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Short Communication

Nitrate ameliorates myelin loss and cognitive impairment in Alzheimer's disease through upregulation of neuronal sialin and subsequent inhibition of TPPP phosphorylation

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive decline. The pathological hallmarks of AD include the deposition of β-amyloid (Aβ) plaques, hyperphosphorylation of tau protein, synaptic loss, and reactive astrogliosis in the brain [1]. As there is still no effective therapy for AD, the development of novel treatment strategies is imperative.

Numerous studies have demonstrated the therapeutic potential of nitrate, a natural dietary component, in treating pulmonary hypertension [2] and hypertension [3], as well as enhancing human exercise capacity [3,4]. In addition, we have previously shown that nitrate administration offers protection against stress-induced gastric injury [5] and salivary gland injury caused by radiotherapy [6]. In the cortex of AD patients, significantly decreased nitrate levels have been found [7]. Moreover, several epidemiological studies have demonstrated an inverse association between the amount of nitrate consumed in the diet and the risk of AD, as well as the rate of cognitive decline in AD patients [8,9], which implies nitrate can have a beneficial effect on AD. However, its exact role in protecting against AD and the underlying mechanism are still unclear.

Nitrate exerts its functions predominantly in two ways. Firstly, via the nitrate–nitrite–nitric oxide (NO) pathway, nitrate is metabolized into NO, which subsequently facilitates vasodilation and molecular signal transduction [10]. Secondly, by upregulating the expression of sialin, a nitrate transport channel, nitrate can modulate various cellular biological functions [10,11]. Sialin is widely distributed in the body [12]. In recent years, our research group has conducted numerous studies on nitrate and sialin [5,6,13]. However, previous research on the biological role nitrate exerts via sialin primarily focused on peripheral tissues and organs, leaving the nitrate/sialin-mediated effects in the central nervous system not fully understood. Mutations in the *SLC17A5* gene, which encodes for sialin, primarily manifest as neurological symptoms such as cognitive impairment, seizures, and hypotonia. MRI scans of patients with *SLC17A5* mutations reveal demyelinating lesions, characterized by diminished white matter volume and corpus callosum thinning [14]. *Slc17a5* knockout (*Slc17a5*$^{-/-}$) mice display comparable symptoms and neuropathological alterations [15], suggesting sialin may be involved in the regulation of the structure and function of the white matter, particularly the myelin sheath.

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However, it is presently unknown whether sialin is involved in the pathogenesis of AD by modulating myelin.

In this study, we explored the role nitrate plays in AD and the possible mechanism by which it can offer protection against this disease. Specifically, we treated 7–8-month-old, male APP/PS1 mice and male, wild-type C57BL/6J mice of the same age with 4 mmol/L sodium nitrate ($NaNO_3$) or saline daily by intragastric administration for two months. The result showed that nitrate treatment resulted in a significant increase in hippocampal cell nitrate levels in both APP/PS1 and wild-type C57BL/6J mice compared with saline-treated mice (Fig. 1a). The novel object recognition (NOR) test showed that nitrate administration significantly alleviated the decrease in discrimination index (DI) values (Fig. 1b, c). Furthermore, the Y-maze test indicated nitrate effectively attenuated the reduction in spontaneous alternation rate (Fig. 1d), with no significant differences in speed among the four groups (Fig. 1e).

Aβ plaque formation is a characteristic pathological feature in AD patients [1], which we confirmed using immunofluorescence microscopy (Fig. 1f, g). The postmortem human hippocampal tissues obtained from AD patients and Non-AD individuals were provided by the National Human Brain Bank for Development and Function, Chinese Academy of Medical Sciences, and Peking Union Medical College in Beijing, China. The study involving human brain samples was approved by the Institutional Review Board of the Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences (approval numbers: 009-2014, 031–2017, 2022125) and the Ethics Committee of the Capital Medical University (approval number: Z2022SY065). All subjects have provided written informed consent. Detailed information on the human brain samples is provided (Table S1 online). Using immunofluorescent staining of $A\beta_{1-42}$ and Thioflavin S histological staining, we found a significantly reduced Aβ plaque count in hippocampal tissue of APP/PS1 mice after nitrate treatment (Fig. 1h, i, Fig. S1a, b online). Additionally, ELISA analysis revealed significantly decreased levels of both soluble and insoluble $A\beta_{1-42}$ in the hippocampal tissue of APP/PS1 mice after nitrate treatment (Fig. S1c, d online). Western blot analysis showed nitrate administration significantly relieved the increase in $oA\beta_{1-42}$ levels in APP/PS1 mice, as well as the decrease in expression of synaptic proteins PSD95 and synaptophysin (Fig. 1j–m). Transmission electron microscopy demonstrated nitrate significantly attenuated the abnormal synaptic ultrastructure in hippocampal tissue of APP/PS1 mice (Fig. S2 online). Taken together, these results suggested nitrate administration alleviates the AD-like pathology, and cognitive impairment in APP/PS1 mice.

To investigate how nitrate alleviates AD-like pathology and cognitive impairment, we performed RNA sequencing analysis of mouse hippocampal tissue. Using Gene Ontology (GO) analysis, we discovered that pathways enriched in genes significantly upregulated in nitrated-treated versus saline-treated APP/PS1 mice were predominantly linked to the myelin sheath (Fig. 1n). This finding indicated that the myelin sheath alterations in APP/PS1 mice can be alleviated by nitrate. In hippocampi of AD patients, we saw a decline in fluorescent intensity of myelin basic protein (MBP) compared with Non-AD individuals (Fig. 2o, p) indicating myelin loss in AD patients. Furthermore, Western blot results showed nitrate effectively ameliorated the decline in the myelin-related proteins myelin oligodendrocyte glycoprotein (MOG) and MBP compared with saline in APP/PS1 mice (Fig. S3a–c online). However, the expression of factors that control oligodendrocyte differentiation, including SRY-related HMG-box gene 10 (SOX10) and oligodendrocyte transcription factor (Olig2), along with the oligodendrocyte progenitor marker platelet-derived growth factor receptor α (PDGFR-α), did not significantly differ between the four mouse groups described earlier (Fig. S3d–f online). Moreover, transmission electron microscopy demonstrated nitrate significantly ameliorated the decline in myelin sheath thickness, as indicated by the g-ratio, in APP/PS1 mice (Fig. S3g–i online). These results suggested that nitrate ameliorates myelin loss in this AD mouse model.

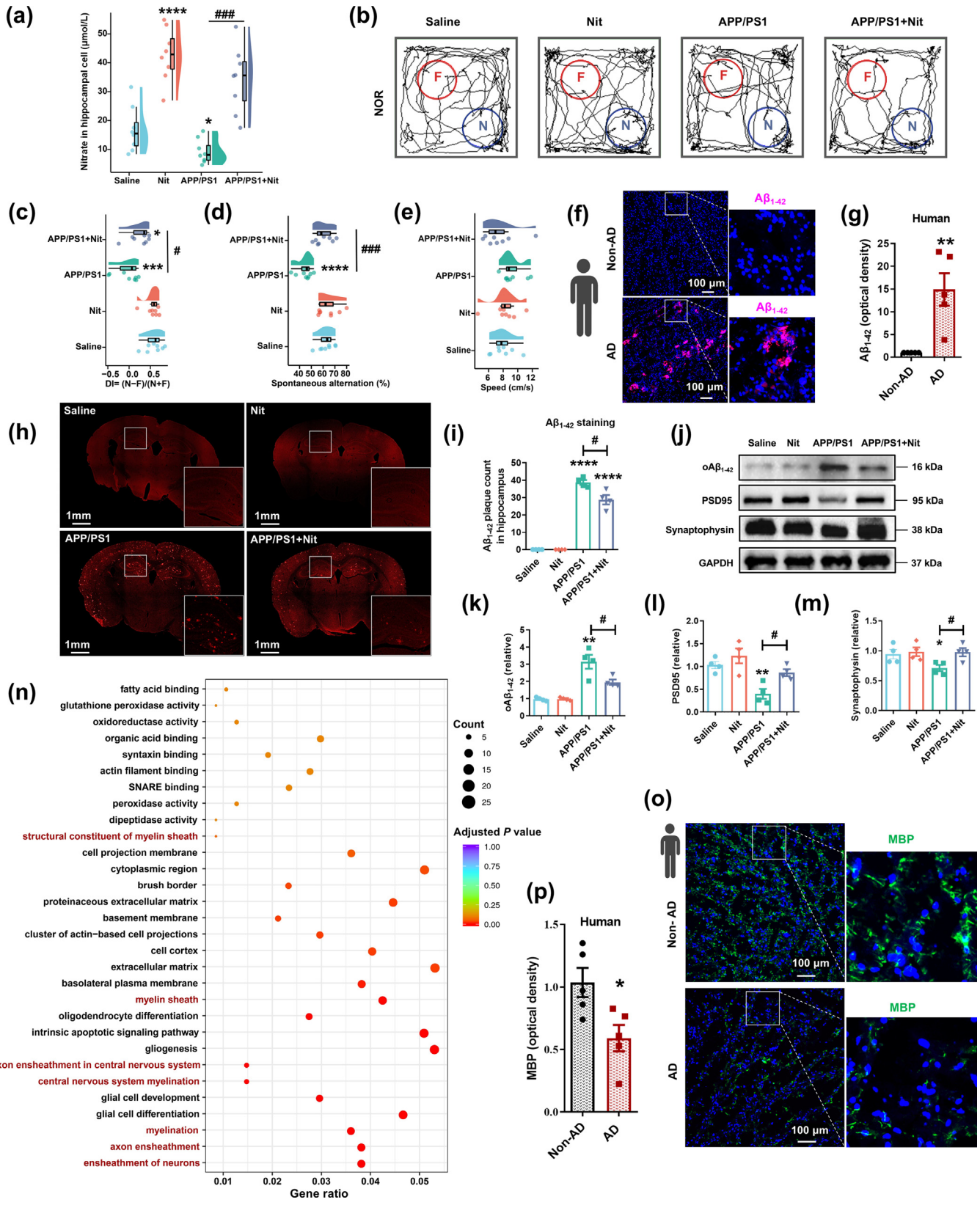
Next, we explored how nitrate ameliorates myelin loss and cognitive impairment in APP/PS1 mice. As nitrate acts in two ways—by serving as an NO donor and by upregulating sialin [10]—we assessed the expression levels of key downstream molecules of nitrate: NO and sialin. We found significantly decreased sialin protein levels in hippocampi of AD patients (Fig. 2a, b) and downregulation of the encoding gene for sialin (*SLC17A5*) in temporal cortices of AD patients (Fig. S4 online). Moreover, nitrate administration rescued the decrease in hippocampal sialin levels in APP/PS1 mice (Fig. 2c, d), but there was no significant difference in hippocampal NO levels between APP/PS1 and wild-type C57BL/6J mice (Fig. S5 online). These results suggested that nitrate can attenuate cognitive deficits by increasing sialin levels.

To elucidate which role sialin plays in different brain regions, we conducted spatial-transcriptomics RNA sequencing analysis of four brain sections from wild-type C57BL/6J and *Slc17a5*$^{-/-}$ mice. We obtained 2559 mean spots with an average of 98,318 reads and a median of 5674 genes per spot. Using the 10x Genomics Space Ranger pipeline, the sequencing reads were aligned to the standard reference genome, and 4 samples that passed alignment quality control were combined using the 10x Genomics Space Ranger aggr. Anatomical annotation of expression clusters was carried out using hematoxylin and eosin (H&E) images and the Allen Brain Atlas, which enabled identification of gene expression clusters that aligned with anatomical layers in the following brain regions: cerebral cortex, hippocampus, striatum, fiber tracts, polymodal association cortex–related thalamus, sensory-motor cortex–related thalamus, hypothalamus, and amygdala (Fig. S6a online). Uniform Manifold Approximation plots showed 17 distinct gene expression clusters (Fig. S6b online).

Next, we identified differentially expressed genes (DEGs) in distinct spatial clusters (Fig. S6c online), and used UpSet graphs to display downregulated DEGs in the cerebral cortex, hippocampal region, striatum, fiber tracts, thalamus, ventricular system, and hypothalamus of *Slc17a5*$^{-/-}$ mice compared with wild-type mice. We found that after the *Slc17a5* knockout, the DEGs in all brain regions showed a reduced expression of myelin-related genes (Fig. S6d online). RT-qPCR analysis also showed that myelin-related mRNA levels of *Mbp*, *Mog*, *Plp1*, *Cnp*, and *Mag* were significantly downregulated in the whole brain of *Slc17a5*$^{-/-}$ mice compared with wild-type mice (Fig. S6e online). Immunofluorescence staining confirmed decreased expression of MBP in hippocampal tissue of *Slc17a5*$^{-/-}$ mice (Fig. S6f, g online). However, mRNA expression of markers for oligodendrocyte precursor cells (*Pdgfra* and *Cspg4*) and mature myelinating oligodendrocytes (*Apc*) remained unchanged (Fig. S6e online). These findings suggested sialin is closely associated with myelination in all brain regions.

We wondered whether sialin is involved in the nitrate-induced amelioration of myelin loss and cognitive impairment we observed in APP/PS1 mice. First, we explored the specific cell type that expresses sialin in the hippocampus. By quantifying sialin levels in different hippocampal cell types, we found significantly increased sialin expression in both human and mouse hippocampal neurons compared with that in astrocytes, microglia, and oligodendrocytes (Fig. 2e–g, Fig. S7a–e online). The results in primary hippocampal cell cultures were consistent with those observed in human and mouse hippocampal tissues (Fig. S7f–h online).

Since sialin is highly expressed in neurons and mutations or knockout of its encoding gene cause loss of myelin and cognitive impairment, we wondered whether overexpression of hippocampal neuronal sialin can alleviate these symptoms. To answer this


(a)
Nitrate in hippocampal cell (μmol/L)
Saline
Nit
APP/PS1
APP/PS1+Nit
(b)
NOR
F
N
(c)
DI= (N−F)/(N+F)
(d)
Spontaneous alternation (%)
(e)
Speed (cm/s)
(f)
Non-AD
AD
100 μm
(g)
Human
Aβ1-42 (optical density)
(h)
1mm
(i)
Aβ1-42 staining
Aβ1-42 plaque count in hippocampus
(j)
oAβ1-42
PSD95
Synaptophysin
GAPDH
16 kDa
95 kDa
38 kDa
37 kDa
(k)
oAβ1-42 (relative)
(l)
PSD95 (relative)
(m)
Synaptophysin (relative)
(n)
fatty acid binding
glutathione peroxidase activity
oxidoreductase activity
organic acid binding
syntaxin binding
actin filament binding
SNARE binding
peroxidase activity
dipeptidase activity
structural constituent of myelin sheath
cell projection membrane
cytoplasmic region
brush border
proteinaceous extracellular matrix
basement membrane
cluster of actin−based cell projections
cell cortex
extracellular matrix
basolateral plasma membrane
myelin sheath
oligodendrocyte differentiation
intrinsic apoptotic signaling pathway
gliogenesis
axon ensheathment in central nervous system
central nervous system myelination
glial cell development
glial cell differentiation
myelination
axon ensheathment
ensheathment of neurons
Gene ratio
Count
Adjusted P value
(o)
MBP
(p)
MBP (optical density)

question, we injected adeno-associated viral (AAV) vectors overexpressing *Slc17a5* (AAV-*Slc17a5*) with the neuron-specific promoter hSyn or empty vector (AAV-Vec1) into the hippocampal region of 7–8-month-old, male, wild-type C57BL/6J and APP/PS1 mice to induce overexpression of hippocampal neuronal sialin (Fig. S7i–l online). After two months, the NOR test revealed overexpression of hippocampal neuronal sialin alleviated the decline in DI values in APP/PS1 mice (Fig. S7m, n online). Additionally, the Y-maze test showed a significant rise in the spontaneous alternation rate in APP/PS1 mice with hippocampal neuronal sialin overexpression (Fig. S7o online).

Next, we examined oAβ_{1-42} and myelin protein levels using Western blotting, which showed hippocampal neuronal sialin overexpression mitigated the increased oAβ_{1-42} levels and decreased MBP levels in APP/PS1 mice (Fig. S7p–s). Together, these results indicated that overexpression of hippocampal neuronal sialin can ameliorate cognitive impairment and myelin loss in APP/PS1 mice.

To establish that nitrate ameliorates cognitive impairment and myelin loss by upregulating hippocampal neuronal sialin, we injected AAV-*Slc17a5*-RNAi with the neuron-specific promoter hSyn to knockdown neuronal *Slc17a5* or empty vector (AAV-Vec2) into the hippocampal region of 7–8-month-old, male, wild-type C57BL/6J mice and APP/PS1 mice. Daily intragastric administration of 4 mmol/L sodium nitrate or saline commenced simultaneously with the AAV injections and continued until the mice were sacrificed (Fig. S8a online). The effectiveness of the *Slc17a5* knockdown was verified at 9–10 month-old (Fig. S8b–d online). The NOR test revealed that two months after knockdown of hippocampal neuronal sialin expression, nitrate's effect on improving DI values in APP/PS1 mice was inhibited (Fig. 2h, i). The Y-maze test showed a significant decrease in the spontaneous alternation rate in nitrate-treated APP/PS1 mice that had been injected with AAV-*Slc17a5*-RNAi compared with nitrate-treated APP/PS1-Vec2 mice (Fig. 2j). In addition, knockdown of hippocampal neuronal sialin expression inhibited nitrate's effect on oAβ_{1-42}, MOG, and MBP levels in APP/PS1 mice (Fig. S8e–h online). Together, these findings showed that knockdown of hippocampal neuronal sialin expression inhibited nitrate's beneficial effects on cognitive impairment and myelin loss in APP/PS1 mice, which suggested nitrate exerts these effects through upregulation of hippocampal neuronal sialin.

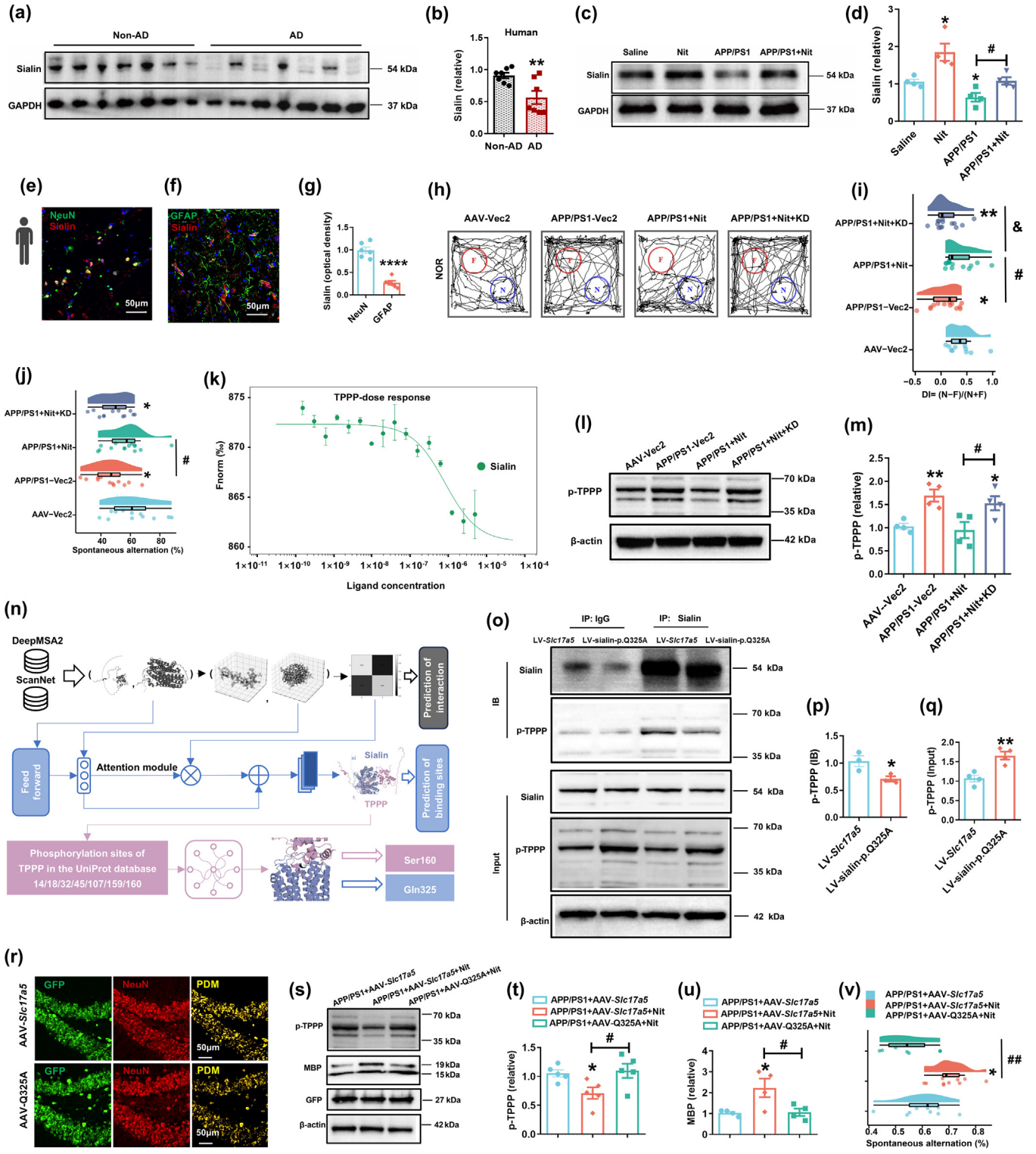
To investigate how nitrate ameliorates myelin loss and cognitive decline through upregulation of sialin, we performed an immunoprecipitation assay targeting two types of sialin antibodies. Subsequently, we utilized mass spectrometry to identify sialin-interacting proteins and found 64 overlapping sialin-interacting proteins (Fig. S9a, Table S2 online). Using GO enrichment analysis, we discovered these 64 interacting proteins are primarily linked to the myelin and cytoskeleton remodeling (Fig. S9b online). To investigate which proteins are located downstream of sialin, we mapped the aforementioned 64 proteins to genes exhibiting changes in *Slc17a5*$^{-/-}$ mice. The results revealed four genes with altered expression levels in *Slc17a5*$^{-/-}$ mice compared with wild-type mice: *Tppp* and *Bin1* displayed decreased expression, whereas *Tpm2* and *Acta2* exhibited increased expression (Fig. S9c online). Previous studies have shown TPPP and bridging integrator 1 (Bin1) are mainly expressed in oligodendrocytes and that they are associated with AD and myelin [16,17]. We evaluated TPPP, Bin1, Tpm2 and Acta2 protein expression levels in hippocampal tissue of nitrate- or saline-treated APP/PS1 and wild-type C57BL/6J mice, but did not find any significant differences among the four groups (Fig. S9d–i online).

TPPP regulates microtubule stability and intracellular trafficking and is closely related to myelination and cognitive function [16]. As phosphorylation of TPPP inhibits its function [18], we evaluated the expression of phosphorylated TPPP (p-TPPP) and saw that nitrate administration significantly inhibited p-TPPP levels in hippocampal tissue of APP/PS1 mice (Fig. S9j, k online). Coimmunoprecipitation analysis showed sialin interacted with p-TPPP (Fig. S9l online), and microscale thermophoresis (MST) analysis revealed TPPP interacted with sialin (Fig. 2k). Moreover, overexpression of neuronal sialin attenuated the elevation in TPPP phosphorylation in APP/PS1 mice (Fig. S9m, n), while neuronal sialin knockdown inhibited nitrate's effect on mitigating the increased TPPP phosphorylation in this AD mouse model (Fig. 2l, m). These results suggested nitrate administration in APP/PS1 mice upregulates neuronal sialin, which binds to TPPP, thereby reducing phosphorylation of TPPP.

To identify the specific site where sialin interacts with p-TPPP, we used a multimodal deep learning framework to predict protein–protein interaction sites and checked the results against sites documented in the UniProt database. The phosphorylation site of TPPP listed in the UniProt database (Ser160) intersected with the Gln325 site of sialin (Fig. 2n). Subsequently, we introduced a mutation at this sialin site by replacing glutamine (Q) with alanine (A), generated lentiviral vectors overexpressing full-length sialin (LV-*Slc17a5*) or mutant sialin (LV-sialin-p.Q325A), and successfully transfected SH-SY5Y cells with these viral vectors (Fig. S10a online). The sialin-p.Q325A mutation resulted in reduced interaction between sialin and p-TPPP, as shown by elevated p-TPPP levels, in SH-SY5Y cells (Fig. 2o–q). Furthermore, this mutation led to collapse of the cytoskeleton in SH-SY5Y cells (Fig. S10b online). This indicated that interaction between the sialin-p.Q325 site and TPPP results in a decrease in TPPP phosphorylation, thereby maintaining the stability of the cytoskeleton.

In addition, while nitrate alleviated the increased p-TPPP levels and decreased MBP levels in APP/PS1 mice, the neuronal sialin-p.Q325A mutation suppressed the nitrate-induced decrease in p-TPPP levels and increase in MBP levels in hippocampal tissue of

Fig. 1. Effects of nitrate administration on myelin and cognitive function in APP/PS1 mice. (a) Quantification of hippocampal cell nitrate levels. Nit: nitrate. $n = 8$. $^{*}P < 0.05$; $^{****}P < 0.0001$ vs. saline; $^{\#\#\#}P < 0.001$ vs. APP/PS1. (b) Representative traces obtained with NOR test. F: familiar object; N: novel object. (c) Quantification of discrimination index (DI) values obtained with NOR test. $n = 8$. $^{*}P < 0.05$; $^{***}P < 0.001$ vs. saline; $^{\#}P < 0.05$ vs. APP/PS1. (d) Quantification of spontaneous alternation rate in Y-maze test. $n = 8$. $^{****}P < 0.0001$ vs. saline; $^{\#\#\#}P < 0.001$ vs. APP/PS1. (e) Quantification of speed in Y-maze test. $n = 8$. (f, g) Representative immunofluorescence staining images (f) and quantitative analysis (g) of amyloid-β_{1-42} (Aβ_{1-42}) expression in postmortem hippocampal tissue of Non-AD individuals and AD patients. $n = 5$. Scale bar = 100 μm. $^{**}P < 0.01$. (h) Representative immunofluorescence images of Aβ_{1-42} expression. Scale bar = 1 mm. (i) Quantification of hippocampal Aβ_{1-42} plaque count. $n = 4$. $^{****}P < 0.0001$ vs. saline; $^{\#}P < 0.05$ vs. APP/PS1. (j–m) Representative Western blot bands (j) and quantitative analysis of expression of oAβ_{1-42} (k), PSD95 (l) and synaptophysin (m). $n = 4$. $^{*}P < 0.05$; $^{**}P < 0.01$ vs. saline; $^{\#}P < 0.05$ vs. APP/PS1. (n) Gene Ontology analysis of upregulated differentially expressed genes in hippocampal tissue of nitrate-treated versus saline-treated APP/PS1 mice. (o and p) Representative immunofluorescence staining images (o) and quantitative analysis (p) of myelin basic protein (MBP) expression in postmortem hippocampal tissue of Non-AD individuals and AD patients ($n = 5$). Scale bar = 100 μm. $^{*}P < 0.05$. Data are expressed as mean ± standard error of the mean (SEM). Student's *t*-test was used to compare two groups and one-way ANOVA followed by a Bonferroni post-hoc test to compare three or more groups.


(a)
Non-AD
AD
Sialin
GAPDH
54 kDa
37 kDa
(b)
Human
Sialin (relative)
Non-AD AD
(c)
Saline
Nit
APP/PS1
APP/PS1+Nit
Sialin
GAPDH
54 kDa
37 kDa
(d)
Sialin (relative)
Saline
Nit
APP/PS1
APP/PS1+Nit
(e)
NeuN
Sialin
50μm
(f)
GFAP
Sialin
50μm
(g)
Sialin (optical density)
NeuN
GFAP
(h)
AAV-Vec2
APP/PS1-Vec2
APP/PS1+Nit
APP/PS1+Nit+KD
NOR
(i)
APP/PS1+Nit+KD
APP/PS1+Nit
APP/PS1−Vec2
AAV−Vec2
DI= (N−F)/(N+F)
(j)
APP/PS1+Nit+KD
APP/PS1+Nit
APP/PS1−Vec2
AAV−Vec2
Spontaneous alternation (%)
(k)
TPPP-dose response
Fnorm (‰)
Sialin
Ligand concentration
(l)
AAV-Vec2
APP/PS1-Vec2
APP/PS1+Nit
APP/PS1+Nit+KD
p-TPPP
β-actin
70 kDa
35 kDa
42 kDa
(m)
p-TPPP (relative)
AAV-Vec2
APP/PS1-Vec2
APP/PS1+Nit
APP/PS1+Nit+KD
(n)
DeepMSA2
ScanNet
Prediction of interaction
Feed forward
Attention module
Sialin
TPPP
Prediction of binding sites
Phosphorylation sites of TPPP in the UniProt database
14/18/32/45/107/159/160
Ser160
Gln325
(o)
IP: IgG
IP: Sialin
LV-Slc17a5
LV-sialin-p.Q325A
IB
Input
Sialin
p-TPPP
β-actin
54 kDa
70 kDa
35 kDa
42 kDa
(p)
p-TPPP (IB)
LV-Slc17a5
LV-sialin-p.Q325A
(q)
p-TPPP (Input)
LV-Slc17a5
LV-sialin-p.Q325A
(r)
AAV-Slc17a5
AAV-Q325A
GFP
NeuN
PDM
50μm
(s)
APP/PS1+AAV-Slc17a5
APP/PS1+AAV-Slc17a5+Nit
APP/PS1+AAV-Q325A+Nit
p-TPPP
MBP
GFP
β-actin
70 kDa
35 kDa
19 kDa
15 kDa
27 kDa
42 kDa
(t)
APP/PS1+AAV-Slc17a5
APP/PS1+AAV-Slc17a5+Nit
APP/PS1+AAV-Q325A+Nit
p-TPPP (relative)
(u)
APP/PS1+AAV-Slc17a5
APP/PS1+AAV-Slc17a5+Nit
APP/PS1+AAV-Q325A+Nit
MBP (relative)
(v)
APP/PS1+AAV-Slc17a5
APP/PS1+AAV-Slc17a5+Nit
APP/PS1+AAV-Q325A+Nit
Spontaneous alternation (%)

APP/PS1 mice (Fig. 2r–u). The Y-maze test showed that the sialin-p.Q325A mutation inhibited the nitrate-induced cognitive recovery in APP/PS1 mice (Fig. 2v). Taken together, these findings verified nitrate ameliorated myelin loss and cognitive impairment in APP/PS1 mice through upregulation of neuronal sialin, which in turn reduced TPPP phosphorylation.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

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Author contributions

Songlin Wang conceived and supervised the project. Xinyue Chen performed most of the studies and data analyses. Geng Hu performed the deep learning analysis. Lirong Chang assisted with the *in vivo* experiments. Xiaoyu Li, Chunmei Zhang and Jinsong Wang assisted with in vitro experiments. Xinyue Chen wrote the manuscript. Songlin Wang, Lirong Chang, Yi Tang, Yan Wu, Ran Zhang and Xue Wang edited the manuscript. All authors reviewed the manuscript.

Data availability

All data are available in the main text or the Supplementary materials. The raw RNA-seq and spatial-transcriptomics RNA-seq data are deposited in the database of CNGB Sequence Archive (CNSA): CNP0006235 and CNP0006246. This study did not generate original code.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scib.2025.03.017.

References

[1] Self WK, Holtzman DM. Emerging diagnostics and therapeutics for Alzheimer disease. Nat Med 2023;29:2187–99.
[2] Tawa M, Nagata R, Sumi Y, et al. Preventive effects of nitrate-rich beetroot juice supplementation on monocrotaline-induced pulmonary hypertension in rats. PLoS One 2021;16:e0249816.
[3] Webb AJ. "Every beet you take": lowering systolic blood pressure and improving vascular function/exercise capacity via the dietary nitrate-nitrite-NO pathway in patients with COPD. Eur Respir J 2024;63:2302238.
[4] Roberts MD, Moulding B, Forbes SC, et al. Evidence-based nutritional approaches to enhance exercise adaptations. Curr Opin Clin Nutr 2023;26:514–20.
[5] Jin L, Qin L, Xia D, et al. Active secretion and protective effect of salivary nitrate against stress in human volunteers and rats. Free Radical Bio Med 2013;57:61–7.
[6] Chang S, Hu L, Xu Y, et al. Inorganic nitrate alleviates total body irradiation-induced systemic damage by decreasing reactive oxygen species levels. Int J Radiat Oncol 2019;103:945–57.
[7] DiCiero MM, de Bruin VM, Vale MR, et al. Lipid peroxidation and nitrite plus nitrate levels in brain tissue from patients with Alzheimer's disease. Gerontology 2000;46:179–84.
[8] de Crom T, Blekkenhorst L, Vernooij MW, et al. Dietary nitrate intake in relation to the risk of dementia and imaging markers of vascular brain health: a population-based study. Am J Clin Nutr 2023;118:352–9.
[9] Morris MC, Wang Y, Barnes LL, et al. Nutrients and bioactives in green leafy vegetables and cognitive decline: prospective study. Neurology 2018;90:e214–22.
[10] Wang S, Qin L. Homeostatic medicine: a strategy for exploring health and disease. Curr Med (Cham) 2022;1:16.

Fig. 2. Nitrate ameliorates myelin loss and cognitive impairment in APP/PS1 mouse through upregulation of neuronal sialin and subsequent inhibition of TPPP phosphorylation. (a, b) Representative Western blot bands and quantitative analysis of sialin expression in postmortem hippocampal tissue of Non-AD individuals and AD patients (n = 7). $^{**}P$ < 0.01. (c, d) Representative Western blot bands and quantitative analysis of sialin expression in hippocampal tissue of the four groups (n = 4). $^{*}P$ < 0.05 vs. saline; #P < 0.05 vs. APP/PS1. (e, f) Representative immunofluorescence images of expression of sialin (red) and NeuN/GFAP (green) in human hippocampal tissue. Scale bar = 50 μm. (g) Quantitative analysis of sialin immunofluorescence optical density in neurons (NeuN) and astrocytes (GFAP) in human hippocampal tissue (n = 6). $^{****}P$ < 0.0001. (h) Representative traces of locomotor activity obtained with NOR test in the four groups. (i) Quantification of DI values obtained with NOR test in the four groups (n = 10–14). $^{*}P$ < 0.05; $^{**}P$ < 0.01 vs. AAV-Vec2; #P < 0.05 vs. APP/PS1-Vec2; $^{\&}P$ < 0.05 vs. APP/PS1+Nit+KD. (j) Quantification of spontaneous alternation rate in Y-maze test in the four groups (n = 12–14). $^{*}P$ < 0.05 vs. AAV-Vec2; #P < 0.05 vs. APP/PS1-Vec2. (k) Microscale thermophoresis analysis showing binding affinity between TPPP and sialin (n = 3). Dissociation rate constant (Kd): 8.1235 × 10^{-7} mol/L; Kd confidence: ± 3.5963 × 10^{-7}; signal-to-noise ratio: 10.795409. (l, m) Representative Western blot bands and quantitative analysis of p-TPPP expression in hippocampal tissue of four groups (n = 4). $^{*}P$ < 0.05; $^{**}P$ < 0.01 vs. AAV-Vec2; #P < 0.05 vs. APP/PS1+Nit. (n) Multimodal deep learning framework was used to predict interaction sites between sialin and p-TPPP. (o) Representative Western blot bands of coimmunoprecipitation analysis showing interaction between sialin and p-TPPP in SH-SY5Y cells transfected with lentiviral vectors expressing full-length sialin (LV-*Slc17a5*) or mutant sialin (LV-sialin-p.Q325A). (p) Quantitative analysis of p-TPPP expression in SH-SY5Y cells transfected with LV-*Slc17a5* or LV-sialin-p.Q325A (n = 3). $^{*}P$ <0.05. (q) Quantitative analysis of expression of p-TPPP (input) in SH-SY5Y cells transfected with LV-*Slc17a5* or LV-sialin-p.Q325A (n = 4). $^{**}P$ < 0.01. (r) Representative immunofluorescence double staining images of expression of GFP (green) and NeuN (red) in hippocampal tissue of AAV-*Slc17a5* and AAV-Q325A groups. Scale bar = 50 μm. (s–u) Representative Western blot bands (s) and quantitative analysis of expression of p-TPPP (t) and myelin basic protein (MBP) (u) in hippocampal tissue of the four groups (n = 4–5). $^{*}P$ < 0.05 vs. APP/PS1+AAV-*Slc17a5*; #P < 0.05 vs. APP/PS1+AAV-*Slc17a5*+Nit. (v) Quantification of spontaneous alternation rate in Y-maze test in the four groups (n = 7–10). $^{*}P$ < 0.05 vs. APP/PS1+AAV-*Slc17a5*; ##P < 0.01 vs. APP/PS1+AAV-*Slc17a5*+Nit. Data are expressed as mean ± SEM. Student's t-test was used to compare two groups and one-way ANOVA followed by a Bonferroni post-hoc test to compare three or more groups.

[11] Zhou J, Liu H, Hu L, et al. Nitrate and body homeostasis. Med Plus 2024;1:100003.
[12] Yarovaya N, Schot R, Fodero L, et al. Sialin, an anion transporter defective in sialic acid storage diseases, shows highly variable expression in adult mouse brain, and is developmentally regulated. Neurobiol Dis 2005;19:351–65.
[13] Pan W, Hu G, Li S, et al. Nanonitrator: novel enhancer of inorganic nitrate's protective effects, predicated on swarm learning approach. Sci Bull 2023;68:838–50.
[14] Gupta J, Reddy N, Mankad K, et al. Teaching neuroImage: a new imaging finding in a boy with salla disease caused by a pathogenic variant in the SLC17A5 gene. Neurology 2023;101:631–2.
[15] Prolo LM, Vogel H, Reimer RJ. The lysosomal sialic acid transporter sialin is required for normal CNS myelination. J Neurosci 2009;29:15355–65.
[16] Fu MM, McAlear TS, Nguyen H, et al. The golgi outpost protein TPPP nucleates microtubules and is critical for myelination. Cell 2019;179:132–46.
[17] Garcia-Agudo LF, Shi Z, Smith IF, et al. BIN1(K358R) suppresses glial response to plaques in mouse model of Alzheimer's disease. Alzheimers Dement 2024;20:2922–42.
[18] Hlavanda E, Klement E, Kokai E, et al. Phosphorylation blocks the activity of tubulin polymerization-promoting protein (TPPP): identification of sites targeted by different kinases. J Biol Chem 2007;282:29531–9.