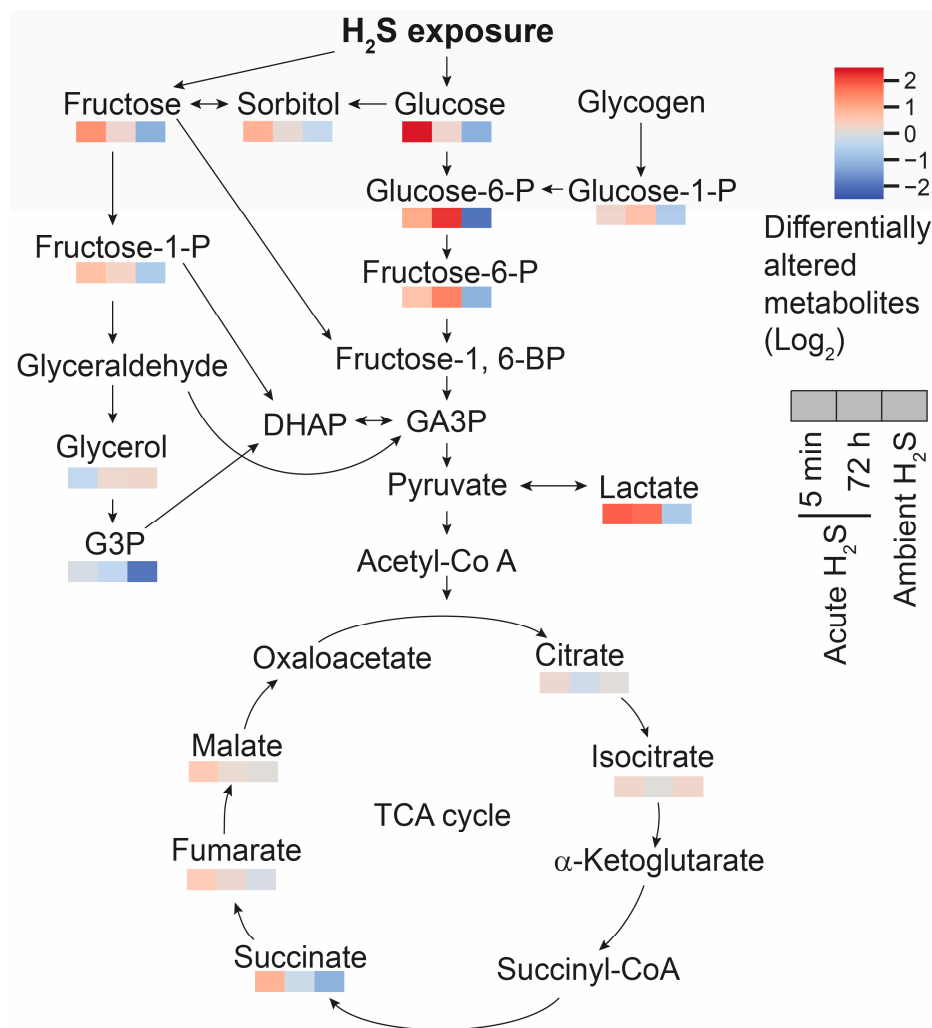


Figure 12. Schematic pathway of glycolysis and TCA cycle. Responses at 5 min and 72 post acute H₂S exposure and also following subchronic ambient H₂S are presented in a heatmap analysis. Fructose, sorbitol, and glucose were significantly increased by acute H₂S exposure but decreased by subchronic ambient H₂S exposures compared to RA control group. Changes in lactic acid are shown in a similar fashion. Succinate, fumarate, and malate concentrations were increased at 5 min post acute H₂S exposure. Differentially altered metabolites are presented in fold changes (log₂ scale). Abbreviations: DHAP: Dihydroxyacetone phosphate; Fructose-1-P: Fructose 1-phosphate; Fructose-1,6-BP: Fructose 1,6-bisphosphate; Fructose-6-P: Fructose 6-phosphate; G3P: Glycerol-3-phosphate; GA3P: Glyceraldehyde 3-phosphate; Glucose-1-P: Glucose 1-phosphate; Glucose-6-P: Glucose 6-phosphate; and TCA cycle: Tricarboxylic acid cycle.

3.6. H₂S Dysregulated Inosine Metabolism

Inosine and its metabolites (hypoxanthine and xanthine) were increased by subchronic ambient H₂S exposure (Figure 13), indicating an increased catabolism of purines under these conditions. Allantoin, an oxidation product of urate and usually considered a marker of oxidative stress, was the only metabolite increased at 5 min post acute H₂S exposure. In contrast, inosine, hypoxanthine, and xanthine were all increased following subchronic ambient H₂S exposure along with guanine and guanosine which are converted to xanthine (Figure 13).

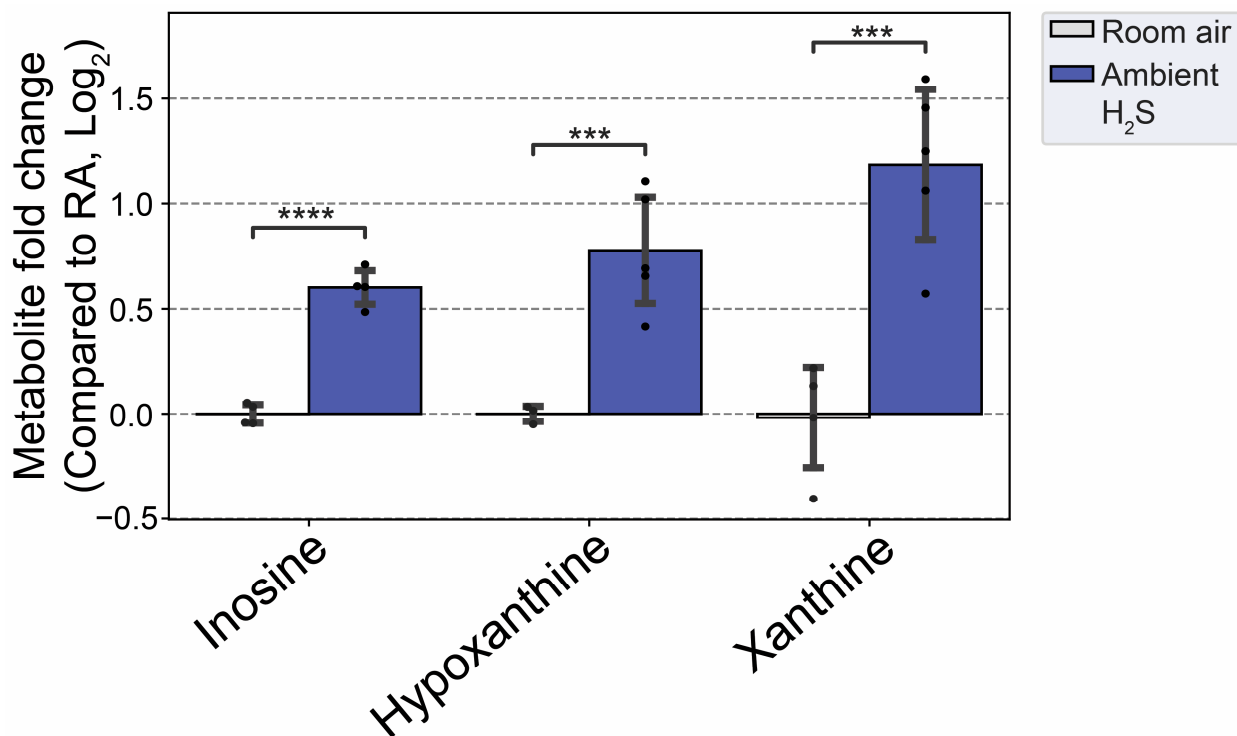


Figure 13. Altered inosine and metabolites following subchronic ambient H₂S exposure. H₂S groups were compared to RA control group. Values are presented as mean \pm standard deviation. ANOVA with post hoc Tukey HSD test was performed for acute H₂S exposure groups, while unpaired Student's *t*-test was performed for subchronic ambient H₂S exposure groups for statistical significance. Asterisks indicate significant differences compared to RA control group. *p* *** < 0.01 and **** < 0.001.

4. Discussion

Hydrogen sulfide is widely reported to impact a broad spectrum of physiological functions. It is also reported to be a highly toxic compound with steep dose–response relationships. Acute high dose exposures are linked to acute death and to permanent neurobehavioral abnormalities and neurodegeneration [28–31], while the impacts of chronic low level H₂S exposure in humans remain ambiguous. Acute H₂S exposure impairs breathing and this is widely cited as the cause of H₂S-induced acute death. The brainstem is an important brain region which regulates autonomous functions including breathing [32]. Despite the significance of this compound in health and disease, the lack of data on the impact of H₂S on the brain metabolism is striking. This is the first comprehensive metabolomic study of acute high dose H₂S exposure and subchronic low dose H₂S exposure on the brain. For the subchronic low dose study, we used 5 ppm H₂S to expose mice only for 2 h per day Monday through Friday for 5 weeks. As a reference, the OSHA TWA exposure guidelines for an 8 h workday, 5 days a week, is 10 ppm. The results showed that both acute and subchronic ambient H₂S significantly altered many classes of metabolites including organic oxygen compounds, organic acids, phenylpropanoids, polyketides, organoheterocyclic compounds, benzenoids, nucleosides, nucleotides, organic nitrogen compounds, lipids, and lipid-like molecules in the brain. Importantly, metabolic changes induced by H₂S exposure were different depending on whether it was a high dose single exposure or subchronic low dose exposure. Some metabolites, however, were increased both by acute and by subchronic exposures, suggesting that such compounds are sensitive indicators of H₂S exposure.

3-Methylhistidine, a derivative of histidine and methionine amino acids, and phosphatidylethanol 18:1_18:1 (PEtOH 18:1_18:1), a glycerophosphoethanol, were both significantly increased following both single H₂S exposure and subchronic ambient H₂S exposure. The functions of these two metabolites in the brainstem are not clear at the moment. Vanillin

was decreased, while vanillin-4-sulfate was increased by acute H₂S exposure. Vanillin has been linked to colorectal cancer. The roles of vanillin and vanillin-4-sulfate and the role of H₂S exposure in altering vanillin and vanillin-4-sulfate remain to be determined. However, these metabolites may serve as potential biomarkers of H₂S poisoning.

Several metabolites were altered at 5 min and 72 h following single acute H₂S exposure. N⁸-acetyl spermidine was increased by 0.8-fold following acute H₂S exposure. It is a polyamine derived from spermidine by deacetylation and is reported to play a role in regulating cell signaling and gene expression, among other functions [33]. It was previously reported that increases in N⁸-acetyl spermidine are caused by inhibiting N⁸-acetylspermidine deacetylase. This compound is linked to the differentiation of neurons and to elevated dopamine [33]. Interestingly, acute H₂S exposure increases dopamine concentration in the brainstem [3]. N⁸-acetyl spermidine is also considered a biomarker for ischemic cardiomyopathy [34]. Interestingly, H₂S is a cytochrome c oxidase inhibitor and causes chemical ischemia [35]. The exact role of N⁸-acetyl spermidine remains to be studied, but it may signal brain ischemia.

Flavin adenine, a derivative of riboflavin or vitamin B₂, is also known as flavin adenine dinucleotide (FAD), and it plays a critical role as a cofactor of many enzymes. FAD was increased more than 1-fold at 5 min and 0.5-fold after 72 h following single acute exposure and is therefore a potential biomarker of acute H₂S exposure in the brainstem. Since FAD plays a significant role as a cofactor in brain energy metabolism, it is possible that a sudden increase in FAD in the brainstem may indicate an imbalance in the redox cellular state (favoring FAD over FADH). FADH is not detectable by any of the mass spectrometers used in this study.

Adenosine and behenic acid were decreased 1-fold and 2-fold at 5 min and 72 h post acute H₂S exposure, respectively. Adenosine is a nucleoside which plays many important roles in energy transfer, in signal transduction, as a neurotransmitter, and as a potent vasodilator [36]. H₂S is well known to reduce ATP generation by inhibiting cytochrome c oxidase in the electron transport chain. Adenosine 5'-monophosphate was significantly decreased by about 1.5-fold following subchronic ambient H₂S exposure. A decrease in adenosine concentration may limit several biological functions in which it is involved. Behenic acid, in contrast, is a very long-chain saturated fatty acid (VLCFA) and was decreased by more than 1.5-fold at 5 min and 72 h post acute H₂S exposure, but not by subchronic H₂S exposure. The reduction in behenic acid concentration may negatively impact energy production and homeostasis, among other effects. These results suggest that both acute and subchronic H₂S exposure alter brainstem metabolism but in different ways. More research is needed to understand the implications of these results on brain function.

The brainstem has been reported to be particularly vulnerable to acute H₂S poisoning. Following exogenous exposure, H₂S was reportedly found in its highest concentrations in this brain region [20]. This brain region is also recognized as the center of regulation for key autonomic functions [37,38]. We have also previously shown that the brainstem is susceptible to H₂S-induced neurodegeneration [3]. In addition, we showed that the inhibition of central respiratory drive (from the brainstem) is a key cause of death in our mouse model of acute H₂S poisoning [3]. Clinical effects of acute H₂S poisoning characterized by seizures and apnea, among others, were not seen in mice subchronically exposed to 5 ppm H₂S for 1 month, indicating that the mechanisms involved were different. In this study, the only clinical effects noted following subchronic H₂S exposure in mice was a loss in body weight.

The results of the metabolite enrichment analysis summarized in Figures 5–7 are very interesting. When the metabolomic profiles of acute and subchronic H₂S exposure in mice are compared to existing databases of metabolomics of the cerebrospinal fluid from patients suffering from different neurological conditions, a pattern of similarities and differences emerges. At 5 min post exposure, the metabolomic enrichment pattern of acute H₂S poisoning matches with many human neurological disorders including early-onset encephalopathy and cortical myoclonus, leukemia, propionic acidemia, anoxia, Rett syn-

drome, epilepsy, Alzheimer's disease (AD), Parkinson's disease (PD), Glut-1 deficiency syndrome, different seizure disorders, and schizophrenia, among others. In this mouse model, we have reported convulsions and neurodegeneration [3,7–9,12,13]. AD and PD are neurodegenerative diseases, while epilepsy matched with convulsions in our mouse model. We have also reported a significant increase in dopamine and serotonin concentrations in this model [3,9,12]. Dopamine is a modulatory neurotransmitter which is both excitatory and inhibitory depending on which receptors it binds to. Serotonin is predominantly inhibitory and has been linked to schizophrenia. The summary of neurotransmitter changes in Figure 8 suggests that acute and subchronic H₂S exposure tilts neurotransmitters toward inhibitory and excitatory effects, respectively. It is probable that the loss of central respiratory drive is linked predominantly to the neurotransmitter status observed following acute H₂S exposure in this study.

H₂S exposure is widely reported to cause hypoxic neuronal injury, which is consistent with its mechanism of action of inhibiting *cytochrome c oxidase* activity [35]. Reduced *cytochrome c oxidase* activity is also reported in Alzheimer's disease [39], and chronic neurodegenerative diseases are characterized by brain energy deficits [1,2]. The metabolomic profiles at 5 min post acute H₂S exposure matched a metabolic profile of neurological disorders observed at 72 h min post acute exposure, including epilepsy, aseptic meningitis, Alzheimer's disease, schizophrenia, and different seizure disorders.

We were surprised to see that subchronic H₂S exposure to 5 ppm, which is within the OSHA guidelines of 10 ppm for an 8 h workday, caused metabolic derangement in the brainstem. This is a significant finding which needs confirmation because of the health implications in the workplace. Strikingly, the metabolome of subchronic ambient H₂S also matched with many human neurological disorders, including Alzheimer's disease, Parkinson's disease, and schizophrenia, which was similar to what we observed after single acute H₂S exposure, with some exceptions. Notably, the metabolome of subchronic ambient H₂S exposure additionally matched that of the CSF from patients with dementia, Canavan disease, alcoholism, and multiple sclerosis. Common themes of both acute and subchronic low dose H₂S exposure revolve around metabolomic similarities between the metabolome of H₂S exposure and that of seizure disorders and neurodegenerative conditions, especially Alzheimer's disease, Parkinson's disease, neuroinflammation, ataxia, and hypoxic-induced injury. This possibly signals that environmental H₂S exposure shifts the brain metabolism, which may predispose or increase susceptibility to these debilitating disease conditions. Indeed, this mouse model with acute H₂S exposure exhibited seizures, knockdown, and acute death [3,6–9,12,13]. These results signal that H₂S-induced metabolomic changes play important roles in H₂S-induced physiological symptoms including seizures and loss of consciousness. Notably, it is interesting that patients predisposed to seizures or neurofibromatosis are very sensitive to acute H₂S poisoning [40]. Also, H₂S aggravated seizure-like events in a rat seizure model [41]. Regarding the discovery of similarities in the metabolomes of acute and subchronic H₂S exposure to those of the CSF of patients with Alzheimer's disease, there is meagre literature on this topic. It is well known that the interaction of genes and the environment plays important roles in many neurological disorders including Alzheimer's disease and Parkinson's disease. Considering that H₂S is an environmental pollutant of occupational concern and that H₂S exposure may be closely linked to many neurological disorders with big health and economic burdens in society, more work should be focused on this area to investigate the potential role of ambient H₂S exposure in AD and/or other neurological conditions summarized in Figures 5–7.

The effects of acute H₂S and subchronic ambient H₂S exposures on neurotransmitters were investigated. Acute H₂S exposure decreased aspartic acid, glutamic acid, and adenosine, while serotonin was increased. Serotonin was significantly decreased by subchronic ambient H₂S exposure. Aspartic acid and glutamic acid are excitatory neurotransmitters, while serotonin is inhibitory [42,43]. Adenosine acts both as an excitatory and inhibitory neurotransmitter. We previously reported that dopamine and serotonin were significantly increased in the brainstem at 5 min post acute H₂S [3], which is consistent with the findings

in this study. Tryptophan is a precursor to serotonin, while phenylalanine and tyrosine are precursors to dopamine. In this study, tyrosine was significantly increased at 5 min post acute exposure in the brainstem, while tryptophan was increased by subchronic ambient H₂S exposure. Other neurotransmitters including acetylcholine, histidine, serine, noradrenaline, γ -aminobutyric acid (GABA), glycine, and histamine were not significantly altered in this study.

Glutamate and aspartate are the main excitatory neurotransmitters in the brain and are released in a Ca²⁺-dependent manner upon electrical stimulation. Serotonin is an inhibitory neurotransmitter and has an impact on a wide range of behavioral effects such as appetite, aggression, memory, fear, and depression. It is also involved in other central nervous system (CNS) functions such as respiration, body temperature, motor control, and bowel movement [44]. It is well known that acute exposure to high concentrations of H₂S induces breathing suppression, a loss of consciousness, and acute death in humans. However, the exact mechanisms behind these effects of acute H₂S-induced toxicity are not known. A decrease in glutamate and aspartate coupled with an increase in serotonin following acute H₂S exposure in the brainstem may play important roles in H₂S-induced inhibition of breathing and/or loss of consciousness, considering that the brainstem regulates essential vital functions including breathing activity and consciousness [32]. Interestingly, we recently reported that sodium sulfide, a H₂S donor, suppressed neuronal activity in vitro by suppressing Ca²⁺ oscillation in primary cortical neurons [6]. An electrical stimulus releases glutamate from synaptic vesicles to the synaptic cleft. The released glutamate is taken up to glial cells by glutamate–aspartate transporters and converted to glutamine, which is then transported back to the neuron. Glutaminase in mitochondria converts glutamine to glutamate and aspartate. Acute H₂S exposure induced increased glutamine at 5 min post acute exposure, indicating that glutamate–glutamine cycling may be dysregulated. A decrease in aspartate may influence the conversion of glutamine to glutamate, leading to a decrease in glutamate upon acute H₂S exposure. Serotonin was also reported to inhibit glutamate release and the action of released glutamate [43]. In addition, an acute surge of serotonin induces focal seizures [45].

Interestingly, subchronic ambient H₂S exposure significantly decreased serotonin concentration. The current OSHA guideline sets the recommended airborne exposure limit (REL) to 10 ppm for hydrogen sulfide in the workplace [22]. To the best of our knowledge, it has not been previously reported that subchronic ambient H₂S exposure dysregulates serotonin. Therefore, this finding is novel. There are many serotonin receptors. It should be interesting to investigate the health impact of decreased serotonin in subchronic ambient H₂S exposure.

Glutamate not only acts as a neurotransmitter but also as an anaplerotic component in tricarboxylic acid (TCA) cycle in energy balance. Glutamate is converted to α -ketoglutarate. In addition to the altered glutamate and glutamine, fumarate and malate were increased by 0.5-fold compared to the RA control mice at 5 min post acute H₂S exposure. Intermediates of the TCA cycle including succinate, fumarate, and malate were increased at 5 min post acute H₂S exposure, while succinate and fumarate were decreased following ambient H₂S exposure.

Glucose is a key compound in glycolysis. Glucose concentration was significantly increased at 5 min post acute H₂S exposure compared to the RA control group but decreased following ambient H₂S exposure. An increase in brain glucose during acute H₂S exposure may suggest that it is spared following the inhibition of cytochrome c oxidase by H₂S. Elevated glucose concentrations can affect multiple biological pathways including the polyol pathway, glycolysis, the PPP, and HBP. Intermediates of the polyol pathway and glycolysis were increased, while the PPP and HBP were not significantly altered at 5 min post acute H₂S exposure, indicating acute H₂S exposure significantly dysregulates energy balance. An accumulation of succinate may be caused by inverse activity of succinate dehydrogenase converting fumarate to succinate [46]. Lactate concentration remained elevated following acute H₂S exposure, which is in line with previous findings that the

treatment of sodium hydrosulfide induced elevation in lactate. It was shown that acute H₂S exposure-induced inhibition of cytochrome c oxidase in the brain, including the brainstem, remained up to 72 h [3]. It is plausible that the inhibition of cytochrome c oxidase subsequently affected the ETC and TCA cycle following acute H₂S exposure, which could not meet the energy demand of the CNS. An accumulation of succinate was shown to produce ROS and induce static epilepticus in a kainic acid rat model [46]. Although the exact effects of ambient H₂S exposure on decreased glucose levels needs to be confirmed, ambient H₂S exposure decreased the glucose level compared to the RA control group.

Fatty acids have multiple important roles in the nervous system, from serving as structural components of membranes, energy sources, signaling molecules, cellular differentiation, and apoptosis to contributing to pathological conditions such as aging and neurodegenerative diseases [47]. The regulation of fatty acids is tightly controlled and in different ways in different brain regions. Behenic acid is a VLCFA. VLCFAs are preferentially metabolized in the peroxisome. The statistically significant reduction in behenic acid observed in this study may indicate altered peroxisome fatty acid beta oxidation. Subchronic ambient H₂S strikingly increased metabolites of both the mono-unsaturated fatty acid (MUFA) and poly-unsaturated fatty acid (PUFA) synthesis pathways in the brainstem, including oleic acid, arachidonic acid (AA), and docosahexaenoic acid (DHA) in the brainstem compared to the RA control group. Arachidonic acid, which was also increased, is pro-inflammatory and is also linked to neurodegenerative diseases such as AD [48]. A deficiency in DHA has been associated with neurodegenerative disorders [49]. We have also previously reported that acute H₂S poisoning causes neuroinflammation and neurodegeneration [3,9,12,13].

Subchronic ambient H₂S also dysregulated inosine metabolism and increased inosine, hypoxanthine, and xanthine. Inosine is metabolized to hypoxanthine, xanthine, and finally to uric acid [50]. Inosine was shown to play important roles in neuroprotection, among other roles, presumably via anti-inflammatory and antioxidant properties [51]. AMP was shown to be metabolized to IMP, hypoxanthine, xanthine, and uric acid to enhance ATP production during acute energy consumption [50]. The administration of inosine was shown to induce hypoglycemia [52]. Indeed, subchronic ambient H₂S decreased glucose concentration more than 1-fold compared to the RA control group. Hypoxanthine and its metabolites were shown to be increased in post mortem tissues over a time span of several hours [53]. In this study, brainstem regions were immediately dissected and flash-frozen. Moreover, the changes in hypoxanthine and its metabolites were compared to those of the RA control group which were handled identically to brain tissues from H₂S exposed mice. This finding shows that subchronic ambient H₂S dysregulates energy homeostasis. More work is needed on this topic to determine whether subchronic ambient H₂S exposure causes a brain energy deficit. Brain energy deficit has been cited a contributing factor in neurodegeneration [1,2]. Potentially, this suggests that ambient H₂S exposure may be an environmental factor predisposing to neurodegeneration.

This study has many potential applications. Understanding the basic mechanisms of H₂S-induced neurotoxicity will lead to development of therapeutic drugs for the treatment of victims of acute and subchronic H₂S poisoning. Hydrogen sulfide is considered a chemical weapon [54,55], and currently, there are no FDA-approved drugs for treating victims of acute H₂S poisoning. Also, environmental pollutants, including those that affect mitochondria, play a role in inducing neurodegeneration. The results of this study will likely contribute to our knowledge on the role that H₂S plays in neurodegeneration and other neurological diseases shown in Figures 5–7. In addition, this work may guide the development of novel diagnostic and forensic biomarkers of acute and subchronic H₂S poisoning.

This study, though yielding interesting results, had some limitations. For example, this study only consisted of male, adult mice. Future studies should include female mice as well so as to determine whether there may be sex differences in brain metabolism following H₂S exposure. As has been reported [9], male mice are more sensitive to acute

H₂S poisoning than females. Therefore, including female mice in this study may shed light on the toxic mechanisms of H₂S. Another limitation of this study was that the acute study only lasted up to 72 h post H₂S exposure. In previous studies, we have shown neurodegeneration to be fully manifested on day 7 in the brainstem and other brain regions [3,5,7–9]. Also, in human victims of single acute H₂S poisoning accidents, a plethora of long-term debilitating neurological sequelae including neuropsychiatric disturbance, sleep disorders, headaches, memory and cognition deficits, and persistent vegetative states are reported [25–28,56]. Therefore, future studies should investigate metabolomic changes in the brainstem several months later to determine the metabolomic profiles of delayed neurotoxic effects. Finally, concentrations of tissue metabolites, especially brain tissue, can be influenced by and vary in sample collection and sample preparation procedures [57,58]. It has been shown that concentrations of brain tissue metabolites will decrease or increase if the post mortem autolysis is not prevented by procedures like microwave fixation before brain dissection [57,58]. In this study, control and H₂S exposed mice were decapitated and the brain was rapidly removed and micro-dissected on ice over a 2–3 min time period before they were immediately stored at –80 °C until analysis. All experiments had suitable control samples and all samples were handled similarly, and comparisons were made between the RA control and H₂S exposed mice. Thus, whereas the absolute concentrations of metabolites would likely be different if samples were microwave fixed before brain microdissection, the outcome of the study should be the same. However, future metabolomic studies will be performed on microwave-fixed mice to completely stop enzymatic activity during tissue collection and sample preparation.

5. Conclusions

In summary, the results of this study identified 3-methylhistidine and PEtOH18:1_18:1 as potential biomarkers of H₂S exposure, because they were significantly increased in the brainstems of mice exposed to H₂S both acutely and subchronically. Twelve metabolites were altered by acute H₂S exposure including N-acetylspermidine, adenosine, behenic acid, flavin adenine, vanillin, vanillin-4-sulfate, caffeic acid, and behenic acid, which were identified as potential biomarkers of acute H₂S exposure. Acute H₂S decreased glutamate and aspartate, while serotonin was increased, suggesting that overall acute H₂S shifts neurotransmitters predominantly toward inhibitory status. Serotonin was decreased by subchronic ambient H₂S exposure and so was adenosine, suggesting that chronic H₂S exposure may predispose neurological excitation. Glucose and fructose were increased in acute H₂S poisoning, while glucose was significantly decreased in subchronic ambient H₂S. MUFAs and PUFAs were increased in subchronic ambient H₂S exposure. Overall, the results of this study provide evidence that both acute and subchronic H₂S exposure alters neurotransmitters and energy metabolism in the brainstem. This has opened novel mechanisms underlying H₂S-induced neurotoxicity. Surprisingly, and more importantly, this study shows that current OSHA guidelines (10 ppm for 40 h/work week) may not be adequately protecting worker health, as a subchronic exposure of mice to 5 ppm H₂S for only a few hours per day significantly altered neurotransmitters and energy metabolism.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/metabo14010053/s1>, Information S1: Primary metabolites by GC-TOF MS, Information S2: HILIC-MS/MS analysis for hydrophilic compounds including biogenic amines, Information S3: RPLC-MS/MS analyses for untargeted lipidomics metabolites.

Author Contributions: Conceptualization. W.K.R.; data curation. D.-S.K.; methodology. D.-S.K., C.M.S.M. and W.K.R.; software. D.-S.K.; investigation. D.-S.K. and C.M.S.M.; project administration. D.-S.K.; resources. W.K.R.; validation. D.-S.K.; formal analysis D.-S.K.; supervision. D.-S.K. and W.K.R.; visualization. D.-S.K.; writing—original draft preparation, D.-S.K. and W.K.R.; writing—review and editing, D.-S.K., C.G. and W.K.R.; funding acquisition, W.K.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Iowa state University internal funds for W.K.R. and start-up fundings by the University of California, Davis for W.K.R.

Institutional Review Board Statement: This study was conducted in accordance with the Institutional Animal Care and Use Committee (IACUC) of the University of California at Davis (UC Davis). The animal study protocol was approved by IACUC with IACUC-18-136 on 25 September 2018 at ISU and with IACUC-21819 on at UC Davis on 19 October 2020.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are openly available in dryad at <https://doi.org/10.5061/dryad.tdz08kq5n> accessed on 21 December 2023.

Acknowledgments: The authors are thankful to the West Coast Metabolomics Center, UC Davis, for their support.

Conflicts of Interest: The authors declare no conflicts of interest, including commercial interests. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Cunnane, S.C.; Trushina, E.; Morland, C.; Prigione, A.; Casadesus, G.; Andrews, Z.B.; Beal, M.F.; Bergersen, L.H.; Brinton, R.D.; de la Monte, S.; et al. Brain energy rescue: An emerging therapeutic concept for neurodegenerative disorders of ageing. *Nat. Rev. Drug Discov.* **2020**, *19*, 609–633. [[CrossRef](#)] [[PubMed](#)]
2. Yin, F.; Sancheti, H.; Patil, I.; Cadenas, E. Energy metabolism and inflammation in brain aging and Alzheimer's disease. *Free Radic. Biol. Med.* **2016**, *100*, 108–122. [[CrossRef](#)] [[PubMed](#)]
3. Santana Maldonado, C.M.; Kim, D.S.; Purnell, B.; Li, R.; Buchanan, G.F.; Smith, J.; Thedens, D.R.; Gauger, P.; Rumbelha, W.K. Acute hydrogen sulfide-induced neurochemical and morphological changes in the brainstem. *Toxicology* **2023**, *485*, 153424. [[CrossRef](#)] [[PubMed](#)]
4. Santana Maldonado, C.; Weir, A.; Rumbelha, W.K. A comprehensive review of treatments for hydrogen sulfide poisoning: Past, present, and future. *Toxicol. Mech. Methods* **2023**, *33*, 183–196. [[CrossRef](#)]
5. Rumbelha, W.K.; Kim, D.S.; Min, A.; Nair, M.; Giulivi, C. Disrupted brain mitochondrial morphology after in vivo hydrogen sulfide exposure. *Sci. Rep.* **2023**, *13*, 18129. [[CrossRef](#)]
6. Kim, D.S.; Pessah, I.N.; Santana, C.M.; Purnell, B.; Li, R.; Buchanan, G.F.; Rumbelha, W.K. Investigations into hydrogen sulfide-induced suppression of neuronal activity in vivo and calcium dysregulation in vitro. *Toxicol. Sci.* **2023**, *192*, 247–264. [[CrossRef](#)] [[PubMed](#)]
7. Kim, D.S.; Anantharam, P.; Padhi, P.; Thedens, D.R.; Li, G.; Gilbreath, E.; Rumbelha, W.K. Transcriptomic profile analysis of brain inferior colliculus following acute hydrogen sulfide exposure. *Toxicology* **2020**, *430*, 152345. [[CrossRef](#)] [[PubMed](#)]
8. Kim, D.S.; Anantharam, P.; Hoffmann, A.; Meade, M.L.; Grobe, N.; Gearhart, J.M.; Whitley, E.M.; Mahama, B.; Rumbelha, W.K. Broad spectrum proteomics analysis of the inferior colliculus following acute hydrogen sulfide exposure. *Toxicol. Appl. Pharmacol.* **2018**, *355*, 28–42. [[CrossRef](#)]
9. Anantharam, P.; Whitley, E.M.; Mahama, B.; Kim, D.S.; Imerman, P.M.; Shao, D.; Langley, M.R.; Kanthasamy, A.; Rumbelha, W.K. Characterizing a mouse model for evaluation of countermeasures against hydrogen sulfide-induced neurotoxicity and neurological sequelae. *Ann. N. Y. Acad. Sci.* **2017**, *1400*, 46–64. [[CrossRef](#)]
10. Rumbelha, W.; Whitley, E.; Anantharam, P.; Kim, D.S.; Kanthasamy, A. Acute hydrogen sulfide-induced neuropathology and neurological sequelae: Challenges for translational neuroprotective research. *Ann. N. Y. Acad. Sci.* **2016**, *1378*, 5–16. [[CrossRef](#)]
11. Tiranti, V.; Viscomi, C.; Hildebrandt, T.; Di Meo, I.; Mineri, R.; Tiveron, C.; Levitt, M.D.; Prella, A.; Fagiolarini, G.; Rimoldi, M.; et al. Loss of ETHE1, a mitochondrial dioxygenase, causes fatal sulfide toxicity in ethylmalonic encephalopathy. *Nat. Med.* **2009**, *15*, 200–205. [[CrossRef](#)] [[PubMed](#)]
12. Anantharam, P.; Whitley, E.M.; Mahama, B.; Kim, D.S.; Sarkar, S.; Santana, C.; Chan, A.; Kanthasamy, A.G.; Kanthasamy, A.; Boss, G.R.; et al. Cobinamide is effective for treatment of hydrogen sulfide-induced neurological sequelae in a mouse model. *Ann. N. Y. Acad. Sci.* **2017**, *1408*, 61–78. [[CrossRef](#)] [[PubMed](#)]
13. Anantharam, P.; Kim, D.S.; Whitley, E.M.; Mahama, B.; Imerman, P.; Padhi, P.; Rumbelha, W.K. Midazolam Efficacy Against Acute Hydrogen Sulfide-Induced Mortality and Neurotoxicity. *J. Med. Toxicol. Off. J. Am. Coll. Med. Toxicol.* **2018**, *14*, 79–90. [[CrossRef](#)] [[PubMed](#)]
14. Guidotti, T.L. Hydrogen sulfide: Advances in understanding human toxicity. *Int. J. Toxicol.* **2010**, *29*, 569–581. [[CrossRef](#)] [[PubMed](#)]
15. Guidotti, T.L. Hydrogen Sulfide intoxication. In *Handbook of Clinical Neurology*, 3rd ed.; Lotti, M., Bleecker, M.L., Eds.; Elsevier: Amsterdam, The Netherlands, 2015; Volume 131.

16. Reiffenstein, R.J. Neurochemical and neurophysiological effects of high dose hydrogen sulphide. In Proceedings of the International Conference on Hydrogen Sulphide Toxicity, Banff, AB, Canada, 18–21 June 1989; Prior, M.G., Roth, S.H., Green, F.H.Y., Hulbert, W.C., Reiffenstein, R., Eds.; The Sulphide Research Network: Banff, AB, Canada, 1989.
17. Reiffenstein, R.J.; Hulbert, W.C.; Roth, S.H. Toxicology of hydrogen sulfide. *Annu. Rev. Pharmacol. Toxicol.* **1992**, *32*, 109–134. [[CrossRef](#)] [[PubMed](#)]
18. Beauchamp, R.O., Jr.; Bus, J.S.; Popp, J.A.; Boreiko, C.J.; Andjelkovich, D.A. A critical review of the literature on hydrogen sulfide toxicity. *Crit. Rev. Toxicol.* **1984**, *13*, 25–97. [[CrossRef](#)]
19. Vicas, I.M.O. Therapeutics and Management of H₂S Poisoning. In Proceedings of the International Conference on Hydrogen Sulphide Toxicity, Banff, AB, Canada, 18–21 June 1989; Prior, M.G., Roth, S.H., Green, F.H.Y., Hulbert, W.C., Reiffenstein, R., Eds.; Sulphide Research Network. Department of Pharmacology, University of Alberta: Banff, AB, Canada, 1989; pp. 217–219.
20. Warenycia, M.W.; Goodwin, L.R.; Benishin, C.G.; Reiffenstein, R.J.; Francom, D.M.; Taylor, J.D.; Dieken, F.P. Acute Hydrogen sulfide poisoning. *Biochem. Pharmacol.* **1989**, *38*, 973–981. [[CrossRef](#)]
21. Sprague, W. Arrive Guidelines 2.0. *Vet. Clin. Pathol.* **2020**, *49*, 378–379. [[CrossRef](#)]
22. Occupational Safety and Health Administration. Hydrogen Sulfide. Available online: <https://www.osha.gov/hydrogen-sulfide/hazards> (accessed on 21 December 2023).
23. Occupational Safety and Health Administration. Hydrogen Sulfide. Available online: <https://www.osha.gov/SLTC/hydrogensulfide/standards.html> (accessed on 10 December 2023).
24. Fiehn, O. Metabolomics by Gas Chromatography-Mass Spectrometry: Combined Targeted and Untargeted Profiling. *Curr. Protoc. Mol. Biol.* **2016**, *114*, 30–34. [[CrossRef](#)]
25. Matyash, V.; Liebisch, G.; Kurzchalia, T.V.; Shevchenko, A.; Schwudke, D. Lipid extraction by methyl-tert-butyl ether for high-throughput lipidomics. *J. Lipid Res.* **2008**, *49*, 1137–1146. [[CrossRef](#)]
26. Tsugawa, H.; Cajka, T.; Kind, T.; Ma, Y.; Higgins, B.; Ikeda, K.; Kanazawa, M.; VanderGheynst, J.; Fiehn, O.; Arita, M. MS-DIAL: Data-independent MS/MS deconvolution for comprehensive metabolome analysis. *Nat. Methods* **2015**, *12*, 523–526. [[CrossRef](#)] [[PubMed](#)]
27. Cajka, T.; Smilowitz, J.T.; Fiehn, O. Validating Quantitative Untargeted Lipidomics Across Nine Liquid Chromatography-High-Resolution Mass Spectrometry Platforms. *Anal. Chem.* **2017**, *89*, 12360–12368. [[CrossRef](#)] [[PubMed](#)]
28. Tvedt, B.; Edland, A.; Skyberg, K.; Forberg, O. Delayed neuropsychiatric sequelae after acute hydrogen sulfide poisoning: Affection of motor function, memory, vision and hearing. *Acta Neurol. Scand.* **1991**, *84*, 348–351. [[CrossRef](#)] [[PubMed](#)]
29. Nam, B.; Kim, H.; Choi, Y.; Lee, H.; Hong, E.S.; Park, J.K.; Lee, K.M.; Kim, Y. Neurologic sequela of hydrogen sulfide poisoning. *Ind. Health* **2004**, *42*, 83–87. [[CrossRef](#)]
30. Matsuo, F.; Cummins, J.W.; Anderson, R.E. Neurological sequelae of massive hydrogen sulfide inhalation. *Arch. Neurol.* **1979**, *36*, 451–452. [[CrossRef](#)] [[PubMed](#)]
31. Snyder, J.W.; Safir, E.F.; Summerville, G.P.; Middleberg, R.A. Occupational fatality and persistent neurological sequelae after mass exposure to hydrogen sulfide. *Am. J. Emerg. Med.* **1995**, *13*, 199–203. [[CrossRef](#)] [[PubMed](#)]
32. Basinger, H.; Hogg, J.P. *Neuroanatomy, Brainstem*; StatPearls Publishing: St. Petersburg, FL, USA, 2023. Available online: <https://www.ncbi.nlm.nih.gov/books/NBK544297/> (accessed on 10 December 2023).
33. Mudumba, S.; Menezes, A.; Fries, D.; Blankenship, J. Differentiation of PC12 cells induced by N8-acetylspermidine and by N8-acetylspermidine deacetylase inhibition. *Biochem. Pharmacol.* **2002**, *63*, 2011–2018. [[CrossRef](#)]
34. Nayak, A.; Liu, C.; Mehta, A.; Ko, Y.A.; Tahhan, A.S.; Dhindsa, D.S.; Uppal, K.; Jones, D.P.; Butler, J.; Morris, A.A.; et al. N8-Acetylspermidine: A Polyamine Biomarker in Ischemic Cardiomyopathy With Reduced Ejection Fraction. *J. Am. Heart Assoc.* **2020**, *9*, e016055. [[CrossRef](#)]
35. Baldelli, R.J.; Green, F.H.; Auer, R.N. Sulfide toxicity: Mechanical ventilation and hypotension determine survival rate and brain necrosis. *J. Appl. Physiol.* **1993**, *75*, 1348–1353. [[CrossRef](#)]
36. Boison, D. Adenosine as a neuromodulator in neurological diseases. *Curr. Opin. Pharmacol.* **2008**, *8*, 2–7. [[CrossRef](#)]
37. Faraguna, U.; Ferrucci, M.; Giorgi, F.S.; Fornai, F. Editorial: The Functional Anatomy of the Reticular Formation. *Front. Neuroanat.* **2019**, *13*, 55. [[CrossRef](#)] [[PubMed](#)]
38. Mangold, S.A.; Das, J.M. *Neuroanatomy, Reticular Formation*; StatPearls: St. Petersburg, FL, USA, 2023. Available online: <https://www.ncbi.nlm.nih.gov/books/NBK556102/> (accessed on 10 December 2023).
39. Cardoso, S.M.; Proenca, M.T.; Santos, S.; Santana, I.; Oliveira, C.R. Cytochrome c oxidase is decreased in Alzheimer’s disease platelets. *Neurobiol. Aging* **2004**, *25*, 105–110. [[CrossRef](#)]
40. Shivanthan, M.C.; Perera, H.; Jayasinghe, S.; Karunanayake, P.; Chang, T.; Ruwanpathirana, S.; Jayasinghe, N.; De Silva, Y.; Jayaweerabandara, D. Hydrogen sulphide inhalational toxicity at a petroleum refinery in Sri Lanka: A case series of seven survivors following an industrial accident and a brief review of medical literature. *J. Occup. Med. Toxicol.* **2013**, *8*, 9. [[CrossRef](#)] [[PubMed](#)]
41. Luo, Y.; Wu, P.F.; Zhou, J.; Xiao, W.; He, J.G.; Guan, X.L.; Zhang, J.T.; Hu, Z.L.; Wang, F.; Chen, J.G. Aggravation of seizure-like events by hydrogen sulfide: Involvement of multiple targets that control neuronal excitability. *CNS Neurosci. Ther.* **2014**, *20*, 411–419. [[CrossRef](#)] [[PubMed](#)]
42. Dingledine, R.; McBain, C.J. Glutamate and Aspartate Are the Major Excitatory Transmitters in the Brain. In *Basic Neurochemistry: Molecular, Cellular and Medical Aspects*, 6th ed.; Lippincott: New York, NY, USA, 1999.

43. Maura, G.; Marcoli, M.; Pepicelli, O.; Rosu, C.; Viola, C.; Raiteri, M. Serotonin inhibition of the NMDA receptor/nitric oxide/cyclic GMP pathway in human neocortex slices: Involvement of 5-HT(2C) and 5-HT(1A) receptors. *Br. J. Pharmacol.* **2000**, *130*, 1853–1858. [[CrossRef](#)] [[PubMed](#)]
44. Berger, M.; Gray, J.A.; Roth, B.L. The expanded biology of serotonin. *Annu. Rev. Med.* **2009**, *60*, 355–366. [[CrossRef](#)] [[PubMed](#)]
45. Almallouhi, E.; Rahwan, M.; Dainton, H.; Bonilha, L. Focal seizure as a manifestation of serotonin syndrome: Case report. *Avicenna J. Med.* **2019**, *9*, 119–121. [[CrossRef](#)]
46. Zhang, Y.; Zhang, M.; Zhu, W.; Yu, J.; Wang, Q.; Zhang, J.; Cui, Y.; Pan, X.; Gao, X.; Sun, H. Succinate accumulation induces mitochondrial reactive oxygen species generation and promotes status epilepticus in the kainic acid rat model. *Redox Biol.* **2020**, *28*, 101365. [[CrossRef](#)]
47. Hussain, G.; Schmitt, F.; Loeffler, J.P.; Gonzalez de Aguilar, J.L. Fattening the brain: A brief of recent research. *Front. Cell. Neurosci.* **2013**, *7*, 144. [[CrossRef](#)]
48. Thomas, M.H.; Pelleieux, S.; Vitale, N.; Olivier, J.L. Arachidonic acid in Alzheimer's disease. *J. Neurol. Neuromed.* **2016**, *1*, 1–6.
49. Kerdiles, O.; Laye, S.; Calon, F. Omega-3 polyunsaturated fatty acids and brain health: Preclinical evidence for the prevention of neurodegenerative diseases. *Trends Food Sci. Technol.* **2017**, *69*, 203–213. [[CrossRef](#)]
50. Johnson, T.A.; Jinnah, H.A.; Kamatani, N. Shortage of Cellular ATP as a Cause of Diseases and Strategies to Enhance ATP. *Front. Pharmacol.* **2019**, *10*, 98. [[CrossRef](#)]
51. Nascimento, F.P.; Macedo-Junior, S.J.; Lapa-Costa, F.R.; Cezar-Dos-Santos, F.; Santos, A.R.S. Inosine as a Tool to Understand and Treat Central Nervous System Disorders: A Neglected Actor? *Front. Neurosci.* **2021**, *15*, 703783. [[CrossRef](#)] [[PubMed](#)]
52. Abdelkader, N.F.; Ibrahim, S.M.; Moustafa, P.E.; Elbaset, M.A. Inosine mitigated diabetic peripheral neuropathy via modulating GLO1/AGEs/RAGE/NF-kappaB/Nrf2 and TGF-beta/PKC/TRPV1 signaling pathways. *Biomed. Pharmacother.* **2022**, *145*, 112395. [[CrossRef](#)] [[PubMed](#)]
53. Kovacs, Z.; Kekesi, K.A.; Bobest, M.; Torok, T.; Szilagyi, N.; Szikra, T.; Szepesi, Z.; Nyilas, R.; Dobolyi, A.; Palkovits, M.; et al. Post mortem degradation of nucleosides in the brain: Comparison of human and rat brains for estimation of in vivo concentration of nucleosides. *J. Neurosci. Methods* **2005**, *148*, 88–93. [[CrossRef](#)] [[PubMed](#)]
54. Binder, M.K.; Quigley, J.M.; Tinsley, H.F. Islamic State chemical weapons: A case contained by its context? *CTC Sentin.* **2018**, *11*, 27–31.
55. HAZMAT/SAFETY. TSA Issues Security Awareness Message on Potential Use of H2S in Terrorist Attack. Available online: <https://www.bulktransporter.com/hazmatsafety/tsa-issues-security-awareness-message-potential-use-h2s-terrorist-attack> (accessed on 10 December 2023).
56. Wasch, H.H.; Estrin, W.J.; Yip, P.; Bowler, R.; Cone, J.E. Prolongation of the P-300 latency associated with hydrogen sulfide exposure. *Arch. Neurol.* **1989**, *46*, 902–904. [[CrossRef](#)]
57. Fomenko, M.V.; Yanshole, L.V.; Tsentalovich, Y.P. Stability of Metabolomic Content during Sample Preparation: Blood and Brain Tissues. *Metabolites* **2022**, *12*, 811. [[CrossRef](#)]
58. Dienel, G.A. Stop the rot. Enzyme inactivation at brain harvest prevents artifacts: A guide for preservation of the in vivo concentrations of brain constituents. *J. Neurochem.* **2021**, *158*, 1007–1031. [[CrossRef](#)]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.