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Farnesol Brain Transcriptomics in CNS Inflammatory Demyelination

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Highlights

- Whole-brain transcriptomics analysis shows that EAE causes dysregulation of neuronal signaling and maturation and a pro-inflammatory profile.
- Treatment with farnesol (FOL) attenuates the expression of immune/inflammation response genes and pathways.
- FOL treatment alters cellular stress response pathways and genome regulation/repair mechanism pathways in EAE Brains

Abstract

Background: Farnesol (FOL) prevents the onset of experimental autoimmune encephalitis (EAE), a murine model of multiple sclerosis (MS). **Objective:** We examined the transcriptomic profile of the brains of EAE mice treated with daily oral FOL using next-generation sequencing (RNA-seq). **Methods:** Transcriptomics from whole brains of treated and untreated EAE mice at the peak of EAE was performed. **Results:** EAE-induced mice, compared to naïve, healthy mice, overall showed increased expression in pathways for immune response, as well as an increased cytokine signaling pathway, with downregulation of cellular stress proteins. FOL downregulates pro-inflammatory pathways and attenuates the immune response in EAE. FOL downregulated the expression of genes involved in misfolded protein response, MAPK activation/signaling, and pro-inflammatory response. **Conclusion:** This study provides insight into the molecular impact of FOL in the brain and identifies potential therapeutic targets of the isoprenoid pathway in MS patients.

Keywords: EAE, Farnesol, isoprenoids, brain, transcriptomics.

1. Introduction

Multiple sclerosis (MS) is a chronic autoimmune disorder that causes loss of the myelin sheath in the CNS. Known pathological hallmarks of MS are demyelination, axonal degeneration, and breakdown of the blood-brain barrier (BBB), but it has an unknown etiology [1]. Hallmark symptoms of MS include muscle weakness, cognitive decline, and vision loss [2]. Around 2.5 million people globally are diagnosed with MS, with a prevalence of 50-300 per 100,000 people [3]. The gut microbiome has increased in interest as a possible therapeutic target for neurodegenerative/ autoimmune disease, with evidence showing that the gut microbiome during active stages of MS is significantly different compared to healthy individuals [4]. Gut composition changes trigger alterations in the circulating Treg/Th17 cell ratio [5]. Th17 cells collected in the brain and spinal cord lesions show an enhanced proliferative response to multiple myelin peptides [6].

Studies have shown that oral administration of isoprenoids protects against CNS inflammatory demyelination [7] and suppresses immune responses [8]. Farnesol (FOL) is a 15-carbon isoprenol derived from farnesyl pyrophosphate, a key intermediate of the cholesterol synthesis pathway. It is a component of essential oils derived from plants (*cis,trans*-farnesol) such as citronella, chamomile, and lemongrass. FOL is also found in mammalian tissues and cells (*trans,trans*-farnesol). Isoprenoids are bioactive compounds shown to regulate various signaling pathways and have reported anti-inflammatory/cancer properties [9]. Earlier studies have reported significant inhibition of neuronal and vascular voltage-gated Ca^{2+} channels by FOL, suggesting potential physiological regulation of Ca2+ signaling in the brain and the vasculature [10] [11]. Oral FOL treatment also reduced neurotoxicity, oxidative stress, and reactive gliosis in an acrylamide-induced neurotoxic mouse model. FOL treatment showed a reduction of pro-inflammatory cytokines tumor necrosis factor-alpha (TNF-α) and interleukin-1β (IL-1β) and

improved motor coordination [12]. Last, we recently showed that oral FOL treatment reduces the infiltration of immune cells into the CNS of EAE mice, protecting the animals against the disease [13].

RNA sequencing (RNA-seq) is a powerful tool for quantitatively analyzing gene expression in cells and tissues. This experimental tool has been used to identify pathways altered by diseases and impacted by treatment [14]. We used RNA-seq to characterize transcriptome alterations of the EAE brain and identify the molecular mechanisms associated with FOL protection against the disease [13].

2. Methods

2.1. Animals and housing conditions

Ten-week-old female C57BL6 NHsd mice weighing approximately 20 grams (Envigo RMS, Inc., Indianapolis, IN, USA) were housed in groups of 5 in wire-top cages with a 12-h light/dark cycle (22 ± 1 ºC; 23-33% humidity). Animals had free access to food and water with all care and procedures following Eastern Washington University institutional policies for animal well-being and health (IACUC protocol 2019-10-12).

2.2. *EAE induction and treatments*

Animals were given one week to acclimate to the EWU housing environment. EAE was induced using Hooke Laboratories Induction kits (Hooke Kit™ EK-2110, Hooke Laboratories, Lawrence, MA). The kits use MOG_{35-55} in emulsion with complete Freund's adjuvant (CFA) and Pertussis toxin (PTX) in a glycerol buffer. On day 0 of EAE induction, the MOG_{35-55}-CFA emulsion was diluted with PTX in phosphate-buffered saline and injected subcutaneously. PTX toxin injection was repeated the following day (day +1). The mice were then randomly allocated to three experimental groups to receive 100 mg/kg/day of FOL (*trans, trans*-farnesol, Sigma-Aldrich, cat.

#277,541) solubilized in corn oil (EAE-FOL; n=10 mice), corn oil only (CO-EAE; n=9), or no treatment (EAE group; n=9). FOL and corn oil were administered daily by gavage, with the dose adjusted weekly according to animal body weights. A group of sex- and age-matched mice was not EAE-induced (Naïve: n = 8) and were used as healthy controls. Brains of naïve mice were collected matching collection daytime of the experimental groups.

2.3. *RNA extraction and RNA sequencing*

Brains were collected aseptically at the EAE disease's peak (day 19) after CO_2 euthanasia. The tissue collection time was selected based on previous EAE experiments for this project [13]. The EAE clinical scores at euthanasia were: EAE: 2.4 ± 1.3; CO-EAE: 1.5 ± 1.0; EAE-FOL: 0.8 ± 1.0 [13]. The brains from healthy animals (Naïve) were collected during the same daytime as the experimental controls. Samples were stored at -80 ºC until RNA extraction, after a quick rinse with ice-cold phosphate-buffered saline and snap freeze in liquid nitrogen. Half brains were homogenized with a tissue disruptor (Fisherbrand Bead Mill 24; Fisher Scientific, Waltham, MA, USA). RNA was then extracted and purified using RNeasy Kits (Qiagen, Germantown, MD, USA). RNA quality and concentration were determined using a nanodrop spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA) and PCR and gel electrophoresis (1% agarose) at the Genomics Core Lab at Washington State University College of Pharmacy). Samples were diluted in nucleotide-free water and stored at -80 ºC until sequencing.

2.4. *Illumina sequencing*

Novogene Corporation Inc. (Sacramento, CA) performed sequencing. RNA purity was rechecked by Novogene using the NanoPhotometer® spectrophotometer (Implen, CA, USA). Next, RNA integrity and quantitation were determined with an RNA Nano 6000 Assay Kit of the Bioanalyzer 2100 system (Agilent Technologies, CA, USA). For sequencing, 1 μg RNA per

sample was used. Libraries were generated using NEBNext® UltraTM RNA Library Prep Kit for Illumina® (NEB, USA). mRNA was purified from total RNA using poly-T oligo-attached magnetic beads. Fragmentation was performed in NEBNext First Strand Synthesis Reaction Buffer. The first strand of cDNA was synthesized using a random hexamer primer and M-MuLV Reverse Transcriptase (RNase H-). The second strand was synthesized with DNA Polymerase I and RNase H. Exonuclease/polymerases were used to generate blunt ends. NEBNext Adaptor with hairpin loop structure was ligated to prepare for hybridization. 150~200 bp in length cDNA fragments were selected with the AMPure XP system (Beckman Coulter, Beverly, USA). USER Enzyme (NEB, USA) (3 μl) was used with size-selected, adaptor-ligated cDNA at 37 °C for 15 min followed by 5 min at 95 °C before PCR. PCR was performed with Phusion High-Fidelity DNA polymerase, Universal PCR primers, and Index (X) Primer. PCR products were purified (AMPure XP system), and library quality was assessed on the Agilent Bioanalyzer 2100 system.

2.5. RNA-seq analysis and statistical analysis

RNA-seq libraries were mapped to a mouse reference genome (Kallisto STAR). Reference genome and gene model annotation files were downloaded from the genome website browser NCBI/UCSC/Ensembl directly. Indexes of the reference genome were built using STAR, and paired-end clean reads were aligned to the reference genome using STAR (v2.5). STAR used the Maximal Mappable Prefix (MMP) method, which can generate a precise mapping result for junction reads. Clean raw reads were calculated into FPKM (fragments per Kilobase of exon model per million reads mapped). We identified differentially expressed genes (DEGs) using the criterion of $P < .05$ and |log2 (fold change)|. Volcano plots, PCA, cluster plots, and DEG diagrams were prepared by Jupyter (Maayanlab. cloud) software [15]. Gene Ontology (GO) enrichment results (up/downregulation) were analyzed using Enrichr datasets and compared the domains of molecular function, cellular component, and biological process. (GO_Biological Process_2018, GO_Molecular_Function_2018, GO_Cellular component_2018). Pathway enrichment analysis

was done with KEGG using Enrichr (Kyoto Encyclopedia of Genes and Genomes) with their respective libraries (KEGG_2016, Reactome_2016, WikiPathways_2016). Transcription factor enrichment analysis analyzed pathways (up/downregulated) using Enrichr, with their libraries (ChEA_2016, ENCODE_FT_ChIP-seq_2015, ARCH_TFs_Coexp). All Significant results using catalogs/libraries were determined using Benjamin-Hochberg correction, then a cut-off P-value<0.1. Fragments Per Kilobase of transcript sequence per Millions of base pairs sequenced (FPKM) were compared by analysis of the variance followed by Tukey's multiple comparisons. Statistical analyses were performed with Rstudio (RStudio Team (2020). RStudio: Integrated Development for R. RStudio, PBC, Boston, MA URL http://www.rstudio.com/) and with GraphPad Prism version 9.4 for Apple (GraphPad Software, San Diego, California USA, www.graphpad.com"). Figures were generated with RStudio and with GraphPad Prism.

3. Results

3.1. Whole brain RNA-seq data quality control and data distribution

All reads were filtered to obtain clean reads by removing reads containing adapters and poly-N sequences, reads with more than 10% of bases that could not be determined, and low-quality reads with a Qscore (Quality value) of over 50% bases being <= 5. The error rate of the whole sequences for all samples was 0.02%, the Q20(%) (Phred values greater than 20 base number contain the percentage of total bases) at least 98%, and the lowest Q30(%) 94.84%. Phred defines the quality of the nucleobase's identification generated by the sequencing. Pearson analysis performed using the sequences of all samples identified one brain sample with a low correlation with all the other samples (EV4, Suppl. Fig. 1). Therefore, EV4 was excluded from all analyses. Figure 1 shows the differential expression patterns as a heatmap, overlapping Venn diagram, Principal Analysis Components (PCAs), and volcano plots. A statistical analysis of all gene counts downregulated and upregulated in the brains of EAE mice vs. naïve mice are shown in Table 1 and Table 2, respectively. The tables also show group-to-group comparisons.

3.2. ***Effect of EAE on gene expression – Untreated EAE vs.*** **healthy (Naïve)** ***comparison***

3.2.1. EAE causes dysregulation of neuronal signaling and maturation.

Gene Ontology (GO) is commonly used to describe and classify gene characteristics on a wide-scale analysis [16]. DEGs associated with “nervous system development” (GO: 0007399) were downregulated in EAE vs. healthy (Naïve) (Suppl. Fig. 10A). *Sema3c*, *Dpysl3* (Suppl. Fig. 13), and *Plxna4* (Fig. 2A), all known regulators of nervous system development, were downregulated. *Sema3c* (Suppl. Fig. 13) product is the co-receptor for *Plxn4a* [17]. GO term enrichment analysis showed 19 DEGs in “regulation of cell differentiation” (GO: 0045595) were downregulated in EAE vs. healthy (Naïve) (Suppl. Fig. 10A). We used the Kyoto Encyclopedia of Genes and Genomes database (KEGG) for further analysis of the genes associated with specific pathways and networks [18]. This analysis showed the downregulation of 13 DEGs related to axon guidance (hsa04360) and downregulation of the “cell adhesion molecules” pathway (CAMs; 12 DEGs associated) (Suppl. Fig. 4A). CAMs are an essential part of axon response to extracellular stimuli and play a crucial role in neuronal morphology [19]. *Slc4a11* was also downregulated in EAE vs. healthy (Naïve) (Fig. 2B), associated with suppressing neuronal excitability [20].

Aside from neurons, genes associated with neuronal development were also downregulated in EAE, including *Smarca4* (Fig. 4A), a requirement for oligodendrocyte differentiation/maturation [21]. Downregulation of *Fabp7* was also reported (Suppl. Fig. 13), a gene required for oligodendrocyte differentiation [22]. This pattern continued with the downregulation of *Myt1l* and *Pou3f2, two genes* implicated in neuronal differentiation (Fig. 4A). In contrast, DEGs associated with “osteoclast differentiation” were upregulated in EAE vs. healthy (Naïve) (Suppl. Fig. 4A).

EAE also decreased the expression of DEGs associated with “GABA synthesis, release, reuptake, and degradation” as well as “Transmission across chemical synapses” (R-HSA-888590) when compared to naïve conditions (Fig. 3B). We found that EAE caused the upregulation of *Gabrr3* (Fig. 5A) but the downregulation of *Gabra2*, *Gabre*, *Gabrg2*, *Gabbr2*, and *Gabra3* (Suppl Fig. 13). GO term enrichment analysis showed downregulation of “transmitter-gated ion channel activity involved in the regulation of postsynaptic membrane potential” (GO: 0099529) and “chlorine channel activity” (GO: 0005254). *Gabra2* and *Gabrg2* were DEGs associated with both pathways described. Last, KEGG analysis showed downregulation of “alanine, aspartate, and glutamate metabolism” in EAE (Suppl. Fig. 4A). The imbalance between glutamate and GABA has been shown to affect fatigue in MS [23].

The comparison between untreated EAE brains and healthy (Naïve) mice brains suggests that at the peak of the disease, neuroinflammation causes dysregulation in neuronal differentiation and development. The data further show that EAE downregulates pathways implicated in GABA-mediated neurotransmission.

3.2.2. EAE causes a pro-inflammatory immune response in the Brain.

We examined the expression of genes involved in pro-inflammatory and immune-related pathways using the Reactome database [24]. In EAE, 116 DEGs associated with “immune response” pathways were upregulated (Fig. 2A). The pathways “chemokine activity” (GO: 008998) and “chemokine receptor binding” (GO:0042379) were also upregulated (Suppl. Fig. 8A). “Cytokine-mediated signaling pathway” (GO: 0019221) and those implicated in the “cellular response to cytokine stimulus” (GO: 0071345) (Suppl. Fig. 10A), and “cytokine receptor activity” (GO: 0004896) (Suppl. Fig. 6A) pathways were also upregulated. Further analysis using Wikipathways revealed upregulation of the “IL-5 signaling” and “IL-3 signaling” pathways (*not shown*) in the EAE brain. Interestingly, GO and KEGG analyses showed upregulation of DEGs in

the “Toll-like receptor Signaling” pathway (Suppl. Fig. 8A & Fig. 2A). This finding was supported by the Reactome analysis that identified upregulation of the “Toll-like receptor cascades” pathway and upregulation of the “TLR4 Cascade” (14 DEGs) pathway (Suppl. Fig. 4B). KEGG also found upregulation of the “TNF signaling pathway" (18 DEGs) and “Complement and coagulation cascade” (11 DEGs) (Suppl. Fig 4A), and “MHC protein binding” (5 DEGs) (GO: 0042287) (Suppl. Fig. 8A).

We also observed increased expression of DEGs associated with “STAT2” (Fig 4A) and the “Jak-STAT signaling” pathways (Suppl. Fig. 4A), and “Negative regulation of viral genome replication” (12 DEGs) (GO: 0045071) (Suppl. Fig. 10A). Genes connected with interferon (IRF) pathways (IRF1, IRF2, IRF5, IRF7, and IRF9 pathways) were also upregulated (Fig. 2A). IRF pathways are well-characterized in the context of viral infections [25]. Interestingly, *Trim23* was downregulated (Fig. 4A). *Trim23* has been associated with viral infection-based autophagy [26]. Last, the involvement of interferon-related pathways in EAE was further supported with the upregulation of 19 “interferon alpha/beta signaling” (19 DEGs) and “interferon gamma signaling” (16 DEGs) (Fig. 2A). These findings are relevant considering that IFN-beta (IFN-β) was the first disease-modifying treatment approved for MS [27].

Last, pathways associated with the immune response to pathogenic microbes were also upregulated in EAE. By KEGG, DEGs related to parasitic “Leishmaniasis” and “Chagas disease,” viral “Herpes simplex infection” and “Measles infection,” and bacterial “*Staphylococcus aureus* infection” were upregulated in EAE vs. healthy (Naïve) (Suppl. Fig. 4A).

In summary, our data show upregulations of both innate and adaptive immune cell activity in the brains at EAE’s clinical peak, including increases in chemokine/cytokine, pro-inflammatory, and toll-like receptor pathways.

3.2.3. EAE impacts sterol synthesis regulatory genes.

Recent literature has highlighted that neuronal cholesterol synthesis is vital for remyelination, with oligodendrocytes increasing cholesterol synthesis after neuronal injury [28]. GO analysis showed downregulation of 12 DEGs associated with both the “cholesterol biosynthesis” in EAE (Fig. 2A); Reactome analysis showed downregulation of several DEGs of the “activation of gene expression by sterol regulatory element binding proteins (SREBP)” pathway (Fig. 2A). These pathways had six overlapping DEGs: *Sqle*, *Hmgcs1*, *Mvd*, *Hmgcr*, *Dhcr7*, *Lss*, and *Fdft1*. *Sqle* and *Hmgcr* are essential enzymes to the mevalonate pathway [29], and *Hmgcs1* is a critical rate-limiting step in cholesterol production in neurons and astrocytes [30]. *Fdft1* is a farnesyltransferase with dysregulation associated with cancer progression [31]; In addition, *Dhcr7* is the last step in the cholesterol synthesis cascade [32]. Overall, highlighting in EAE is a downregulation of gene expression associated with several phases of cholesterol synthesis. KEGG analysis also showed the downregulation of seven DEGs in the “steroid biosynthesis” pathway in EAE mice (Suppl. Fig. 4A). Four DEGs associated with “cholesterol synthesis” also correlated with the “steroid biosynthesis” pathway: *Sqle*, *Dhcr7*, *Lss*, and *Fdft1*. R Studio analysis verified the downregulation of both *Sqle* and *Dhcr7* in EAE vs. naïve mice (Suppl. Fig. 14).

3.3. Effect of FOL on gene expression - EAE-FOL vs. untreated EAE comparisons

3.3.1. FOL attenuates the expression of immune/inflammation response genes and pathways.

The anti-inflammatory activity of FOL has been attributed in part to its ability to control cytokine release [9] [33]. Brain samples from the EAE-FOL group showed upregulation of 8 “positive regulation of leukocyte migration” DEGs. Four of these DEGs were affiliated with “leukocyte aggregation.” They were significantly upregulated compared to the Naïve group (healthy mice) (Suppl. Fig. 10C). GO analysis revealed a downregulation of DEGs in the “antigen

processing and presentation" pathway in EAE-FOL brains (Suppl. Fig. 5B) and KEGG reported downregulation of genes of the "TNF signaling pathway" (Suppl. Fig. 5B). The downregulated DEGs of the "TNF signaling pathway" were *Nfkbia*, *Mmp14*, *Atf6b*, *Akt2*, *Pik3r2*, *Fos*, *Junb*, *Cxcl1*, and *Map3k5*. In addition, there was a trend ($p < 0.01$) for FOL-induced *Fas* downregulation (Suppl. Fig. 12). This is notable considering that *Fas* over-expression in CD8 T cells correlates with exacerbated EAE [34]. KEGG analysis further showed the downregulation of genes of the "human T-lymphocyte virus 1" pathway in the brains of FOL-treated EAE mice compared to those from untreated EAE animals (Suppl. Fig. 5B). Of note is the downregulation of *Crem* and *Tcf7* (Fig. 4B), two genes considered markers of pro-inflammatory Th17 cells and believed to play a role in MS immunopathology [35] [36]. MAP kinase pathways were downregulated in FOL-treated EAE mice, the "negative regulation of MAP kinase activity" pathway (GO: 0043407; Suppl. Fig. 11B) and the Reactome "MAPK targets/Nuclear events mediated by MAP kinases" pathway (5 DEGS, Fig. 4A), with three overlapping genes (*Dusp4*, *Dusp3*, and *Dusp6*). FOL also showed downregulation in the "glucocorticoid receptor binding" pathway (5 DEGs) (Suppl. Fig. 9B), a pathway that has been the target of anti-inflammatory steroids used in MS treatments [37].

3.3.2. FOL treatment alters cellular stress response pathways in EAE brains

Current literature supports that endoplasmic reticulum (ER) and oxidative stress are critical regulators of neuronal apoptosis [38]. Several ER/oxidative stress pathways were found to be downregulated in brains of EAE mice treated with FOL: "unfolded protein response" pathway (Reactome analysis; Fig. 3), "response to ER stress" pathway (13 DEGs, GO: 0034976, Suppl. Fig. 11B), "protein processing in the ER" pathway (29 DEGs, Suppl. Fig. 5B), "ubiquitin protein ligase binding" pathways (GO: 0031625, Suppl. Fig. 9B), and the "endoplasmic-reticulum-associated-protein degradation" pathway (GO: 0036503, Suppl. Fig. 11 B). Interestingly in EAE-FOL, the "ubiquitin-like-protein ligase binding" pathway (18 DEGs) (GO:0031625) was downregulated when compared to the healthy (Naïve) group (Suppl. Fig. 8C). Other ER/oxidative

stress pathways showed downregulation of gene expression: “Endoplasmic-reticulum-associated protein degradation” (ERAD) pathway (10 DEGs, Suppl. Fig. 11B) and “Heat Shock Response” pathway. Heat shock factors (HSF) are regulated by heat shock proteins (HSP) and function to help with protein folding or signal for degradation [39]. The “cellular stress response” (Fig. 3A) and the “cellular response to heat stress” pathways were both downregulated in FOL-treated mice (Fig. 3B). The cofactor *Hspa5* and *Chord1* were downregulated, comparable to healthy (Naïve) levels (Fig 5A). Finally, genes of the “N-glycan trimming in the ER and Calnexin/Calreticulin response” pathway, a key pathway that ensures proper protein folding [40], were also downregulated in FOL-treated mice (Fig. 3A).

Taken together, the treatment with FOL reduced the expression of pathways associated with oxidative, heat-induced stress and genes linked to protein misfolding, upregulated in EAE brains. Our findings suggest an effect of FOL attenuating oxidative stress during EAE.

3.3.3. FOL impacts cancer-associated pathways

Several studies have established the anti-cancer properties of FOL [9,41]. A recent study found that MS patients had higher prostate, colorectal/anal, and lung cancer risks than population controls [42]. We performed a more in-depth analysis of the cellular pathways associated with cancer-associated DEGs impacted by FOL treatment. KEGG analysis revealed a downregulation of several “pathways in cancer” genes (Suppl. Fig. 5B), a downregulation of “Longevity regulating pathway” genes (Suppl. Fig. 5B), and a downregulation of genes of the “apoptosis-related network due to altered *Notch3* in ovarian cancer” pathway (*not shown)*. Downregulation of “pathways in cancer” and “longevity regulating pathway” genes was not observed in the EAE-Veh group when compared to the untreated EAE group (Suppl. Fig. 5A). This suggests that the effect of FOL on cancer-related pathways is specific to FOL and independent from corn oil, its vehicle.

3.3.4. FOL impacts genome regulation/repair mechanism pathways in EAE Brains

GO analysis revealed that 36 DEGs associated with the “negative regulation of transcription from RNA polymerase II promoter” pathway were downregulated by FOL (Suppl Fig 9B). Interestingly, the treatment with FOL impacted the expression of 2 genes (downregulation of *Mtf2* and upregulation of *Mga*, Fig 2B), two genes implicated in regulating the activity of RNA polymerase II and DNA transcription [43,44]. FOL also downregulated several genes of the “negative regulation of transcription, DNA templated” pathway (GO: 0000122 & 0045892; Suppl. Fig. 11B), with 15 DEGs not related in both pathways and upregulated several genes of the “Y-form DNA binding” pathway (GO: 0000403; Suppl Fig. 9B). An upregulation was also seen with *Cdkn2aip* (Fig 4B), a gene implicated in DNA damage responses, apoptosis and cell proliferation [45]. Last, FOL changed the expression of DEGs associated with chromosome repair/maintenance (upregulation of *Kntc1* and chromatin condenser *Ncor1* and downregulation of *Suz12*; Fig. 4B).

FOL treatment impacted several other pathways implicated in genome regulation and repair mechanisms. Upregulation was observed for “U2-type spliceosomal complex” (GO: 0005684) and “U2-type pre-spliceosome” (GO: 0005684, Suppl. Fig. 6C). In contrast, the following were downregulated: “RNA splicing, via transesterification reactions with bulged adenosines as nucleophiles” (GO: 0000377, Supp Fig 11C), “spliceosome” pathway (KEGG analysis, Supp Fig 5C), “U6 snRNA binding” (GO: 0017070, Supp Fig 9C), “mRNA splicing, via spliceosome” (Suppl Fig 11C), “mRNA splicing – Major pathway” and “processing of capped intron-containing pre-mRNA” (Suppl Fig 3B).

4. Discussion

Our previous work shows that daily oral FOL administration delayed the onset and significantly attenuated the clinical severity of murine experimental autoimmune encephalitis (EAE). EAE is a well-established model of multiple sclerosis characterized by the crossing of immune cells through the blood-brain barrier and profound neuroinflammation [13]. FOL reduced the infiltration of immune cells to spinal cord parenchyma while modifying the composition of the gut microbiome [13]. While the anti-inflammatory and neuroprotective activities of FOL had previously been reported [9,12], the molecular basis of these activities, especially in the EAE brain, had not been investigated. To fill this knowledge gap, we performed a transcriptomics analysis (RNA-seq) of the brains of EAE mice treated with FOL. The data were compared to relevant control transcriptomics profiles, namely those of brains collected from untreated EAE and vehicle (corn oil)-treated mice and healthy mice. The data show that oral FOL treatment ameliorated pathways associated with an immune response and pathways associated with protein misfolding in the brain. Additionally, we found an upregulation of positive immune cell regulation in the brain. Compared to untreated EAE animals, which showed upregulation of various pathways associated with immune cell activity/activation.

The brain of FOL-treated EAE mice showed downregulation of pathways associated with “cellular stress response” and “unfolded protein response” (Suppl. Fig 11B). A recent study showed that dietary supplementation with FOL protected a Parkinson’s mouse model (CamK-PARIS) [46]. Dietary treatment with FOL increased in approximately 50% the amounts of the isoprenoid in the brain compared to untreated controls. FOL treatment reduced hydrogen peroxide concentrations and prevented dopaminergic neuron loss *in vivo* [46]. Our RNA-seq study shows that the oral treatment with FOL resulted in the downregulation of the “parkin-ubiquitin proteasomal system pathway” (DEGs associated: *Hspa8, Hspa5, Tubb3, Siah2, Stub1, Tuba4a, Hspa1b*) when compared to untreated EAE mice. FOL treatment increased the expression of *Sod2* in EAE-FOL compared to EAE and EAE-Veh (Fig 5B). In addition, *Sfpq* expression was

comparable to naïve levels with FOL treatment (Fig. 5A). Another study found that FOL ameliorated Charcot-Marie-Tooth disease (C22 mice), caused by degeneration of the peripheral axon or demyelination of Schwann cells; With mutations in *Hsp22/27* also being reported [47]. FOL treatment *in vitro/in vivo* improved the integrity of Schwann cells, myelination defects, and overall increased locomotor function [47]. Our data indicate that FOL treatment downregulates the "cellular response to heat stress" pathway (Fig. 3A) and associated cofactors *Hspa5* and *Chord1* (Fig. 5A) compared to untreated EAE.

We observed that the relative abundances of antigen-presenting cells and $CD4^+$ T cells infiltrating the CNS of EAE mice treated with FOL were significantly reduced compared to untreated EAE mice and vehicle-treated EAE mice [13]. Interestingly, while FOL treatment reduced the abundance of infiltrated $CD4^+$ T cells, an increase in $Foxp3^+$Tregs was observed in the spinal cords. Our study suggests that FOL treatment could promote immunoregulation in the context of neuroinflammation. No study has determined whether FOL has immunosuppressive effects on pro-inflammatory Th17 cells as Th1 cells. However, the exposure of immature dendritic cells (DCs) to FOL results in dysfunctional function, causing a failure of Th1 activation and a lack of Th1-promoting cytokines, such as IL-12, *in vitro.* Interestingly, although FOL activated innate immune cells in this study, the responses triggered could not help mount adaptive immunity efficiently; instead, the responses induced immunosuppression [33]. Our results support this, with EAE-FOL showing downregulation of "antigen processing and presentation" (Supp Fig 5B). A FOL-infused chow diet was also used for an asthma mouse model (OVA-challenged mice) to find that the FOL diet slightly (not significant) decreased IL-4 cytokine levels in the spleen of mice [48]. In our RNA-seq analysis, we observed a significant downregulation of IL-4 DEGs in the brains of FOL-treated EAE mice, strengthening the idea that FOL treatment results in overall inflammatory response reductions and FOL's potential as a neurotherapeutic for neuroinflammatory conditions.

FOL is a known intermediate of the mevalonate pathway [49]. The treatment with pitavastatin, a known inhibitor of HMG-CoA reductase in the mevalonate pathway, reduced EAE severity with a significant downregulation of *Il17a*, *Csf2*, *Il21, Il23*, *Il1b*, *Il6*, and *Ccr2* expression in spinal cords [50]. Though we did not find a downregulation of *Ccr2* specifically, we observed the downregulation of *Ccr5* after FOL treatment. Both genes (*Ccr2* and *Ccr5*) are reported to be upregulated in Th17 and downregulated in Tregs in pediatric MS patients [51]. Another study found that when *Hmgcs1* expression was promoted in astrocytes, it reduced inflammatory infiltration and demyelination [52]—showing that cholesterol synthesis/regulation is a critical factor in neuroinflammatory disease. Our RNA-seq results support that oral FOL treatment ameliorates multiple immune response pathways in the brain. In addition, FOL treatment reduced the MAPK pathway cytokine pathways associated with autoreactive immune cells targeted to the brain.

FOL significantly impacted several genes implicated in regulating the cholesterol synthesis pathway, which is impaired in the EAE brain [49]. However, FOL did not fully restore the expression of cholesterol synthesis genes to the level observed in naïve/healthy animals, thus raising the possibility that higher daily doses are needed to normalize cholesterol synthesis transcriptomics fully. It is also well-established that HMGCR inhibition reduces the viability of many cell types. A study done with PC-12 cells showed that FOL had a protective effect against atorvastatin-dependent cytotoxicity (ATR), a cytotoxicity model of Alzheimer's disease. Their results showed farnesol reversed ATR and MβCD-toxicity of AβPP-sw cells to their control levels. Farnesyl transferase inhibitor reduced the cell viability to statin treatment levels [53]. To date, little data support evidence for a direct effect of farnesol on neurons. However, the study by Roullet and colleagues showed that farnesol is a potent blocker of neuronal N- and L-type Ca^{2+} channels, channels that are implicated in neurotransmission but also in brain calcium overload and ensuing neuronal death [10,28]. CNS calcium overload has been proposed as a pathogenic factor in MS [54], and the therapeutic benefits of manipulating calcium channel blockers in MS have been

reported [55,56]. With this, it is tempting to speculate that the neuroprotective activity of FOL in EAE is partly the consequence of FOL action on CNS voltage-gated calcium channels.

Corn oil was used as a vehicle by solubilizing FOL and facilitating oral administration, as previously done by others [57]. We observed significant differences in the average clinical scores between FOL and vehicle treatments [13]. However, a vehicle effect was also observed, and corn-oil treatment also reduced the severity of EAE [13]. Interestingly, vehicle and FOL treatments resulted in the downregulation of "response to unfolded protein" (GO: 0006986) (Suppl Fig 11B). The *p*-value for EAE-FOL (p = 6e-14) was lower than EAE-Veh (p = 6e-09). Another study also supported that exogenous FOL increases transcription of small heat shock proteins of *Candida auris in vitro*, supporting that this effect is farnesol induced [58]. This was also supported in our findings, with FOL-treated mice showing downregulation of "unfolded protein response," "cellular response to stress," and "cellular response to heat stress" (Fig. 3A/B).

Our published work showed how FOL treatment modifies the composition of the gut microbiome, significantly impacting the Firmicutes:Bacteroidetes ratio [13]. FOL is a quorum-sensing molecule reported to regulate microbial biofilm formation. It has also been reported to be an antimicrobial agent, specifically for gram-positive bacteria such as *Staphylococcus aureus* [59] [60]. Multiple recent studies suggest MS favors a dysbiotic microbiome [61]. However, the molecular mechanisms by which the microbiome could regulate neuroinflammatory/autoimmune diseases remain to be elucidated. Our previous works have demonstrated that the interactions between the gut microbiota and CNS inflammatory demyelination occur in both directions [62,63]. The present study indicates that EAE activates major inflammatory pathways, including TNF signaling. TNF-α is a significant regulator of epithelial barrier integrity, including in the intestine [64]. EAE upregulation of TNF signaling pathways could result in increased intestinal barrier disruption, an effect that has been previously published [65]. It is possible that EAE's impact on

the intestinal barrier could result in the microbiota changes observed during the disease. Further studies will address our hypothesis.

Supporting the concept of the bidirectional nature of inflammatory pathways associated with EAE and the inflammation triggered by microbes, it was previously shown that peptidoglycan, a main component of the bacterial cell wall, aggregates to MS lesions and is associated with the activation of innate immune response genes [66]. Brain presence of bacterial lipopolysaccharide has also been found to impact EAE severity [67]. Our study describes specific immune-related pathways that are affected by EAE. When comparing the transcriptomic profile of EAE-FOL and EAE brains, a significant downregulation of "*Vibrio cholerae* infection *Homo sapiens*" was found (Supp. Fig, 4C and 5B). Although the direct impact of microbes and microbial products on the CNS is a possible mechanism by which the gut could regulate disease, immunomodulation triggered in response to gut microbes is also possible. For example, polysaccharide A (PSA) produced by *Bacteroides fragilis* promotes immunoregulatory responses that protect against EAE [68–71]. Our findings suggest that oral farnesol attenuates neuroinflammation in EAE through several mechanisms, including activation and modulation of immune pathways in the brain and modification of the gut microbiome.

It is also possible that farnesol impacted immune cells in the EAE spleen. Interestingly, oral administration of Berberine (BB), a quaternary ammonium salt which, like farnesol has quorum-sensing and immunomodulatory activities, was shown to reduce the severity of experimental autoimmune uveitis, an animal model of human endogenous uveitis [72]. These studies further showed, using RNA-seq, that BB downregulated the "antigen processing and presentation" pathways [72]. This pathway is also downregulated in FOL-treated EAE mice (Suppl Fig 5B) together with the "MAPK targets/Nuclear events mediated by MAP kinases" pathway (Fig 4A) and *Akt2*, a key gene implicated in TNF signaling [73]. These two pathways and the *Akt* gene are also downregulated in the spleen of mice treated orally with Tiepishihu Xiyangshen, a traditional

Chinese medicine with immunomodulatory activity [74,75]. Overall, we can surmise that the transcriptomics profile of peripheral immune cells will be comparable to what we found in the brain, with downregulation of pathways/DEGs associated with immune cell activation/pro-inflammatory responses. However, this hypothesis must be confirmed in future studies specifically designed to assess the functional effects of FOL treatment on circulating and tissue immune cell subpopulations in EAE mice.

Our transcriptomics findings were not validated with complementary quantitative PCR and protein expression analyses. Unfortunately, we no longer have access to the samples, and such validations will have to be performed in future studies. As discussed in the previous paragraph, the characterization of the transcriptomics profile of peripheral immune cells would have provided information critical to the understanding of the immunomodulatory activity of FOL. In our previous work [13], We reported that FOL did not significantly affect the splenic T cell subpopulations. In contrast, we observed a significant reduction in the proportion of T cells in the CNS and an increase in Tregs frequencies [13]. Based on these findings, we focused on the brain instead of the spleen to perform transcriptomics investigations. Last, we did not analyze FOL's pharmacokinetic-pharmacodynamic properties. Future studies will measure brain FOL levels and correlate these levels with clinical efficacy and gene expression.

Notwithstanding these limitations, our transcriptomics study stands out as the first comprehensive investigation of the immunomodulatory activity of oral FOL in experimental multiple sclerosis. It also confirms the involvement of several immune/neurological pathways and genes in EAE pathogenesis reported by others and thus provides not only a molecular understanding of the disease but also a molecular roadmap to developing targeted therapies.

Our initial report of the preventive activity of FOL in EAE highlighted how daily farnesol gavage significantly changed the gut microbiome [13]. However, it also confirmed the

neuroprotective activity of vegetable oils (the FOL vehicle in our study) in this model reported by others [76,77]. Our transcriptomics analysis provides molecular clues to the therapeutic activity of these oils (see Figs. 5, and Suppl. Figs. 5, 7, 9, and 11) and calls for studies to delineate the role of FOL and that of the vehicle in preventing EAE onset. Such studies would require using a biologically neutral vehicle for oral administration or investigating the efficacy of other routes of FOL delivery. These issues have begun to be addressed, and reports of using FOL-infused chow or vehicle-free intranasal administration have recently been published [46,78].

Testing the efficacy of non-oral routes of FOL delivery is important and needed. However, the efficacy of the oral route demonstrated in our earlier report raises the possibility of an interaction of FOL with the gut microbiota [13], an interaction which secondarily would impact the brain. Hence, future studies will be needed need to investigate if the observed FOL-induced changes in the brain transcriptomics are replicated using non-oral routes of administration and test the hypothesis of a gut-brain axis-mediated mechanism of action for FOL.

Conclusion

In our whole-brain transcriptomics analysis performed in EAE mice at the peak of disease, we observed that FOL treatment alters transcriptional and pre-translational pathways. Our data highlight that the effects of FOL treatment are not restricted to one part of genome regulation. Farnesol alters various immune pathways, alleviates cellular stress signals, and increases the expression of genome repair pathways/mechanisms.

Data Sharing

The data discussed in this publication are accessible through GEO Series accession number GSE233583 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE233583).

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Declaration of interest

The authors have no financial interests to disclose.

Declaration of Competing Interest

Authors have no competing interests to disclose.

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Tables

Table 1. Analysis of the Variance of gene counts downregulated in EAE mice vs healthy (naïve) by Kruskal-Wallis, followed by multiple comparisons between all groups (Adjusted *p* values)*.

	All groups	Naïve vs. EAE	Naïve vs. EAE – Veh	Naïve vs. EAE – FOL	EAE vs. EAE – Veh	EAE vs. EAE – FOL	EAE – Veh vs. EAE – FOL
Lars2	1.18E-04	3.27E-02	5.30E-02	1.96E-01	9.96E-01	4.27E-05	8.27E-05
Dnajc18	1.82E-04	2.89E-07	6.65E-06	4.72E-04	6.30E-01	2.62E-02	3.11E-01
Gm23935	2.25E-04	4.68E-02	5.82E-02	2.28E-01	1.00E+00	9.04E-05	1.22E-04
Sod2	2.64E-04	8.62E-07	2.50E-07	1.32E-03	9.63E-01	2.86E-02	8.48E-03
Ankrd34c	2.75E-04	1.01E-05	5.05E-06	1.40E-03	9.93E-01	2.04E-01	1.23E-01
Dpysl3	3.03E-04	1.10E-07	1.78E-06	6.02E-06	7.03E-01	3.25E-01	9.24E-01
Cyp51	3.48E-04	5.03E-06	9.80E-05	1.10E-07	6.81E-01	5.51E-01	8.33E-02
Gnl3l	3.95E-04	3.71E-08	7.33E-08	2.77E-06	9.93E-01	2.57E-01	3.93E-01
Dhcr7	4.24E-04	6.54E-08	4.95E-07	6.74E-09	8.56E-01	8.80E-01	4.17E-01
Itm2a	4.64E-04	1.26E-06	2.06E-05	3.44E-05	7.16E-01	4.99E-01	9.87E-01
Sema3c	5.06E-04	1.49E-06	1.12E-07	4.20E-08	7.47E-01	6.05E-01	9.97E-01
Fabp7	5.09E-04	2.49E-02	1.85E-03	4.14E-06	7.06E-01	1.14E-02	1.38E-01

Gabra2	6.72E-04	2.56E-04	2.29E-06	5.85E-05	3.02E-01	9.70E-01	5.20E-01
Copg2	7.86E-04	1.96E-06	1.92E-05	9.50E-04	8.24E-01	7.90E-02	3.83E-01
Sqle	8.05E-04	3.36E-07	1.35E-06	6.55E-08	9.49E-01	9.64E-01	7.35E-01
Pcdh20	9.17E-04	8.19E-07	7.42E-06	1.85E-06	8.35E-01	9.63E-01	9.81E-01
Olfml2a	1.09E-03	1.53E-06	4.91E-05	2.33E-06	5.63E-01	9.88E-01	7.36E-01
Gm22009	1.09E-03	1.55E-03	1.61E-02	9.90E-01	7.75E-01	2.08E-04	3.13E-03
Gabrg2	2.27E-03	3.37E-04	1.45E-02	1.49E-02	4.51E-01	3.78E-01	1.00E+00
Gabbr2	4.22E-03	2.97E-01	1.20E-02	3.53E-03	3.79E-01	1.77E-01	9.73E-01
Gabrb3	5.65E-03	1.69E-01	6.84E-03	1.92E-02	4.44E-01	7.37E-01	9.52E-01
Gabrg1	6.67E-03	4.68E-04	4.37E-02	9.56E-02	2.62E-01	1.04E-01	9.68E-01
Gabra3	1.17E-02	3.60E-02	3.97E-03	5.40E-03	7.83E-01	8.76E-01	9.96E-01
Gabra5	3.58E-02	3.48E-01	4.02E-02	1.24E-01	6.31E-01	9.32E-01	9.20E-01
Gabre	4.19E-02	2.60E-02	2.64E-02	1.57E-01	1.00E+00	7.67E-01	7.72E-01

*, Adjusted *p* values shown, by Tukey's multiple comparisons (in red font)

Table 2. Analysis of the Variance of gene counts upregulated in EAE mice vs. healthy (naïve) by Kruskal-Wallis, followed by multiple comparisons between all groups (Adjusted *p* values)*.

	All groups	**Naïve vs. EAE**	**Naïve vs. EAE – Veh**	**Naïve vs. EAE – FOL**	**EAE vs. EAE – Veh**	**EAE vs. EAE – FOL**	**EAE – Veh vs. EAE – FOL**
Hnrnpm	2.24E-05	1.86E-09	4.01E-09	5.51E-05	9.88E-01	9.01E-04	2.25E-03
Creld2	8.58E-05	5.94E-07	6.92E-02	7.82E-01	3.58E-04	1.55E-06	2.91E-01
Gm43860	2.14E-04	3.21E-06	4.52E-02	5.28E-03	3.71E-03	2.62E-02	8.21E-01
Fas	2.88E-04	3.43E-06	2.44E-05	6.29E-04	8.80E-01	1.67E-01	5.29E-01
Ythdc1	2.99E-04	3.75E-06	8.31E-04	3.72E-02	1.95E-01	3.59E-03	3.50E-01
Sfpq	3.07E-04	1.03E-05	1.87E-02	7.08E-01	3.09E-02	5.39E-05	1.24E-01
Hspa5	3.59E-04	8.20E-06	6.09E-01	9.79E-01	1.09E-04	4.98E-07	3.02E-01
Cep135	3.70E-04	2.43E-05	1.82E-05	2.44E-07	1.00E+00	3.78E-01	4.40E-01
Chordc1	4.83E-04	8.88E-05	6.24E-02	1.52E-01	5.54E-02	1.34E-02	9.51E-01
Pfkfb3	5.25E-04	1.55E-05	1.28E-01	1.43E-01	4.45E-03	2.52E-03	9.99E-01
Sbno2	8.83E-04	1.02E-08	2.05E-08	1.97E-08	9.92E-01	9.64E-01	9.98E-01
Itih4	1.04E-03	2.71E-04	1.23E-03	2.90E-04	9.37E-01	9.99E-01	9.68E-01
Plaur	1.30E-03	2.67E-03	1.31E-03	2.87E-02	9.92E-01	6.95E-01	5.20E-01
Zc3h11a	2.40E-03	2.16E-03	2.35E-04	6.42E-03	8.28E-01	9.49E-01	5.01E-01

ADAM17	1.45E-02	8.23E-02	8.66E-02	2.29E-01	1.00E+00	9.20E-01	9.29E-01
Fabp4	1.79E-02	6.18E-03	1.20E-01	9.43E-02	5.32E-01	5.56E-01	1.00E+00
Gabrr3	2.41E-02	6.36E-01	1.00E+00	3.45E-02	5.72E-01	3.03E-01	2.00E-02
Gabrr2	3.00E-02	2.19E-01	1.77E-01	8.27E-03	9.99E-01	4.25E-01	5.02E-01

*, Adjusted *p* values shown, by Tukey's multiple comparisons (in red font)

Figure legends:

Figure 1. Differential gene expression analysis from normalized reads of whole brain RNA-seq analysis of healthy (Naïve; N), untreated EAE (EAE), and EAE mice treated with farnesol (EAE-FOL), at the peak of disease (17 days post-induction). A) Differential expression by heatmap that displays gene expression for each sample. Rows represent each gene organized by clusters of genes, while the columns are organized by sample IDs clustered by treatment. Each cell displays normalized gene expression values. B) Venn diagram indicating the number of genes overlapping among treatments. C) Principal Component Analysis results showing a three-dimensional scatter plot of the first three Principal Components (PCs). D) Volcano Plots showing the log2-fold changes and statistical significance of genes, with red points indicating significantly upregulated genes and blue points indicating significantly downregulated genes. Sample size (mice): N = 8; EAE = 9; EAE-Veh = 8; EAE-FOL = 10.

Figure 2. EAE and the treatment with farnesol (FOL) modifies the profile of transcription factors identified in brains. Pathway Enrichment Analysis by comparison of normalized reads to the ChEA (experimentally validated targets (ChEA), experimentally validated targets (ENCODE) and co-expressed genes (ARCHS4) databases. A) Bar charts with pathway enrichment analysis of EAE vs. healthy (naïve). B) Bar charts with pathway enrichment analysis of EAE – FOL versus EAE. The x-axis shows the -log10(P-value) for each pathway. In red, comparisons show statistical significance (with adjusted *p* values < 0.05).

Figure 3. EAE induction promotes the up- and-downregulation of biological pathways in mouse brains. Pathway Enrichment Analysis by comparison of normalized reads to the Reactome database and genes associated with most significant pathways. A) Bar charts with pathway enrichment analysis of EAE vs. healthy (Naïve). The x-axis shows the -log10(P-value) for each pathway. Pathways show statistical significance (with adjusted p values < 0.05) in red. B)

Histograms showing enriched pathways in the columns and genes associated in the rows organized by *p* value.

Figure 4. Treatment with farnesol (FOL) up-and downregulation of biological pathways in EAE brains. Pathway Enrichment Analysis by comparison of normalized reads to the Reactome database and genes associated with most significant pathways. A) Bar charts with pathway enrichment analysis of EAE – FOL versus EAE. The x-axis shows the -log10(P-value) for each pathway. Pathways show statistical significance (with adjusted p values < 0.05) in red. B) Histograms showing enriched pathways in the columns and genes associated in the rows organized by *p* value.

Figure 5. Farnesol treatment regulates some genes up- or downregulated after EAE induction. Fragments Per Kilobase of transcript sequence per Millions base pairs sequenced (FPKM) up-regulated (A) and down-regulated (B) in EAE mice vs. naïve. Significant adjusted *p* values after Tukey's comparisons indicated with asterisks (Tables 1-2 for values). *, *Adj p* < 0.05; **, *Adj p* < 0.01; ***, *Adj p* < 0.001.

Supplementary figures

Supplementary figure 1. Analysis of correlation between samples by Pearson's analysis. Highlighted within a red box, sample EV (EAE – Veh) 4 (EV4) discarded for analysis due to low correlation with other samples. The brain from EV4 was obtained from a moribund mouse.

Supplementary Figure 2. Pathway Enrichment Analysis by comparison of normalized reads to the Reactome database. A) Brains of EAE mice treated with vehicle (corn oil) (EAE – Veh) and healthy (naïve) brains. B) Brains of EAE mice treated with farnesol (EAE – FOL) and healthy (Naïve) brains. The x-axis shows the -log10(P-value) for each pathway. Pathways show statistical significance (with adjusted p values < 0.05) in red.

Supplementary Figure 3. Pathway Enrichment Analysis by comparison of normalized reads to the Reactome database. A) Brains of EAE mice treated with vehicle (corn oil) (EAE – Veh) and EAE brains. B) Brains of EAE mice treated with farnesol (EAE – FOL) and EAE - Veh brains. The x-axis shows the -log10(P-value) for each pathway. Pathways show statistical significance (with adjusted p values < 0.05) in red.

Supplementary Figure 4. Pathway Enrichment Analysis by comparison of normalized reads to the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. A) Brains of EAE mice treated with vehicle (corn oil) (EAE – Veh) and healthy (Naïve) brains. B) Brains of EAE mice treated with vehicle (corn oil) (EAE – Veh) and naive brains C) Brains of EAE mice treated with farnesol (EAE – FOL) and naive brains. The x-axis shows the -log10(P-value) for each pathway. Pathways show statistical significance (with adjusted p values < 0.05) in red.

Supplementary Figure 5. Pathway Enrichment Analysis by comparison of normalized reads to the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. A) Brains of EAE mice treated with vehicle (corn oil) (EAE – Veh) and EAE brains. B) Brains of EAE mice treated with farnesol

(EAE – FOL) and EAE brains. C) Brains of EAE mice treated with farnesol (EAE – FOL) and EAE - Veh brains. The x-axis shows the -log10(P-value) for each pathway. Pathways show statistical significance (with adjusted p values < 0.05) in red.

Supplementary Figure 6. Gene Ontology (GO) enrichment analysis of cellular components. A) Brains of EAE mice treated with vehicle (corn oil) (EAE – Veh) and healthy (Naïve) brains. B) Brains of EAE mice treated with vehicle (corn oil) (EAE – Veh) and naive brains C) Brains of EAE mice treated with farnesol (EAE – FOL) and naive brains. The x-axis shows the -log10(P-value) for each pathway. Pathways show statistical significance (with adjusted p values < 0.05) in red.

Supplementary Figure 7. Gene Ontology (GO) enrichment analysis of cellular components. A) Brains of EAE mice treated with vehicle (corn oil) (EAE – Veh) and EAE brains. B) Brains of EAE mice treated with farnesol (EAE – FOL) and EAE brains. C) Brains of EAE mice treated with farnesol (EAE – FOL) and EAE - Veh brains. The x-axis shows the -log10(P-value) for each pathway. Pathways show statistical significance (with adjusted p values < 0.05) in red.

Supplementary Figure 8. Gene Ontology (GO) enrichment analysis of molecular functions. A) Brains of EAE mice treated with vehicle (corn oil) (EAE – Veh) and healthy (Naïve) brains. B) Brains of EAE mice treated with vehicle (corn oil) (EAE – Veh) and naive brains C) Brains of EAE mice treated with farnesol (EAE – FOL) and naive brains. The x-axis shows the -log10(P-value) for each pathway. Pathways show statistical significance (with adjusted p values < 0.05) in red.

Supplementary Figure 9. Gene Ontology (GO) enrichment analysis of molecular functions. A) Brains of EAE mice treated with vehicle (corn oil) (EAE – Veh) and EAE brains. B) Brains of EAE mice treated with farnesol (EAE – FOL) and EAE brains. C) Brains of EAE mice treated with farnesol (EAE – FOL) and EAE - Veh brains. The x-axis shows the -log10(P-value) for each pathway. Pathways show statistical significance (with adjusted p values < 0.05) in red.

Supplementary Figure 10. Gene Ontology (GO) enrichment analysis of biological processes. A) Brains of EAE mice treated with vehicle (corn oil) (EAE – Veh) and healthy (Naïve) brains. B) Brains of EAE mice treated with vehicle (corn oil) (EAE – Veh) and naive brains C) Brains of EAE mice treated with farnesol (EAE – FOL) and naive brains. The x-axis shows the -log10(P-value) for each pathway. Pathways show statistical significance (with adjusted p values < 0.05) in red.

Supplementary Figure 11. Gene Ontology (GO) enrichment analysis of biological processes. A) Brains of EAE mice treated with vehicle (corn oil) (EAE – Veh) and EAE brains. B) Brains of EAE mice treated with farnesol (EAE – FOL) and EAE brains. C) Brains of EAE mice treated with farnesol (EAE – FOL) and EAE - Veh brains. The x-axis shows the -log10(P-value) for each pathway. Pathways show statistical significance (with adjusted p values < 0.05) in red.

Supplementary Figure 12. Genes upregulated after EAE induction that are not affected by vehicle or farnesol treatments. Fragments Per Kilobase of transcript sequence per Millions of base pairs sequenced (FPKM) upregulated in EAE mice vs. naïve. Significant adjusted *p* values after Tukey's comparisons are indicated with asterisks (Tables 1-2 for values). *, *Adj p* < 0.05; **, *Adj p* < 0.01; ***, *Adj p* < 0.001.

Supplementary Figure 13. Genes downregulated after EAE induction that is not affected by vehicle or farnesol treatments. Fragments Per Kilobase of transcript sequence per Millions of base pairs sequenced (FPKM) downregulated in EAE mice vs. naïve. Significant adjusted *p* values after Tukey's comparisons are indicated with asterisks (Tables 1-2 for values). *, *Adj p* < 0.05; **, *Adj p* < 0.01; ***, *Adj p* < 0.001.