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RESEARCH ARTICLE

DL-3-n-butylphthalide enhances synaptic plasticity in mouse model of brain impairments

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Abstract

Synaptic impairment results in cognitive dysfunction of Alzheimer's disease (AD). As a plant extract, it is found that DL-3-n-butylphthalide (L-NBP) rescues abnormal cognitive behaviors in AD animals. However, the regulatory effects of L-NBP on synaptic plasticity remains unclear. APP/PS1 mice at 12 months old received oral L-NBP treatment for 12 weeks. A water maze test assessed cognitive performances. In vitro patch-clamp recordings and in vivo field potential recordings were performed to evaluate synaptic plasticity. The protein expression of AMPA receptor subunits (GluR1 and GluR2) and NMDA receptor subunits (NR1, NR2A, and NR2B) was examined by Western blot. In addition, glutaminase activity and glutamate level in the hippocampus were measured by colorimetry to evaluate presynaptic glutamate release. L-NBP treatment could significantly improve learning and memory ability, upregulate NR2A and NR2B protein expressions, increase glutaminase activity and glutamate level in the hippocampus, and attenuate synaptic impairment transmission in the AD mice. L-NPB plays a beneficial role in AD mice by regulating NMDA receptor subunits' expression and regulating presynaptic glutamate release.

Keywords: *synaptic plasticity*; *L-3-n-butylphthalide*; *glutamate*; *Alzheimer's disease*; *learning and memory ability*

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Learning and memory impairment is one of the typical symptoms of Alzheimer's disease (AD). Synaptic function underlies the mechanism of memory formation and storage (1), and synaptic impairment was suggested to be the basis of memory dysfunction in AD (2). AD patients showed loss of synapses in the hippocampus (3). Synaptic changes were also identified in AD mice, such as APP/PS1 mice and Tg2576. These changes included synaptic loss, decreased spine density, and long-term potentiation (LTP) impairments (4). On the other hand, chemicals that were shown to attenuate learning and memory deficits in experimental AD animals could also prevent the impairment of synaptic function (5–7). Thus, targeting synaptic dysfunction was considered as a potential intervention in AD (8, 9).

DL-3-n-butylphthalide (L-NBP), as a seed extract obtained from Apium graveolens Linn, was found to improve mice spatial learning and memory (10) and in a triple transgenic mouse model of AD (11). L-NBP was also reported to prevent synaptic plasticity impairment in a rat model with cerebral ischemia (12). Previous studies found that a derivative of L-NBP, potassium 2-(1-hydroxypentyl)-benzoate, could enhance the LTP of APP/PS1 mice (13). Furthermore, L-NBP could rescue hippocampal synaptic loss in aged APP/PS1 mice (14). However, it is not clear whether L-NBP modulates synaptic plasticity. Herein, we sought to determine the effects of L-NBP on synaptic plasticity of APP/PS1 mice and investigate the possible underlying mechanisms.

Methods

Animals and drug treatment

In our study, 200 male APPswe/PSEN1ΔE9 (APP/PS1) mice (Jackson Laboratory, Bar Harbor, ME, USA) and their littermates of wild-type (WT) were used totally.

Mice were aged 12 months and weighted at about 40 g at the beginning of the experiment. They were divided into four groups: 1) WT (vehicle treatment); 2) WT+L-NBP (L-NBP treatment); 3) Tg (APP/PS1, vehicle treatment); 4) Tg+L-NBP (APP/PS1, L-NBP treatment). Mice in the L-NBP (Shijiazhuang Group NBP Pharmaceuticals, China, dissolved in vegetable oil, 3 mg/mL) treatment groups took L-NBP orally at a 15 mg/kg gavage dose, while mice in control groups received an equal amount of vegetable oil. L-NBP or vegetable oil was administrated 5 days per week for 3 months. At the end of drug treatment, mice were at the age of 15 months. Animal handles were performed following the Guide of the Ethics Committee of Xi'an International Medical Center Hospital.

Water maze test

A 120-cm diameter white tank with a 10-cm diameter transparent escape platform was named the water maze. We added white food coloring to the water. The platform was invisible to the mice, which was 0.5 cm below the water surface. The tank was considered to be segmented into four quadrants, and the platform was fixed in the center of one quadrant during training trials (Fig. 1a). We placed the mice in the water from a start position in training trials and allowed 60 sec to navigate to escape to the platform. If the platform were not found in 60 sec, the experimenter would guide the mouse to the platform. Four start positions were used, with one for each quadrant, and the start position was randomly chosen from the four start positions in each training trial. The training lasted for 5 days, and each mouse received six trials per day. A probe test proceeded on the 6th day. First, each mouse was placed in the water opposite the target quadrant (where the platform was previously located). After removing the platform from the tank, they could swim freely in the water for 1 min. Time in the target quadrant was recorded. Animal tracking was analyzed by commercial software (JiLiang Software Technology, Shanghai, China).

In vivo field potential recordings

After being anesthetized by urethane (1.5 g/kg), the mouse's head was fixed in a stereotaxic apparatus. Following surgical procedures, a stimulating electrode was inserted and located at the Schaffer collaterals, and a recording electrode was inserted into the stratum radiatum.

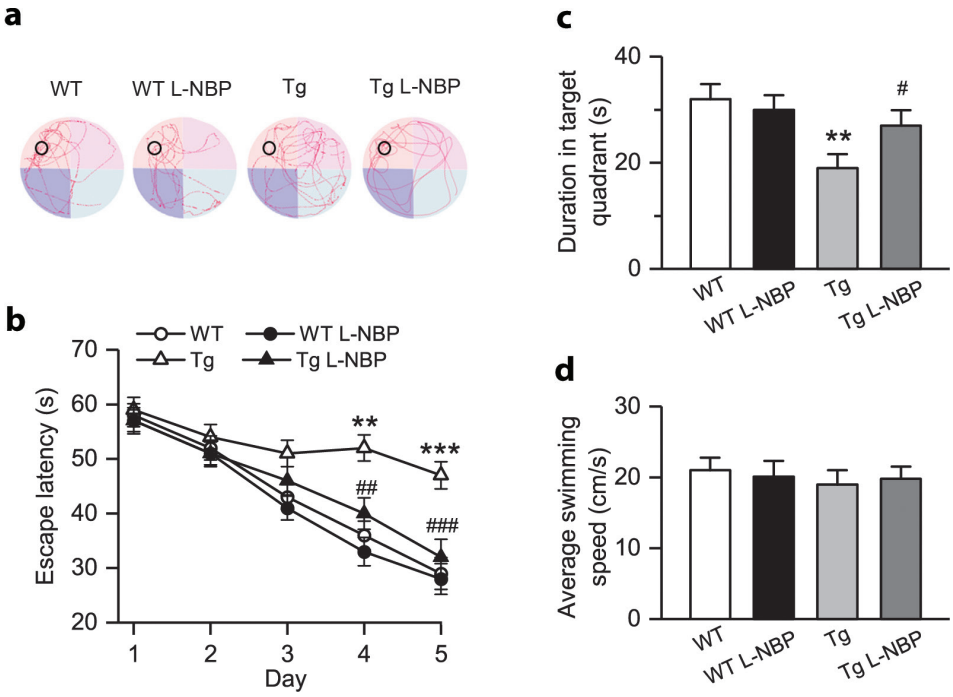

Fig. 1. L-NBP treatment ameliorates spatial learning and memory deficits in APP/PS1 mice. (a) The Morris water maze test assessed mice (n = 11 mice per group) for spatial learning and memory. Sample swim traces are shown. (b) Longer escape latency to reach the platform during training and (c) shorter duration time in the target quadrant during the probe trial were in the Tg group than in the WT group. L-NBP treatment ameliorated this effect, shortened the escape latency, and prolonged the duration in the target quadrant. (d) Average swimming speeds had no significant difference among the four groups. WT group: $^{**}P < 0.01$ and $^{***}P < 0.001$; Tg group: $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$. Vertical bars represent one standard error.

The input–output curves were plotted by stepping stimulation intensity from 0 to 1.5 mA. Two paired stimuli evoked paired-pulse facilitation (PPF) at varying inter-pulse intervals (20–300 ms). After testing for baseline, LTP was evoked by three sets of high-frequency stimulation (HFS, 10 trains of 20 stimuli, 200 Hz, inter-train interval 2 sec) with an interset interval of 5 min.

Patch-clamp recordings

At the end of the experiments, the brain of the sacrificed mouse was quickly removed. The hippocampus was isolated, split into 400-μm thick horizontal sections, incubated in oxygenated artificial cerebrospinal fluid at 22°C for 1 h, and then transferred to the recording chamber. The whole-cell recording was performed on the CA1 pyramidal neurons, visualized by a differential interference contrast (DIC) microscope (Nomarski). The borosilicate glass electrodes (5–7 MΩ) filled with intracellular solution. Stimulating pulses in 0.1 Hz were used to evoke excitatory postsynaptic currents (EPSCs). At +40 mV holding potential, NMDA-EPSCs were recorded with the presence of picrotoxin (GABA antagonist, 50 μM) and NBQX (AMPA antagonist, 10 μM). At -60 mV, AMPA-EPSCs were recorded with picrotoxin (50 μM) and ±APV (NMDA antagonist, 100 μM).

Western-blotting

After the experiments, the protein of the hippocampal tissues was extracted by using radioimmunoprecipitation buffer (Beyotime Biotechnology). Sodium dodecyl sulfate–polyacrylamide gel electrophoresis separated protein samples (30 μg for each lane) and then transferred pieces to a polyvinylidene fluoride membrane. The membranes were blocked by bovine serum albumin (5%) for 1 h and incubated with the indicated primary antibodies at 4°C overnight. The primary antibodies were anti-NR1 (#5704S, 1:800, Cell Signaling Technology, Danvers, MA, USA), anti-NR2A (#4206S, 1:700, Cell Signaling Technology), anti-NR2B (#4207S, 1:700, Cell Signaling Technology), anti-GluR1 (ab183897, 1:800, Abcam, Cambridge, MA, USA), anti-GluR2 (ab206293, 1:800, Abcam), and anti-β-actin (sc-8432, 1:900, Santa Cruz, Dallas, TX, USA). Horse radish peroxidase-conjugated secondary antibodies were further used to incubate the membranes, following visualized by Chemiluminescent Substrate (Thermo Fisher, Waltham, MA). Results were analyzed using the Image J software.

Measurement of glutamate level and glutaminase activity

The homogenates of the hippocampal tissues were centrifuged at 4°C for 10 min (at 8,000 rpm for glutaminase activity measurement and at 2,500 rpm for glutamate level measurement), and the supernatants were collected. The glutaminase activity and glutamate level were measured by colorimetry using commercial kits (A124 and A074, respectively, Jiancheng Bioengineering Institute, Wuhan, China).

Statistics

Data were shown as mean ± standard error of mean (SEM). Two-way analysis of variance (ANOVA) followed by a post hoc Bonferroni test was used to analyze statistical differences. *P*-value less than 0.05 was considered as a statistical difference.

Results

L-NBP ameliorated cognitive deficits

As shown in Fig. 1, on the 4th and 5th day of training, Tg mice had obviously longer latency to reach the platform than WT mice. However, Tg mice treatment with L-NBP could remarkably reduce the escape latency (Fig. 1a and b). In the probe test, the durations in the target quadrant among four groups were significantly different ($P < 0.01$). It indicated that WT mice spent considerably more time in the target quadrant than Tg mice ($P < 0.01$), while Tg mice treatment with L-NBP could dramatically increase the time in the target quadrant ($P < 0.05$) (Fig. 1c). However, there was no significant difference in the average swimming speeds of the four groups ($P > 0.05$) (Fig. 1d). It meant that L-NBP did not influence motor behavior in the mice.

L-NBP treatment ameliorated the impairment of LTP in the hippocampal CA1 area

The input/output curves indicated that the fEPSP slope evoked by high-intensity currents was lower in Tg mice than WT mice (Fig. 2a). We found that HFS could evoke LTP in all groups (Fig. 2b). However, the amplitude of LTP in Tg mice was significantly smaller compared with WT mice ($P < 0.001$, Fig. 2b–d). In addition, the LTP amplitude in Tg+L-NBP mice was substantially larger than in Tg mice ($P < 0.01$, Fig. 2b–d).

L-NBP treatment restored NMDA receptor-mediated EPSCs

A one-way ANOVA was separately performed to assess whether amplitudes of AMPA receptor or NMDA-mediated EPSCs. As shown in Fig. 3, amplitudes of AMPA or AMPA receptor-mediated EPSCs, as well as the NMDA/AMPA ratio, were remarkably larger in WT mice than Tg mice ($P < 0.001$, $P < 0.05$ and $P < 0.05$, respectively) (Fig. 3a–c). L-NBP treatment robustly boosted NMDA-EPSCs amplitude ($P < 0.01$, Fig. 3a, b) and NMDA/AMPA ratio ($P < 0.05$) (Fig. 3c) in Tg mice. However, it had no significant influence on the AMPA-EPSCs amplitude ($P > 0.05$, Fig. 3a, 3b). It might result in an increased NMDA/AMPA ratio in Tg mice ($P < 0.05$, Fig. 3c).

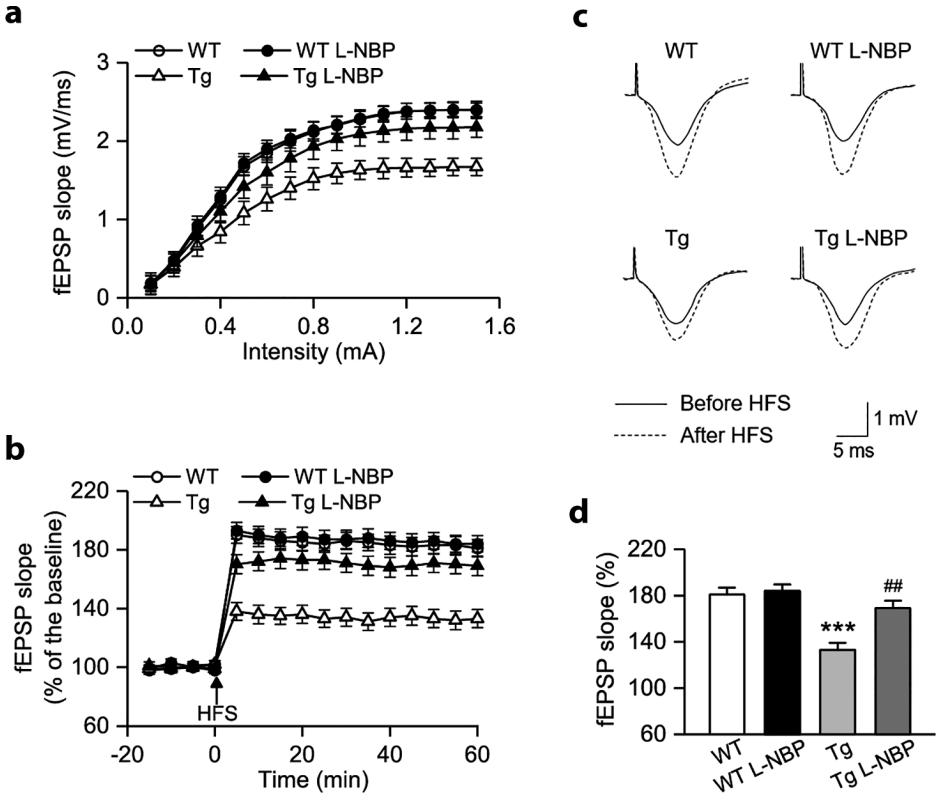

Fig. 2. L-NBP treatment ameliorates the impairment of long-term potentiation (LTP) in the hippocampal CA1 area of APP/PS1 mice. (a) Input/output curves showed the field excitatory postsynaptic potential (fEPSP) slopes as a function of stimulus current intensities in the four groups (n = 14 mice per group). (b) The fEPSP slope assessed the magnitude of LTP after high-frequency stimulation (HFS) (n = 14 mice per group). The arrow indicates the onset of HFS. (c) Sample waveforms of fEPSPs before and after HFS. (d) The fEPSP slope at 60 min was less increased in the Tg group than in the WT group. This suppression of LTP was reversed by L-NBP treatment. WT group: $^{***}P < 0.001$; Tg group: $^{\#\#}P < 0.01$. Vertical bars represent one standard error.

L-NBP treatment restored protein expressions of NR2A and NR2B receptors

Compared to WT mice, NR2A, NR2B, and GluR1 protein expressions were significantly decreased in the hippocampus of Tg mice ($P < 0.01$, $P < 0.01$ and $P < 0.001$, respectively) (Fig. 4a, b). Conversely, L-NBP treatment in Tg mice significantly increased NR2A and NR2B protein expressions ($P < 0.05$ and $P < 0.01$, respectively) but failed to affect the expression of GluR1 ($P > 0.05$, Fig. 4a, b).

L-NBP treatment restored presynaptic function and transmitter synthesis and release

The peak PPF ratio (at 40 ms inter-pulse interval) of Tg mice was remarkably smaller than in WT mice ($P < 0.05$, Fig. 5a–c). Compared with WT mice, the Tg mice also showed significantly lower glutaminase activity and glutamate level ($P < 0.05$ and $P < 0.01$, respectively) (Fig. 5d, e). L-NBP treatment reversed the impairment of PPF ($P < 0.05$) and increased the glutaminase activity and glutamate level in Tg mice ($P < 0.05$ and $P < 0.05$, respectively) (Fig. 5a–e).

Discussion

The present study reveals that L-NBP improved cognitive performances in AD mice, which is consistent with previous findings that L-NBP could enhance learning and memory through Morris water maze test in various experimental AD models, including Aβ intracerebroventricularly infused rats, double transgenic APP/PS1 mice, and triple transgenic 3xTg-AD mice (10, 11, 15). These results demonstrated a beneficial effect of L-NBP in AD models. Furthermore, considering that synaptic plasticity is known as the base of learning and memory, the impact of L-NBP on synaptic plasticity was explored in the current study.

LTP was known to correlate with learning and memory, and it is well accepted as an electrophysiological measure of synaptic plasticity (16). A previous study revealed an

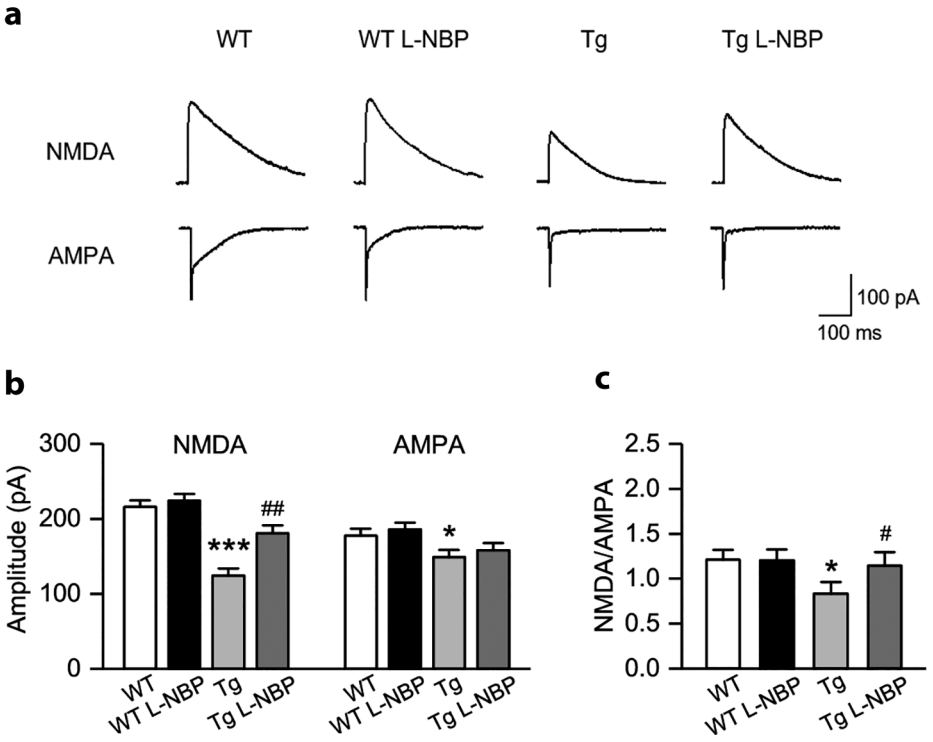

Fig. 3. L-NBP treatment restores NMDA receptor-mediated synaptic transmission in APP/PS1 mice. (a) Excitatory synaptic transmission was assessed by recording AMPA and NMDA receptor-mediated excitatory postsynaptic currents (EPSCs) in the hippocampal CA1 area (n = 12 mice per group). Sample waveforms of evoked EPSCs are shown. (b) Amplitudes of AMPA and NMDA receptor-mediated EPSCs were considerably lower in the Tg group compared with the WT group. The decreased amplitude of NMDA receptor-mediated EPSCs and (c) the ratio of NMDA receptor-mediated EPSCs to AMPA receptor-mediated EPSCs in the Tg group were considerably increased by L-NBP treatment. WT group: $^{*}P < 0.05$ and $^{***}P < 0.001$; Tg group: $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$. Vertical bars represent one standard error.

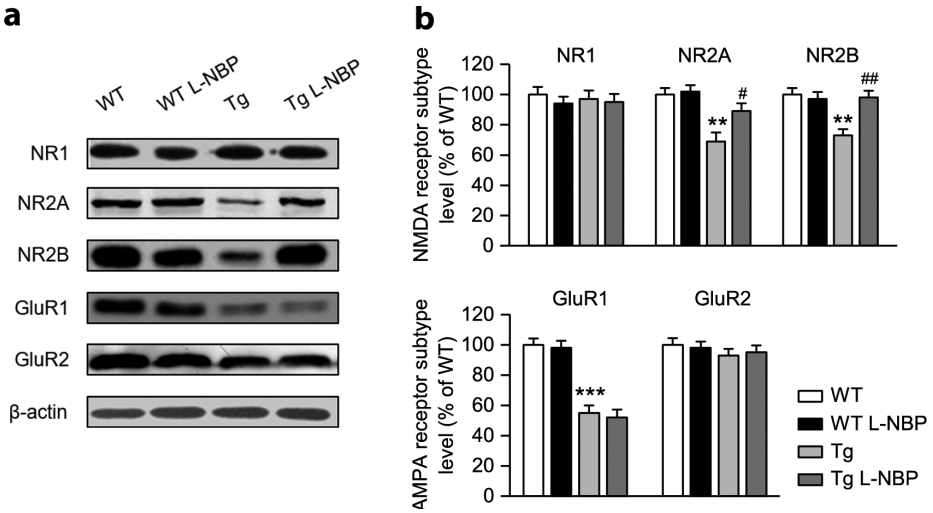

Fig. 4. L-NBP treatment restores the expression of NR2A and NR2B receptors in the APP/PS1 mice. (a) Sample images of Western blot of AMPA receptor subtypes (GluR1 and GluR2) and NMDA receptor subtypes (NR1, NR2A, and NR2B) during the four groups (n = 12 mice per group). (b) NR2A, NR2B, and GluR1 were significantly lower in the Tg group than in the WT group. The decreased levels of NR2A and NR2B were reversed by L-NBP treatment. Quantified results were normalized to levels of β-actin. Values were expressed as percentages compared to the WT group. WT group: $^{**}P < 0.01$ and $^{***}P < 0.001$; Tg group: $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$. Vertical bars represent one standard error.

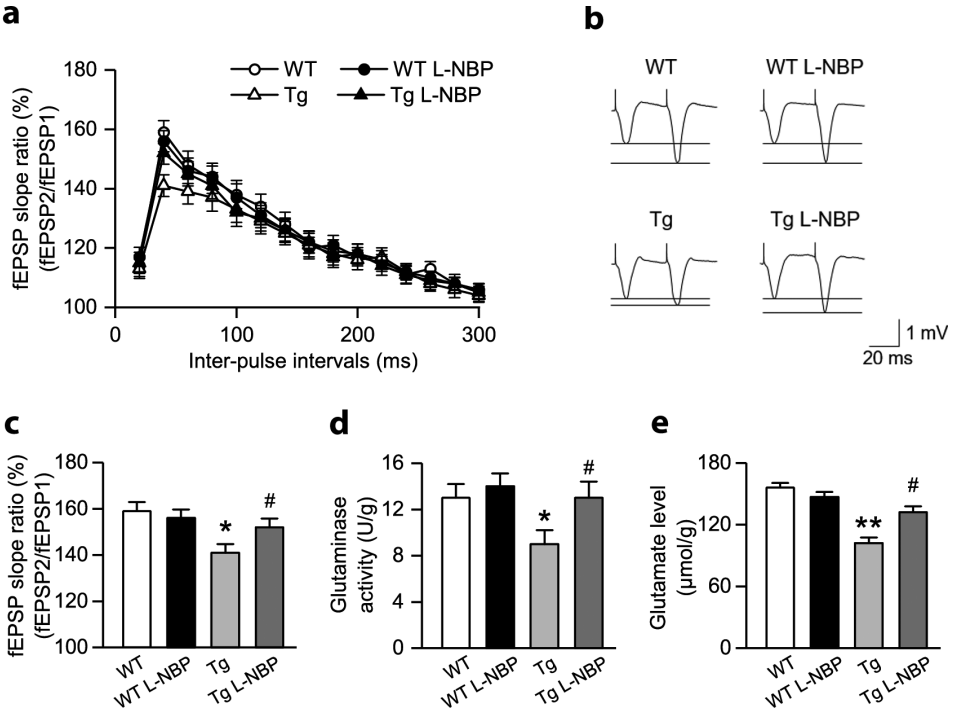

Fig. 5. L-NBP treatment restores presynaptic transmission and transmitter synthesis and releases in APP/PS1 mice. (a) Paired-pulse facilitation (PPF) was recorded at varying interpulse intervals in the hippocampal CA1 area (n = 14 mice per group). (b) Sample waveforms of fEPSPs evoked by paired pulses. (c) PPF ratio (fEPSP2/fEPSP1) at 40 ms of the interpulse interval, (d) glutaminase activity, and (e) glutamate level of the Tg group, which was significantly lower than in the WT group, were improved by L-NBP treatment. For the glutaminase activity and glutamate level assays, data were collected from 11 mice in each group. WT group: $^{*}P < 0.05$ and $^{**}P < 0.01$; Tg group: $^{\#}P < 0.05$. Vertical bars represent one standard error.

impairment of LTP in APP/PS1 mice that were more than 8 months old (17). Consistent with this report, our results of in vivo LTP recording showed that impaired LTP was found in APP/PS1 mice aged 15 months. Furthermore, L-NBP was found to attenuate LTP impairment in other animal models with cognitive impairment, including chronic cerebral ischemia and diabetes with cognitive dysfunction (12, 18). Similarly, L-NBP could also attenuate LTP impairment in the AD mice and cognitive impairment through restoring synaptic plasticity.

NMDA and AMPA receptors are vital components underlying the LTP mechanism and are crucial for controlling synaptic plasticity. These two receptors are coexpressed in the synapse, and the ratio of currents through the two types of channels (NMDA/AMPA ratio) is relatively fixed (19). Therefore, the balance of the NMDA/AMPA ratio is crucial for the formation of synaptic plasticity (20). A previous study observed a reduction of the NMDA/AMPA ratio in the AD mice (21). In our study, the mice also had a significantly lower NMDA/AMPA ratio than their WT littermates. Treatment with L-NBP was found to rescue the decrease of the NMDA/AMPA ratio in our study. As our results showed that L-NBP recovered the decrease in NMDA-EPSCs amplitude but not AMPA-EPSCs amplitude, the effect of L-NBP in NMDA/AMPA ratio might be via restoring NMDA receptor-mediated synaptic transmission.

It is well documented that the signaling properties of these receptors would be influenced by the subunit composition of NMDA and AMPA receptors (19). NMDA receptors are heteromers composed of subunits: NR1, NR2 (A to D), and NR3 (A and B), and those consisting of NR1 subunit and NR2A or NR2B subunit are identified as the primary type of functional NMDA receptors in the brain (22, 23). AMPA receptors are heteromers composed of combinations of subunits GluR1–GluR4, and those containing subunits (GluR1 and GluR2) are involved in synaptic transmission in the brain (24). Postmortem studies have found reductions of NR2A/NR2B mRNA levels of the hippocampus and reduced GluR1 protein levels in the dentate gyrus in AD patients (25, 26). Decreased levels of NR2A and NR2B were also observed in APP/PS1 mice (27). Another study found that APP/PS1 mice showed reduced mRNA expression of NR2B and GluR1 when they developed cognitive dysfunction (28). In accordance with these results, NR2A, NR2B, and GluR1 protein expressions were significantly decreased. Treatment with L-NBP could restore the NR2A and NR2B expressions

but not GluR1. These results might support that the effect of L-NBP on LTP was via repairing NMDA receptor-mediated synaptic transmission. The impact of L-NBP in upregulating NR2B was previously reported in an animal model of diabetes with cognitive dysfunction (18). In accordance with that study, our result showed a striking effect of L-NBP on NR2B level, which almost returned to normal level after L-NBP treatment. Previous evidence suggested that selective reduction in NR2B participated in the cognitive impairments in AD animal models and patients (27). These results indicated that the impact of L-NBP in advancing learning and memory should be partially via upregulating NR2B level.

Furthermore, we examined the effect of L-NBP on presynaptic functions, which is another critical component of synaptic plasticity. PPF is a short-term synaptic plasticity that is evoked by two closely spaced stimuli. The mechanism underlying PPF involves increased presynaptic Ca^{2+} concentration and neurotransmitter release (29). Thus, PPF is commonly used to measure presynaptic functions. Previous studies found that the PPF ratio was reduced in the mice at 15 months (17). Consistently, we demonstrated that the peak PPF ratio was significantly reduced, which indicated an impaired presynaptic function in the hippocampus of this AD model. In addition, glutamate levels decreased in AD patients' brains (30, 31).

Meanwhile, postmortem studies found a reduced level of glutaminase and loss of glutaminase-positive neurons in AD brains (32, 33), which might explain the decrease of glutamate levels. Glutamate level was also reduced in the hippocampus (34). Consistent with these reports, we found that the glutaminase activity and glutamate levels were dramatically reduced in the mice, indicating a reduced presynaptic glutamate release in this model. L-NBP treatment reversed the impairment in PPF and increased the glutaminase activity and glutamate level in the mice in our study. It indicated that L-NBP could restore presynaptic function by restoring glutamate-mediated synaptic transmission, which might underlie the role of L-NBP in alleviating learning and memory deficits in APP/PS1 mice.

Conclusion

In summary, this study revealed that L-NBP treatment could enhance the protein levels of NR2A and NR2B, glutaminase activity, and glutamate levels and attenuate impairments of synaptic transmission, which might underlie the effect of L-NBP in improving learning and memory in APP/PS1 mice.

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Conflict of interest and funding

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References

1. Takeuchi T, Duszkiewicz AJ, Morris RGM. The synaptic plasticity and memory hypothesis: encoding, storage and persistence. Philos Trans R Soc B Biol Sci 2014; 369(1633): 20130288. doi: 10.1098/rstb.2013.0288
2. Shankar GM, Walsh DM. Alzheimer's disease: synaptic dysfunction and Aβ. Mol Neurodegener 2009; 4(1): 48. doi: 10.1186/1750-1326-4-48
3. Scheff SW, Price DA, Schmitt FA, DeKosky ST, Mufson EJ. Synaptic alterations in CA1 in mild Alzheimer disease and mild cognitive impairment. Neurology 2007; 68(18): 1501–8. doi: 10.1212/01.wnl.0000260698.46517.8f
4. Pozueta J, Lefort R, Shelanski ML. Synaptic changes in Alzheimer's disease and its models. Neuroscience 2013; 251: 51–65. doi: 10.1016/j.neuroscience.2012.05.050
5. Cavanagh C, Wong TP. Preventing synaptic deficits in Alzheimer's disease by inhibiting tumor necrosis factor alpha signaling. IBRO Rep 2018; 4: 18–21. doi: 10.1016/j.ibror.2018.01.003
6. Sheng C, Xu P, Zhou K, Deng D, Zhang C, Wang Z. Icariin Attenuates Synaptic and Cognitive Deficits in an A β1-42-Induced Rat Model of Alzheimer's disease. BioMed Res Int 2017; 2017: 12. doi: 10.1155/2017/7464872
7. Xu ZP, Li L, Bao J, Wang ZH, Zeng J, Liu EJ, et al. Magnesium protects cognitive functions and synaptic plasticity in streptozotocin-induced sporadic Alzheimer's model. PLoS One 2014; 9(9):e108645. doi: 10.1371/journal.pone.0108645
8. Bereczki E, Branca RM, Francis PT, Pereira JB, Baek J-H, Hortobágyi T, et al. Synaptic markers of cognitive decline in neurodegenerative diseases: a proteomic approach. Brain 2018; 141(2): 582–95. doi: 10.1093/brain/awx352
9. Anand R, Gill KD, Mahdi AA. Therapeutics of Alzheimer's disease: past, present and future. Neuropharmacology 2014; 76: 27–50. doi: 10.1016/j.neuropharm.2013.07.004
10. Peng Y, Hu Y, Xu S, Li P, Li J, Lu L, et al. L-3-n-butylphthalide reduces tau phosphorylation and improves cognitive deficits in AβPP/PS1-Alzheimer's transgenic mice. J Alzheimers Dis 2012; 29: 379–91. doi: 10.3233/JAD-2011-111577
11. Peng Y, Sun J, Hon S, Nylander AN, Xia W, Feng Y, et al. L-3-n-butylphthalide improves cognitive impairment and reduces amyloid-beta in a transgenic model of Alzheimer's disease. J Neurosci 2010; 30(24): 8180–9. doi: 10.1523/JNEUROSCI.0340-10.2010
12. Xu J, Wang Y, Li N, Xu L, Yang H, Yang Z. l-3-n-butylphthalide improves cognitive deficits in rats with chronic cerebral ischemia. Neuropharmacology 2012; 62(7): 2424–9. doi: 10.1016/j.neuropharm.2012.02.014
13. Li PP, Wang WP, Liu ZH, Xu SF, Lu WW, Wang L, et al. Potassium 2-(1-hydroxypentyl)-benzoate promotes long-term potentiation in Abeta1-42-injected rats and APP/PS1 transgenic mice. Acta Pharmacol Sin 2014; 35(7): 869–78. doi: 10.1038/aps.2014.29
14. Zhang Y, Huang L-J, Shi S, Xu S-F, Wang X-L, Peng Y. L-3-n-butylphthalide rescues hippocampal synaptic failure and attenuates neuropathology in aged APP/PS1 mouse model of Alzheimer's disease. CNS Neurosci Ther 2016; 22(12): 979–87. doi: 10.1111/cns.12594

15. Peng Y, Xing C, Xu S, Lemere CA, Chen G, Liu B, et al. L-3-n-butylphthalide improves cognitive impairment induced by intracerebroventricular infusion of amyloid-beta peptide in rats. Eur J Pharmacol 2009; 621(1–3): 38–45. doi: 10.1016/j.ejphar.2009.08.036
16. Song S, Wang X, Sava V, Weeber EJ, Sanchez-Ramos J. In vivo administration of granulocyte colony-stimulating factor restores long-term depression in hippocampal slices prepared from transgenic APP/PS1 mice. J Neurosci Res 2014; 92(8): 975–80. doi: 10.1002/jnr.23378
17. Gengler S, Hamilton A, Holscher C. Synaptic plasticity in the hippocampus of a APP/PS1 mouse model of Alzheimer's disease is impaired in old but not young mice. PLoS One 2010; 5(3):e9764. doi: 10.1371/journal.pone.0009764
18. Li J, Zhang S, Zhang L, Wang R, Wang M. Effects of L-3-n-butylphthalide on cognitive dysfunction and NR2B expression in hippocampus of streptozotocin (STZ)-induced diabetic rats. Cell Biochem Biophys 2015; 71(1): 315–22. doi: 10.1007/s12013-014-0200-5
19. Rao VR, Finkbeiner S. NMDA and AMPA receptors: old channels, new tricks. Trends Neurosci 2007; 30(6): 284–91. doi: 10.1016/j.tins.2007.03.012
20. Luscher C, Malenka RC. NMDA receptor-dependent long-term potentiation and long-term depression (LTP/LTD). Cold Spring Harb Perspect Biol 2012; 4(6): a005710. doi: 10.1101/cshperspect.a005710
21. Tozzi A, Sclip A, Tantucci M, de Iure A, Ghiglieri V, Costa C, et al. Region- and age-dependent reductions of hippocampal long-term potentiation and NMDA to AMPA ratio in a genetic model of Alzheimer's disease. Neurobiol Aging 2015; 36(1): 123–33. doi: 10.1016/j.neurobiolaging.2014.07.002
22. Belayev L, Lu Y, Bazan NG. Chapter 35 – brain ischemia and reperfusion: cellular and molecular mechanisms in stroke injury A2 – Brady, Scott T. In: Siegel GJ, Albers RW, Price DL, eds. Basic neurochemistry (Eighth Edition). New York: Academic Press; 2012, pp. 621–42.
23. Miller SL, Yeh HH. Chapter 3 – neurotransmitters and neurotransmission in the developing and adult nervous system A2 – Conn, P. Michael. In: Michael P, ed. Conn's translational neuroscience. San Diego, CA: Academic Press; 2017, pp. 49–84.
24. Banerjee A, Borgmann-Winter KE, Ray R, Hahn CG. Chapter 8 – the PSD: a microdomain for converging molecular abnormalities in schizophrenia. In: Abel T, Nickl-Jockschat T, eds. The neurobiology of schizophrenia. San Diego, CA: Academic Press; 2016, pp. 125–47.
25. Bi H, Sze C-I. N-methyl-d-aspartate receptor subunit NR2A and NR2B messenger RNA levels are altered in the hippocampus and entorhinal cortex in Alzheimer's disease. J Neurol Sci 2002; 200(1): 11–18. doi: 10.1016/S0022-510X(02)00087-4
26. Wakabayashi K, Narisawa-Saito M, Iwakura Y, Arai T, Ikeda K, Takahashi H, et al. Phenotypic down-regulation of glutamate receptor subunit GluR1 in Alzheimer's disease☆. Neurobiol Aging 1999; 20(3): 287–95.
27. Huang H-J, Liang K-C, Ke H-C, Chang Y-Y, Hsieh-Li HM. Long-term social isolation exacerbates the impairment of spatial working memory in APP/PS1 transgenic mice. Brain Res 2011; 1371: 150–60. doi: 10.1016/j.brainres.2010.11.043
28. Dickey CA, Loring JF, Montgomery J, Gordon MN, Eastman PS, Morgan D. Selectively reduced expression of synaptic plasticity-related genes in amyloid precursor protein + presenilin-1 transgenic mice. J Neurosci 2003; 23(12): 5219–26.
29. Zucker RS, Regehr WG. Short-term synaptic plasticity. Annu Rev Physiol 2002; 64(1): 355–405. doi: 10.1146/annurev.physiol.64.092501.114547
30. Fayed N, Modrego PJ, Rojas-Salinas G, Aguilar K. Brain glutamate levels are decreased in Alzheimer's disease: a magnetic resonance spectroscopy study. Am J Alzheimers Dis Other Demen 2011; 26(6): 450–6. doi: 10.1177/1533317511421780
31. Antuono PG, Jones JL, Wang Y, Li SJ. Decreased glutamate + glutamine in Alzheimer's disease detected in vivo with (1) H-MRS at 0.5 T. Neurology 2001; 56(6): 737–42. doi: 10.1212/WNL.56.6.737
32. Burbaeva G, Boksha IS, Tereshkina EB, Savushkina OK, Prokhorova TA, Vorobyeva EA. Glutamate and GABA-metabolizing enzymes in postmortem cerebellum in Alzheimer's disease: phosphate-activated glutaminase and glutamic acid decarboxylase. Cerebellum 2014; 13(5): 607–15. doi: 10.1007/s12311-014-0573-4
33. Akiyama H, McGeer PL, Itagaki S, McGeer EG, Kaneko T. Loss of glutaminase-positive cortical neurons in Alzheimer's disease. Neurochem Res 1989; 14(4): 353–8. doi: 10.1007/BF01000038
34. González-Domínguez R, García-Barrera T, Vitorica J, Gómez-Ariza JL. Region-specific metabolic alterations in the brain of the APP/PS1 transgenic mice of Alzheimer's disease. Biochim Biophys Acta 2014; 1842(12, Part A): 2395–402. doi: 10.1016/j.bbadis.2014.09.014

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