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Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

For ICP-MS, the samples were injected into a PerkinElmer NexION 2000C ICP-MS instrument. The LA-ICP-MS spectrometer consisted of a laser ablation system (213 nm Nd:YAG, Cetac Technologies, Omaha, Nebraska, USA) connected to a Perkin Elmer NexION 2000C ICP-MS (Perkin Elmer, Norwalk, CT, USA).

To acquire immunofluorescence images, an Olympus FluoViewTM LV3000 confocal microscope and 10x, 20x or 40x objectives were used. For DAB-stained sections, pictures were acquired using a brightfield microscope coupled to a camera. Transmission electron microscopy images were acquired using either a JEOL 1200EX or a Tecnai G² Spirit BioTWIN transmission electron microscope.

Real time PCR data was collected using Bio Rad's CFX Connect Real-Time PCR detection system.

Behavioral data was collected by monitoring the mice in real time, using the TopScanLite software from CleverSys Inc., coupled to a camera. Behavioral data was collected automatically for each animal (TopScanLite), except the latency to reach the visible platform in the Morris water maze, which was recorded manually using a stopwatch.

For snRNA-seq, nuclei encapsulation and sequencing library preparation were performed at the Harvard Single Cell Core, according to the 10X Genomics manual. The size and quality of the prepared libraries were confirmed on Agilent high sensitivity TapeStation, and the library was independently quantified by qPCR. The prepared libraries were sequenced by Nova-Seq S4 at Harvard Biopolymers Facility.

For microglia RNA-seq, total microglial RNA was extracted from MACs-purified microglia and quantified using an Agilent Tapestation 4200 instrument, with a corresponding Agilent Tapestation High Sensitivity RNA assay. Agilent Bioanalyzer High Sensitivity DNA assay (was used to quantify cDNA concentration. Libraries were obtained using the Illumina NexteraXT kit. Purified libraries were run on an Agilent 4200

Tapestation instrument, with a corresponding Agilent D5000 ScreenTape assay to check the size and concentration of each library. Samples were loaded onto an Illumina NovaSeq 6000 instrument, with a single lane of an S4 flowcell to obtain Paired-End 100bp reads.

For Li-orotate RNA-seq, hippocampal RNA was purified using the Direct-zol RNA Mini Prep kit (Zymo Research), RNA integrity and concentration were assayed using an Agilent 2100 Bioanalyzer instrument, and libraries were prepared using Illumina TruSeq Stranded mRNA sample preparation kits from 500 ng of purified total RNA. dsDNA libraries were quantified using Qubit fluorometer, Agilent TapeStation 2200, and RT–qPCR using the Kapa Biosystems library quantification kit. Uniquely indexed libraries were pooled and sequenced on an Illumina NextSeq 500 with paired-end 75-bp reads by the Dana-Farber Cancer Institute Molecular Biology Core Facilities.

For mass spectrometry proteomic analysis, the fractions were processed at the Harvard Center for Mass Spectrometry; each fraction was submitted for a single LC-MS/MS experiment that was performed on Q Exactive HF-X High Resolution Orbitrap (Thermo Fisher, Waltham, MA) coupled with Ultimate 3000 nanoLC (Thermo Fischer, Waltham, MA).

The conductivity of Li salt solutions was measured using a ST300C conductivity meter (OHAUS, Catalog No. 83033964) equipped with a STCON7 electrode (OHAUS, Catalog No. 30080693) calibrated with potassium chloride conductivity standards. For each lithium salt, three independent solution replicates (n = 3) were prepared. Conductivity values are reported in µS/cm at 25°C.

Data analysis

ICP-MS signal data was analyzed using GeoPro 2010 Software (Cetac Technologies) and LA-ICP-MS data was analyzed using the Iolite Software 2018 (Iolite).

Aβ plaque burden and p-tau pathology were analyzed using Fiji/ImageJ 2.9.0. Fluorescent image analysis was also performed using MetaMorph NX 2.5 (Meta Series, Molecular Devices).

Quantification of myelin sheath thickness, axon diameter, and g-ratios was performed using Metamorph software (Meta Series). A total of 1,376 axons (CTRL group) and 1,396 axons (Li-deficient group) were analyzed from 8 randomly selected fields per animal (4800x magnification) spanning the corpus callosum.

For Golgi labeling, dendritic spine density was quantified using the Fiji software 2.9.0.

For snRNA-seq, the demultiplexed raw sequencing reads were aligned to the mouse genome (mm10 from 10X genomics) using Cell Ranger (6.1.2), with –include-introns option, to account for nuclear pre-mRNAs. The generated counts table was loaded to Seurat (v4) to generate Seurat objects. Cells with more than 10 percent of reads being attributed to mitochondrial transcripts were filtered out. Cells that expressed less than 200 features (low-quality cells) or higher than 8,000 features (apparent doublets) were also filtered out. These thresholds are determined by visual inspection of the distribution of features among cells (Seurat, VlnPlot) and generally consistent with other reports. The cells that passed quality controls were log normalized using NormalizeData function from Seurat with a scale factor of 10,000. Variable features were identified using FindVariableFeatures function from Seurat with vst selection method and 2000 features. Data was scaled using ScaleData, and PCA was performed using RunPCA with the identified variable features using Seurat. Nearest neighbors were found using FindNeighbors using dimension 1:30, which was determined by ElbowPlot following Seurat's manual. The number of clusters was determined by FindClusters function using Seurat v4. UMAP and TSNE were performed using RunUMAP or RunTSNE using Seurat with dimensions 1:30 and do.fast=TRUE parameter. Potential doublets were removed using DoubletFinder (v3) following the default parameters. After filtration, cluster-specific markers were determined by FindAllMarkers function (Seurat v4), with parameters only.pos = F, min.pct = 0.25 and max.cell.per.ident = 500. Cell types were identified by cross-referencing the transcriptome of each individual cell to the Mouse Cell Atlas and independently validated by confirming the expression of established cell type-specific markers, on a cluster-to-cluster basis. Cell type-specific abundance and differential gene expression analyses were performed on the major cell types (excitatory neurons, inhibitory neurons, granule cells, microglia, astrocytes, oligodendrocytes, oligodendrocyte precursor cells and endothelial cells). Clusters of the same cell types were combined to increase the statistical power, and clusters with mixed cell types (<80% homogenous) were removed for cell type-specific analyses. Relative abundance of each cell type was computed by dividing the number of cells of the particular cell type by the total number of cells. Two-tailed unpaired Student's t-tests were performed to determine if there were significant differences in the relative abundance of each cell type between the control and lithium deficient conditions. Differentially expressed genes (DEGs) were computed by the MAST test in Seurat. Genes expressed in less than 1% of the cells in each cell type were filtered out.

For microglia RNA-seq, raw RNA-sequencing data in FASTQ format was subjected to quality assessment using FastQC (version 0.11.9) and sequencing reads are aligned to mouse genome (mm10) using STAR aligner with the following options: --outFilterMismatchNmax 999 --outFilterMismatchNoverLmax 0.04 --alignSJDBoverhangMin 1 --alignSJoverhangMin 8 --outFilterMultimapNmax 20 --outFilterType BySJout --alignIntronMin 20 --alignIntronMax 1000000 --alignMatesGapMax 1000000. Microglia RNAseq yielded an average of 148 million uniquely mapped reads; gene expression levels were quantified using htseq-count. Differential gene expression analysis was conducted using DESeq2 to identify DEGs between Li-def and control microglia, with an adjusted p-value cutoff of 0.05. Upregulated and downregulated DEGs from Li-deficient WT and 3xTg microglia were further analyzed for overlapping DEGs and the overlapping DEGs were subjected to gene ontology enrichment analysis using Metascape v3.5.20240101.

For Lithium orotate RNA-seq, quality control of sequencing reads was performed with FastQC version 0.11.5. Reads were aligned to the Mouse GRCm38 genome with GENCODE M21 gene models using STAR version 2.7.0f with options --outSAMunmapped Within --alignSJDBoverhangMin 1 --alignSJoverhangMin 8 --outFilterMultimapNmax 20 --outFilterType BySJout --alignIntronMin 20 --alignIntronMax 5000000 --alignMatesGapMax 5000000 --twopassMode Basic. The expression of genes was quantified as gene counts using STAR at the same time as alignment with option --quantMode GeneCounts. Gene counts were input to edgeR. Genes were deemed expressed if at least n=9 samples (where n is the group size) had >1 CPM. Genes not satisfying these criteria were removed, keeping the original library sizes. This filtering retained n=14,862 expressed genes out of 55,536 annotated genes for subsequent analyses. Counts were then normalized using the TMM method in edgeR. To adjust gene expression for covariates we fit the linear regression model for each gene and cohort separately using lm() in R: gene expression ~ group + covariates where gene expression is log(CPM), and using the group and covariates: factor, two levels: Lithium Orotate and Water (reference level), covariates (sequencing batch (factor, two levels) and 1 RUVr covariate (continuous). The final normalized and adjusted gene expression values were derived from adding the regression residuals to the estimated effect of the group level to preserve the effect of the group on expression. These normalized and adjusted gene expression values were used to perform gene–gene regression analysis and gene–gene group regression analysis, and to visualize gene expression. To adjust for technical variation, we used the RUV with residuals (RUVr) method implemented in the RUVSeq version 1.18.0 Bioconductor package. We performed a first pass edgeR analysis as described in section "Differential expression analysis" up to and including the glmFit() step with the covariates listed above

excluding the RUVr covariates. Then we used residuals() with argument type='deviance' to obtain a matrix of deviance residuals. The specified number of unwanted factors (RUVr covariates) used in final analyses were then estimated by the RUVr function using log(CPM) expression values and the residuals. The number of unwanted factors was selected based on separation of groups in principal components plots using normalized and adjusted gene expression values and checking that histograms of differential expression P values showed a uniform or anti-conservative pattern. Differential expression analysis between groups with covariate adjustment using the covariates listed above was performed for expressed genes using edgeR (estimateDisp, glmFit, and glmLRT with default arguments) in R. Genes were considered differentially expressed if FDR < 0.05. Gene ontology enrichment analysis was performed separately for upregulated genes and downregulated genes using Metascape v3.5.20240101.

To conduct the GWAS-DEG enrichment analysis, we converted the mouse gene symbols to human orthologs, using a two-step process. First, the "alias2SymbolTable" function within the "Limma" R package 3.58.1 was utilized to map any gene aliases to their corresponding main symbols. Subsequently, the resulting gene symbols were converted to human orthologs using the MGI ortholog table. If there were multiple mapping candidates, all possible conversions were applied. For example, if a mouse gene has multiple human orthologs, records with all relevant human gene symbols were generated. This standardized gene nomenclature facilitated cross-species comparisons in subsequent analyses. To conduct the GWAS-DEG enrichment analysis microglia isolated from Li-deficient mice, we utilized MAGMA v1.10. Gene-set analysis was conducted using the default MAGMA settings, with multiple testing correction applied to account for the number of gene sets tested. Enrichment results were considered significant at a false discovery rate (FDR) of 0.05.

To examine the overlap of human DEGs and mouse DEGs derived from our transcriptomic analyses, we first converted mouse gene symbols to human orthologs, as described above. We matched the cell types analyzed in our mouse studies with those analyzed in humans. To assess the statistical significance of the overlap between the two DEG sets, we conducted a Fisher's exact test. To control for multiple comparisons arising from the analysis of different cell types and DEG directions (upregulated and downregulated), we adjusted the p-values using the Benjamini-Hochberg procedure. Two sets of adjusted p-values were calculated: One set for the overlap of genes upregulated in both datasets (indicated in red), and another for the overlap of genes downregulated in both datasets (indicated in blue).

The analyze the proteomics data, raw data was submitted for analysis in Proteome Discoverer 3.0 software (Thermo Scientific). The MS/MS Data was searched against the UniProt reviewed Mus musculus (Mouse) database along with known contaminants such as human keratins and common lab contaminants. Sequest HT searches were performed using the following guidelines: a 10 ppm MS tolerance and 0.02 Da MS/MS tolerance; Trypsin digestion with up to two missed cleavages; carbamidomethylation (+57.021 Da) on cysteine, TMT 6-plex tags on peptide N-termini and lysine residue (+229.163 Da) were set as static modification; oxidation (+15.995 Da) of methionine set as variable modification; minimum required peptide length set to ≥ 6 amino acids. At least one unique peptide per protein group was required for identifying proteins. Of 13,404 proteins identified, only n=3,392 proteins were identified with high confidence (MS2 spectra assignment FDR<0.01 on both protein and peptide level by applying the target-decoy database search by Percolator) and were included in the statistical analysis, as per standard procedures at the Harvard Center for Mass Spectrometry. The sample labels were control (samples 1,3,5,7) and Li-deficient (samples 2,4,6,8) and were all 3xTg homozygous females, age 15 months (duration of the treatment: from 6 to 15 months). An ANOVA followed by Tukey's post hoc test was used to assess differences in protein abundance between Li-deficient and control samples. P-values were adjusted for multiple comparisons using the Benjamini-Hochberg method to control the false discovery rate. Proteins with an adjusted P-value < 0.05 were considered differentially abundant. T

Statistical analysis was performed using the GraphPad software v 10.3.0 (507). Statistical tests used are noted in the figure legends or in the relevant Methods section. Throughout the paper, all tests are two sided and unpaired unless stated otherwise. A significance level of 0.05 was used to reject the null hypothesis unless stated otherwise. The sample size, the age and sex of experimental animals, as well as the summary of each statistical test (including degrees of freedom, confidence intervals and P-values) can be found in the Source Data file.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The snRNA-seq, microglia RNA-seq and LiO RNA-seq data have been deposited in the Gene Expression Omnibus repository (GSE272344, GSE275326 and GSE295788).

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD063039. The proteins identified with high confidence and included in the statistical analysis are listed in Supplementary Table 7. All other proteins identified with lower confidence can be accessed from the files deposited at the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD063039. MS/MS proteomic data were searched against the UniProt Mus musculus protein database (FASTA format, including isoforms), downloaded on July 17, 2022.

For GWAS-DEG enrichment analysis using MAGMA, summary statistics for Alzheimer's disease (Accession ID: MONDO_0004975) were obtained from the GWAS Catalog (https://www.ebi.ac.uk/gwas/efotraits/MONDO_0004975).

Clinico-pathological data on postmortem human samples from ROSMPAP can be requested at https://www.radc.rush.edu.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	Both male and female participants were included in the human studies. In both sexes, individuals with MCI and AD showed reduced cortical-to-serum Li ratios and lower total cortical Li levels. Mouse experiments also included both sexes, and results were consistent between males and females.
Reporting on race, ethnicity, or other socially relevant groupings	Donor sex was self-reported and provided by Rush Medical Center, which conducted the ROSMAP longitudinal study, as well as by other tissue sources including Massachusetts General Hospital, Duke University, and Washington University.
Population characteristics	The cohort analyzed in this study consisted of 40.2% males and 59.8% females. Among the subgroups, NCI cases comprised 40.8% males and 59.2% females; MCI cases had 42% males and 58% females; and AD cases included 36.4% males and 63.6% females. Both males and females in the MCI and AD groups exhibited reduced Li cortex-to-serum ratio and lower total cortical Li levels. For ROSMAP: To assess cognitive function, 21 cognitive function tests were used, 19 were in common and 11 used to inform on clinical diagnoses, as previously described (references 60,61). Brain tissue obtained from Massachusetts General Hospital, Duke University, and Washington University had a confirmed pathological diagnosis of AD or NCI.
Recruitment	Our study did not involve any recruitment or interaction with the donors. We only analyzed postmortem tissue, which was provided by Rush University Medical Center, as well as the brain banks at Massachusetts General Hospital, Duke University and Washington University. Samples were randomly selected by the source institutions based on tissue availability and alignment with the requested diagnostic categories (NCI, MCI, AD). Within each diagnostic group, samples were matched for age and sex to ensure group comparability.
Ethics oversight	Postmortem human brain and serum samples were obtained in accordance with institutional guidelines and with approval from the Harvard Medical School Institutional Review Board. All procedures complied with relevant ethical regulations. All postmortem human brain and serum samples were fully deidentified prior to receipt, and no identifiable private donor information was accessible to the researchers. As such, informed consent was not applicable. Informed consent, an Anatomic Gift Act, and a repository consent were obtained and the studies were approved by an Institutional Review Board (IRB) of Rush University Medical Center. The Religious Orders Study and Rush Memory and Aging Project (ROSMAP) were approved by an IRB of Rush University Medical Center.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

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☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No statistical methods were used to pre-determine sample size. Sample sizes were chosen based on prior literature that used similar approaches (see Martorell et al. Cell 177(2):256-271 (2019); Zullo et al. Nature 574, 359-364 (2019); laccarino et al. Nature 540(7632):230-235 (2016); Sevigny et al. Nature 537(7618):50-6 (2016); Oddo et al. Neuron 39(3):409-21 (2003)). The sample size was determined to be adequate based on the magnitude and reproducibility of observed differences between the different groups.
Data exclusions	Statistical outliers were identified using the ROUT method (Q=1%, corresponding to FDR<1%) in GraphPad Prism and excluded from the statistical analysis where appropriate. No data were excluded from the study.
Replication	The ICP-MS findings in postmortem human were replicated as follows. First, reduced lithium content in the cortex of AD patients was observed using 2 independent methods – after measurement of total lithium levels in frozen cortical material of cases from both ROSMAP (Fig. 1d) and other sources (Fig. 1e), as well as after fractionation and removal of amyloid plaques (Fig. 1g). Second, decreased Li levels in the AD vs. NCI frontal cortex (P=2x10-3) were also independently confirmed when n=60 NCI and AD cases were processed and analyzed by ICP-MS in a different laboratory (Spectroscopy Core Facility at the University of Nebraska, Lincoln). Third, decreased Li levels in the AD vs. NCI frontal cortex (P=3x10-3) were also confirmed when n=48 NCI and AD cases were processed using an alternative protocol. Frozen samples were thawed and dried to a constant weight by incubating in a dry oven at 60°C for 48 hours. The dried tissue was then digested in 1 mL of 67% nitric acid using a heating block at 95°C for 3 hours. After digestion, 0.3 mL of 30% hydrogen peroxide was added, and the mixture was heated for an additional 3 hours. Finally, the samples were diluted and analyzed using ICP-MS. In agreement with previous findings, the Li levels we measured in the frontal cortex (FC) of aging NCI cases (ROSMAP cases: mean 2.36 ± 1.23 ng/g, range 0.52-6 ng/g; and non-ROSMAP cases: mean 3.5 ± 2.27 ng/g, range 0.89-9.94 ng/g; Figure 1d,e) were similar to those measured in

a previous study [Ramos, P. et al. J Trace Elem Med Biol 38, 174-182 (2016)] which found that Li levels were 4.1 ± 1.7 ng/g in the frontal cortex of aged non-diseased cases (age 71 ± 12 years). Similarly, the Li levels we measured in the cerebellum (ROSMAP cases: mean 2.9 ± 1.69 ng/g, range 0.58-8.4 ng/g; Extended Data Fig. 1b) were similar to those measured in the previous study by Ramos et al. (2.9 ± 1.3 ng/g). Finally, consistent with previous studies, we also observed significantly elevated levels of sodium and zinc, along with reduced copper levels, in the AD frontal cortex (Figure 1a,b and Supplementary Table 1). [References: For sodium: Graham, S. F. et al. J Alzheimers Dis 44, 851-857 (2015). For zinc: Religa, D. et al. Elevated cortical zinc in Alzheimer disease. Neurology 67, 69-75 (2006). For copper: Squitti, R. et al. Biomolecules 11 (2021). https://doi.org/10.3390/biom11070960].

The effects of lithium deficiency on amyloid pathology were replicated in three mouse models: 3xTg, J20, and WT mice. All models exhibited elevated Aβ42, and a marked acceleration of amyloid deposition observed in AD models (3xTg and J20). In 3xTg mice, accelerated amyloid pathology was evident at both short-term (5 weeks) and long-term (9 months) durations of a lithium-deficient diet. Similarly, in J20 mice, accelerated amyloid pathology was observed after both 3 and 9 months of lithium deficiency. Additionally, in WT mice, we noted increased Aβx-42 production at two different time points (20 and 26 months of age) in the hippocampus and cortex.

The effects of Li deficiency on tau pathology were observed after both short (5 weeks) and longer (9 months) treatment durations in 3xTg mice, using two markers (pSer202 tau, CP13; as well as pSer396/Ser404 tau, PHF1) of tau phosphorylation. Moreover, Thioflavin S labeling allowed the detection of tangle-like profiles in Li-deficient 3xTg hippocampus.

The effects of Li deficiency on synapses were substantiated as follows: 1) by snRNA-seq analysis; 2) using labeling of synaptic markers (PSD-95 and Synaptophysin); 3) by unbiased proteomic analysis; and 4) using Golgi labeling, which showed a significant loss of dendritic spines in both WT and 3xTg Li-deficient mice.

Myelin and white matter disruption by lithium deficiency was observed using Fluoromyelin labeling, labeling for Myelin Basic Protein (MBP) and labeling of both mature oligodendrocytes (using Aspartoacylase as a marker) and oligodendrocyte precursor cells (using PDGFRa as a marker). In addition, transmission electron microscopy confirmed the myelin distruption in Li deficient mice at the ultrastructural level.

The effects of Li deficiency on microglia were observed in WT, 3xTg and J20 mice. In WT and 3xTg mice, Li deficiency altered the microglial transcriptome. Immunolabeling showed more reactive microglia in both 3xTg and J20 Li-deficient mice. Moreover, Li-deficient microglia secreted more pro-inflammatory cytokines, and had an impaired ability to clear amyloid beta.

The effects of Li deficiency on memory were replicated in 2 mouse lines: 3xTg and WT. Li deficiency accelerated memory loss in both lines.

The potentiating effects of lithium deficiency on GSK3β were demonstrated through network modeling (Ingenuity Pathway Analysis), proteomics, increased GSK3β levels in the Li-deficient 3xTg hippocampus, and immunolabeling for total and active GSK3β (pTyr216). Enhanced GSK3β activity under lithium-deficient conditions was further supported by both in vivo and in vitro experiments using GSK3β inhibitors, which reversed the effects of lithium deficiency on Aβ deposition, tau phosphorylation, microglial activation, cytokine secretion, amyloid clearance, and myelin integrity.

The plaque-evading abilities of Li orotate (LiO) were demonstrated in both aged J20 and 3xTg mice, at advanced stages of amyloid pathology. Moreover, Li binds human Aβ1-42 fibrils and oligomers in vitro, over a range of concentrations. LiO binds less avidly to amyloid beta fibrils and oligomers compared to the clinical standard lithium carbonate (LiC), further suggesting that it may evade amyloid plaques in vivo.

The therapeutic effects of lithium orotate (LiO) were demonstrated in both 3xTg and J20 mouse models of Alzheimer's disease. In 3xTg mice, LiO showed both preventive and restorative effects on amyloid and tau pathology. Furthermore, LiO outperformed lithium carbonate (LiC), as evidenced by reduced amyloid and phosphorylated tau accumulation, improved synaptic integrity, attenuated microgliosis and astrogliosis, modulation of GSK3β and β-catenin signaling, and improved performance in the Morris water maze test.

The beneficial effects of LiO treatment during aging in wild-type (WT) mice were demonstrated by improved performance in memory tests, preservation of dendritic spines, and reduced neuroinflammation. These findings align with the accelerated cognitive decline observed in Li-deficient WT mice, collectively supporting a physiological role for lithium in maintaining brain function during normal aging.

Randomization

For all mouse studies, animals were randomly assigned to experimental groups.

Blinding

For ICP-MS analysis of human samples, all measurements were performed under double-blind conditions. A team member not involved in the study re-labeled the samples and maintained the key linking original and blinded identifiers. Unblinding was conducted after data acquisition, in the presence of both the study investigators and the independent team member. For all other analyses involving human tissue, investigators were blinded to sample identity during both data collection and analysis. In mouse studies, genotypes and treatment conditions remained blinded to investigators throughout data collection and analysis.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

To detect different tau species, we used two antibodies: (1) a mouse monoclonal antibody that recognizes phosphorylated Ser 202 tau (CP13 clone; generous gift from Peter Davies, Albert Einstein College of Medicine, NY; dilution 1:150), and (2) a mouse monoclonal antibody raised against phosphorylated Ser 396/Ser404 tau (PHF1 clone; from Peter Davies, dilution 1:200). To detect transgenically expressed human Aβ in mouse models, we used a rabbit monoclonal anti-Aβ antibody (clone D54D2; Cell Signaling, catalog No. 8243, dilution 1:250).

The following primary antibodies were also used: anti-Aspartoacylase [N1C3-2] (GeneTex, GTX113389; rabbit polyclonal, dilution 1:200), anti-Aspartoacylase (clone D-11; Santa Cruz Biotechnology, sc-377308, dilution 1:50), anti-β-catenin (clone E247; Abcam, ab32572; rabbit recombinant monoclonal, dilution 1:250), anti-β-catenin (clone 1B8A1; PTGlab, 66379-1-Ig, mouse monoclonal, dilution 1:200), anti-CD68 (clone KP1; Abcam, ab955; mouse monoclonal, dilution 1:200), anti-GFAP (Sigma Aldrich, G9269; rabbit polyclonal, dilution 1:200), anti-GSK3β (clone 3D10; Novus Bio, NBP1-47470SS; mouse monoclonal, dilution 1:200), anti-pTyr216-GSK3β (Millipore Sigma, SAB4300237; rabbit polyclonal, dilution 1:100), anti-pSer9-GSK3β (Abcam, ab131097; rabbit polyclonal, dilution 1:100), anti-Iba1 (clone EPR16588; Abcam ab178846; rabbit recombinant monoclonal, dilution 1:2,000), anti-PSD-95 (clone K28/43; Biolegend, 810401; mouse monoclonal, dilution 1:250), anti-Synaptophysin (clone SY38; Millipore Sigma, mouse monoclonal, MAB5258-I; dilution 1:200), anti-Neurofilament (pan axonal marker; clone SMI-312; Biolegend, 837904; mouse monoclonal, dilution 1:200), anti-GPNMB (clone 2B10B8; PTGlab, 66926-1-Ig; mouse monoclonal, dilution 1:200), anti-LPL (Novus Bio, AF7197-SP; goat polyclonal, dilution 1:200), anti-MAP2 (Phosphosolutions, 1099-MAP2; goat polyclonal, dilution 1:500), anti-Myelin Basic Protein (MBP) (clone D8X4Q; Cell Signaling, 78896; rabbit monoclonal, dilution 1:200) and anti-PDGFRα (R&D Systems, AF1062; goat polyclonal, dilution 1:200).

Secondary antibodies were used at a 1:300 dilution: donkey anti-Goat Alexa 594 (Invitrogen, A-11058), donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed antibody, Alexa 488 (ThermoFisher Scientific, A21206), donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed antibody, Alexa 594 (ThermoFisher Scientific, A21203), donkey anti-Mouse Alexa 647 (Invitrogen, A-31571), donkey anti-Rabbit Alexa 647 (Invitrogen, A-31573), donkey-anti-Goat Alexa 488 (Invitrogen, A-11055).

Validation

The tau antibodies obtained from Peter Davies, raised against pSer202 tau (CP13 clone) and pSer396/Ser404 tau (PHF1), have been extensively validated and cited in the field. In the lab, we have confirmed that both CP13 and PHF1 recognize tau species at the expected molecular weight in western blotting.

The anti-Aβ antibody (clone D54D2; Cell Signaling, catalog No. 8243) is a recombinant rabbit monoclonal antibody that recognizes human Aβ and has been validated for use in WB, IP, IF, and IHC-P. It has been cited 167 times.

The anti-Aspartoacylase antibody [N1C3-2], (GeneTex, GTX113389), rabbit polyclonal, has been validated for use in immunofluorescence, as well as WB, IHC-P, and IHC-Fr and has been cited 19 times.

The anti-Aspartoacylase antibody (clone D-11; Santa Cruz Biotechnology, sc-377308) is a mouse monoclonal antibody that has been validated for use in WB, IP, IF, IHC-P and ELISA. It has been cited 8 times.

The anti-β-catenin antibody (clone E247; Abcam, ab32572), rabbit recombinant monoclonal antibody, is a ChIP Grade antibody that has been validated for use in ChIP, ICC/IF, IHC-P, IP and Western blot. Specificity was confirmed in a knockout line by abcam. The antibidy has been cited 1,408 times.

The anti-β-catenin antibody (clone 1B8A1; PTGlab, 66379-1-Ig), mouse monoclonal antibody, has been validated for use in WB, IHC, IF/ICC, IF-P, flow cytometry, IP, and ELISA. The antibody is knockout-validated and has been cited 108 times.

The anti-CD68 (clone KP1; Abcam, ab955), mouse monoclonal antibody, has been cited 620 times and has been validated for use in IHC-P, ICC/IF, WB by Abcam.

The anti-GFAP (Sigma Aldrich, G9269), rabbit polyclonal, has been validated for use in immunofluorescence, as well as IHC (P) and WB and has been cited 386 times.

The anti-GSK3β antibody (clone 3D10; Novus Bio, NBP1-47470SS), mouse monoclonal, has been cited 10 times, and validated for use in WB, ICC/IF, IHC, ELISA, and flow cytometry. In previous work, we also found that the antibody recognizes a single major GSK3β band in WB.

The anti-pTyr216-GSK3β Millipore Sigma, SAB4300237), rabbit polyclonal, is an affinity purified antibody which has been validated by Millipore Sigma for use in immunofluorescence (using breast cancer samples), and shows a unique band at the expected molecular weight in WB.

The anti-pSer9-GSK3β (Abcam, ab131097), rabbit polyclonal, has been validated for use in immunofluorescence, as well as WB, IHC-P, ICC/IF and has been cited 78 times.

The anti-Iba1 (clone EPR16588; Abcam, ab178846), rabbit recombinant monoclonal, has been validated for use in immunofluorescence, as well as WB, IHC-P, ICC/IF and has been cited 873 times.

The anti-PSD-95 antibody (clone K28/43; Biolegend, 810401), mouse monoclonal, is quality tested by Sigma, and has been validated for use in immunofluorescence; it has been cited 12 times.

The anti-Synaptophysin (clone SY38; Millipore Sigma, MAB5258-I), mouse monoclonal, has been validated for use in immunofluorescence, as well as flow cytometry, IHC-P and has been cited 255 times.

The anti-Neurofilament marker (clone SMI-312; pan axonal marker; Biolegend, 837904) is a mouse monoclonal antibody which has been validated for use in immunofluorescence, is IHC-P in-house quality tested, and has been cited over 100 times.

The anti-GPNMB (clone 2B10B8; PTGlab, 66926-1-Ig), mouse monoclonal, has been validated for use in immunofluorescence, as well as WB, IHC, IF/ICC, and ELISA and has been cited 4 times.

The anti-LPL (Novus Bio, AF7197-SP), goat polyclonal, has been validated for use in immunofluorescence, as well as WB, IHC, and ICC and has been cited 11 times.

The anti-MAP2 (PhosphoSolutions, 1099-MAP2), goat polyclonal, detects human, mouse, and rat MAP2 and is Protein G purified. It is recommended for use in WB, ICC and IHC. It has been cited 3 times.

The anti-Myelin Basic Protein (MBP) (clone D8X4Q; Cell Signaling, 78896), is a rabbit IgG monoclonal that recognizes endogenous levels of total myelin basic protein in mouse, rat and human. It is recommended for WB, ICC, IF and IHC-P and has been cited 114 times.

The anti-PDGFRα (R&D Systems, AF1062) is an affinity-purified goat polyclonal IgG that recognizes the mouse antigen in WB, ELISA and IHC. It has been cited 322 times.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	The BV2 cell line has been available in the Yankner laboratory for over 20 years; we used original stocks stored at -180 deg. Celsius to derive the lines used in this study.
Authentication	The cells are positive for the microglial markers CD11b and Iba1, and exhibit a small, round to slightly elongated shape with a clear cytoplasm and sometimes short processes, consistent with microglial morphology.
Mycoplasma contamination	Not tested.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this study.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	Mus musculus was used to model brain aging and Alzheimer's disease (AD), employing both wild-type (WT) and transgenic mouse lines. WT mice were on a C57BL/6J background. The 3xTg mice17 carry APPSwe and tauP301L mutant transgenes, as well as a PS1 knock-in mutation, and were in a hybrid C57BL/6J and 129Sv/Ev background. The J20 mice16 express transgenic mice express a mutant form of the human amyloid protein precursor bearing both the Swedish (K670N/M671L) and the Indiana (V717F) mutations (APPSwInd) in a C57BL/6J background. To assess treatment effects on disease onset and progression, animals were treated either before pathology emerged (5–6 months for 3xTg; 3 months for J20) or after pathology was established (starting at 9 months for 3xTg and 17 months for J20). To investigate age-related effects in wild-type (WT) mice, chronic treatments were initiated in adulthood (10–12 months) and continued for 10–14 months during aging.
Wild animals	No wild animals were used in this study.
Reporting on sex	Both male and female mice were included in the study, and the major findings were consistent across sexes. Details on the number and sex of animals used in each experimental group are provided in the Source Data file.
Field-collected samples	No field collected samples were used in this study.

Ethics oversight	Animal housing and experimental procedures were approved by the Institutional Animal Care and Use Committee of Harvard Medical School.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A