



The GSK-3 β / β -Catenin Signaling–Mediated Brain–Derived Neurotrophic Factor Pathway Is Involved in Aluminum-Induced Impairment of Hippocampal LTP In Vivo

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Abstract

The neurotoxic effects of aluminum (Al) are associated with the impairment of synaptic plasticity, the biological basis of learning and memory, the major form of which is long-term potentiation (LTP). The canonical glycogen synthase kinase-3 β (GSK-3 β)/ β -catenin signaling–mediated brain–derived neurotrophic factor (BDNF) pathway has been suggested to play important roles in memory. Thus, Al may affect LTP through this pathway. In this study, a Sprague-Dawley rat model of neurotoxicity was established through intracerebroventricular (i.c.v.) injection of aluminum maltol (Al(mal)₃), which was achieved by preimplantation of a cannula into the lateral ventricle. The rats in the control and Al-treated groups received a daily injection of SB216763, an inhibitor of GSK-3 β . Electrophysiology and western blot analysis were used to investigate the regulatory effect of the GSK-3 β / β -catenin signaling–mediated BDNF pathway on LTP impairment induced by Al(mal)₃. The results confirmed that i.c.v. injection of Al(mal)₃ significantly suppressed the field excitatory postsynaptic potential (fEPSP) amplitude, as indicated by a decrease in BDNF protein expression, which was accompanied by dose-dependent decreases in β -catenin protein expression and the phosphorylation of GSK-3 β at Ser9. Rats that received SB216763, a GSK-3 β inhibitor, exhibited higher fEPSP amplitudes than control rats. Furthermore, SB216763 treatment upregulated the hippocampal protein expression of BDNF and β -catenin while increasing the ratio of p-GSK-3 β /GSK-3 β . From the perspective of the identified β -catenin–BDNF axis, Al impairs hippocampal LTP, possibly through the GSK-3 β / β -catenin signaling–mediated BDNF pathway.

Keywords Aluminum · Long-term potentiation · Glycogen synthase kinase 3 β · β -Catenin · Brain-derived neurotrophic factor

Introduction

Aluminum (Al), a neurotoxic metal that is found at abundant levels on Earth and has high melting and boiling points and a low density and exhibits resistance to corrosion, is commonly and widely used in various industrial fields and in consumer products such as vaccines, antacids, foods, skin care products, cosmetics, and cookware [1]. It has been shown that Al can be transported across the blood-brain barrier (BBB) [2]. Moreover, a study of isotopically labeled ²⁶Al suggested Al

has a long half-life in the brain and indicated that it was present in the fetal rat brain [3]. Notably, a large number of epidemiological investigations have shown that long-term exposure to Al mainly harms the nervous system [1], is closely related to diseases associated with cognitive dysfunction [4, 5], and can cause neurological symptoms in the population, such as impaired learning and memory abilities and mild cognitive dysfunction [6, 7]. Moreover, studies have shown that exposure to Al can cause memory impairment in animal models and modify hippocampus-related signal pathways [8, 9] that are crucial to synaptic plasticity.

Synaptic plasticity, the basis of learning and memory [10], is a physiological phenomenon that is involved in neural activity and the consequential changes in synaptic efficacy and neural excitability [11], and the hippocampus plays a dominant and important role in synaptic plasticity [12]. In the brains of mammals, long-term potentiation (LTP) and long-term depression (LTD) are two major manifestations of synaptic plasticity [13]. LTP, which is long-term continuous enhancement of synaptic transmission efficiency caused by

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high-frequency stimulation (HFS) of presynaptic afferent fibers [14] in the cornu ammonis (CA) 1 area of hippocampus can be used to reliably study synaptic learning and memory functions [13]. Our previous studies confirmed that AI impairs LTP in vivo [15]. The ability of AI to inhibit LTP suggests that AI may impair synaptic transmission.

Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family, regulates synaptic transmission and plays a crucial role in cognitive functions [16, 17], which depend on the establishment, maintenance, strengthening, or regulated elimination of synapses between neurons. In addition, inhibition of endogenous BDNF production using gene knockout or antisense RNA impairs spatial learning and memory, suggesting that BDNF plays an important role in learning and memory in the hippocampus [18, 19]. BDNF is one of the most potent mediators of synaptic plasticity, as it is secreted during LTP induction. BDNF can act on receptors in the hippocampus and enhance synaptic transmission efficiency by strengthening the activity of neurotransmitters and receptors [19–21]. In BDNF knockout mice, hippocampal LTP is significantly reduced. Moreover, LTP is increased by reversing exogenous BDNF expression or increasing BDNF expression. Other studies have confirmed that BDNF is functionally essential for the signaling cascades that lead to LTP [22, 23]. Furthermore, in our previous rat model of chronic AI-induced learning and memory impairment, AI-induced LTP impairment was accompanied by a decrease in the expression level of BDNF in the hippocampus [24]. However, the detailed mechanisms by which AI exerts its effect on LTP in the hippocampal CA1 region are only partially understood.

In recent years, glycogen synthase kinase-3 β (GSK-3 β)/ β -catenin signaling has been proven to play important roles in hippocampal memory [25, 26]. GSK-3 β , a ubiquitous serine/threonine protein kinase that is specifically expressed in the central nervous system [27], is constitutively active under basal conditions. The phosphorylation of GSK-3 β at Ser9 inhibits the activity of GSK-3 β in response to upstream signals [28]. GSK-3 β plays a very important role in axonal growth and polarity formation and is related to nervous system dysfunction [29, 30]. Overexpression of GSK-3 β inhibits LTP, and activation of GSK-3 β promotes induction of LTD [31–33]. A study reported that downregulation of the expression of β -catenin, which is a transcription factor in the hippocampus, impairs memory consolidation [34, 35]. The level of β catenin is regulated by GSK-3 β . GSK-3 β activation reduces the stability of β -catenin and leads to its degradation through ubiquitination, whereas GSK-3 β inhibition stabilizes β -catenin and increases its ability to regulate synaptic plasticity [36]. Another study reported that the β -catenin signaling pathway and BDNF regulate neural stem cell development in mice and that the BDNF pathway may engage in crosstalk with the β -catenin pathway [37]. Furthermore, researchers have confirmed that BDNF is a direct target of the β -catenin

transduction pathway [38] and that BDNF expression is regulated by β -catenin [39]. Hence, whether the GSK-3 β / β -catenin signaling-mediated BDNF pathway is involved in the negative effects of AI on LTP is still not known, and few reports on synaptic plasticity in this context have been published to date.

The characteristics of AI, i.e., its small size, near maximal charge, and high affinity for the metal binding sites of enzymes [40], may contribute to its effects on LTP and lead to the involvement of the GSK-3 β / β -catenin signaling-mediated BDNF pathway. Here, Sprague-Dawley rats were treated with aluminum maltol (Al(mal)₃), and changes in LTP in the hippocampal CA1 region induced by AI were analyzed. The ability of the GSK-3 β inhibitor SB216763, a potent irreversible and cell-permeable pharmacological inhibitor of GSK-3 that is structurally distinct from other inhibitors and is highly selective for GSK-3 β [41, 42], to regulate the phosphorylation of GSK-3 β and the expression of β -catenin and BDNF during AI-induced changes in LTP is discussed.

Materials and Methods

Animals and Surgery

Two- to 3-month-old male Sprague-Dawley rats (weighing 250–300 g) supplied by the Laboratory Animal Center of Shanxi Medical University (certificate no. SCXK (Jin) 2009-0001, Taiyuan, China) were housed on a 12-h light/dark cycle (8:00–20:00 h), and the room temperature maintained at approximately 22 °C \pm 2 °C. The rats were provided with food and drinking water ad libitum. Our studies were performed in accordance with the regulations of the Institutional Animal Care and Use Committee of Shanxi Medical University.

The surgical procedure was performed as described previously [43]. Animals were anesthetized with chloral hydrate (350 mg/kg) and placed in a stereotaxic apparatus, and a midline sagittal incision was made in the scalp. A small hole was drilled using the parameters reported by Paxinos et al. [44] for intracerebroventricular (i.c.v.) injection (0.8-mm posterior to bregma, 1.5-mm lateral to the midline, and 4.0 mm below the dura mater) for implantation of a guide cannula. A stainless steel guide cannula (RWD Life Science, Shenzhen, China) into which a stainless steel stylet was inserted was placed in the hole in the skull and secured to the skull with dental acrylic.

Chemicals and Treatment

According to the methods described in our previous study [45], AlCl₃6H₂O (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in distilled water at concentrations of 4 and 20 mM. Maltolate (Sigma-Aldrich, USA) was dissolved in phosphate-

buffered saline at concentrations of 12 and 60 mM. Al(mal)₃ was freshly prepared for each experiment by mixing these two solutions in equal volumes, adjusting the pH to 7.4 with NaOH and filtering the mixture through a 0.22 μ mol/L syringe filter. SB216763 (Abcam, UK), a selective GSK-3 β inhibitor, was dissolved in DMSO and further diluted in phosphate-buffered saline (PBS) [46] to a final concentration of 2 μ M, as described in a previous study [47]. Ten to 14 days after surgery, each rat received an i.c.v. injection of 5 μ l Al(mal)₃ or SB216763.

Before the formal experiment, we established two groups, namely, the no cannula implantation group and the cannula implantation group, to observe whether lateral ventricular cannula implantation had an impact on the rats. Then, the experiment was divided into two parts. First, to study the effects of Al exposure on LTP and related molecules, the rats were randomly divided into four groups (6 rats/group): the control group (0.9% normal saline) and three Al(mal)₃ exposure groups (0 mM, 2 mM, and 10 mM). Second, to determine if the GSK-3 β inhibitor SB216763 antagonizes the Al-induced suppression of hippocampal LTP and downstream signaling molecules, the rats were divided into four groups (6 rats/group): the control group, the GSK-3 β inhibitor group (2 μ M SB216763), the Al(mal)₃ group (10 mM), and the GSK-3 β inhibitor + Al(mal)₃ group (2 μ M SB216763 and 10 mM Al(mal)₃). Each rat was administered an i.c.v. injection of 5 μ l saline, Al(mal)₃ and/or SB216763 at a rate of 1 μ l/min through the stainless steel guide cannula with a microinjector (Gao, Shanghai, China). Drug administration was performed once every 2 days for 10 days.

In Vivo Electrophysiological Studies of the Hippocampus

Electrophysiology was performed using a methods previously reported by our group [15]. Rats from each treatment group were anesthetized with intraperitoneally (i.p.) administered 1.5 g/kg urethane (Sigma-Aldrich, St. Louis, MO, USA) and fixed on a stereotaxic device (Narishige Group). Two small holes were drilled on the right side of the skull at appropriate locations for electrode insertion. A bipolar stimulating electrode (FHC, Bowdoin, ME, USA) was inserted into the Schaffer collateral commissural pathway (4.2-mm posterior to bregma and 3.8-mm lateral to the midline), and a monopolar recording electrode (FHC) was inserted in the stratum radiatum of the CA1 region (3.8-mm posterior to bregma and 2.9-mm lateral to the midline). Stimulating electrodes and recording electrodes were inserted into preset locations in the hippocampus with a micropropulsion unit (Narishige Group). The electrodes were slowly lowered into the CA1 region of the hippocampus until field excitatory postsynaptic potentials (fEPSPs) were observed. Baseline fEPSPs were elicited

by test stimuli with an intensity that elicited 50% of the maximal response at an interval of 30 s and were monitored for 30 min. LTP was induced by a high-frequency train.

Western Blotting

Tissues from the hippocampal CA1 region were homogenized and sonicated on ice using ristocetin-induced platelet agglutination lysis buffer (Wuhan Boster Biological Technology, Wuhan, China) to extract protein. The samples were loaded and separated on 10% SDS-polyacrylamide electrophoresis gels to detect GSK-3 β , pGSK-3 β , β -catenin, BDNF, and GAPDH. The proteins were transferred to PVDF membranes (EMD Millipore, Bedford, MA, USA), which were incubated with rabbit antiGSK3 β (Ser9) (1:1000, Cell Signaling Technology, USA), rabbit antiGSK3 β , rabbit anti β -catenin (1:1000, Cell Signaling Technology, USA), rabbit antiBDNF (1:1000, Cell Signaling Technology, USA), or mouse antiGAPDH (1:3000, CoWin Biotech) overnight at 4 °C. The next day, the membranes were rinsed with Tris-buffered saline containing Tween (TBST; 20 mM Tris-HCl, pH 7.4; 225 mM NaCl; and 0.05% Tween 20). After three washes in TBST, the blots were then incubated with one of the following HRP-conjugated antibodies: antirabbit IgG (Wuhan Boster Biological Technology) or antimouse IgG (CoWin Biotech). Then, the immunolabeled protein bands were detected using an ECL western blot detection kit (CoWin Biotech). Images were obtained with a chemiluminescence imaging system (Bio-Rad). Densitometric quantification of the immunoblots was performed using Quantity One software (Bio-Rad).

Data Analysis

All data are expressed as the mean \pm S.D. and were analyzed with SPSS 17.0. Differences were analyzed by one-way analysis of variance (ANOVA), and post hoc comparisons were performed using the least significant difference (LSD) test (equal variances assumed) or Dunnett's *C* test (equal variances not assumed). *P* < 0.05 was considered statistically significant.

Results

Al Treatment Significantly Suppressed LTP in the CA1 Area

As shown in Fig. 1 a and b, after a stable baseline was established for 30 min, HFS resulted in successful

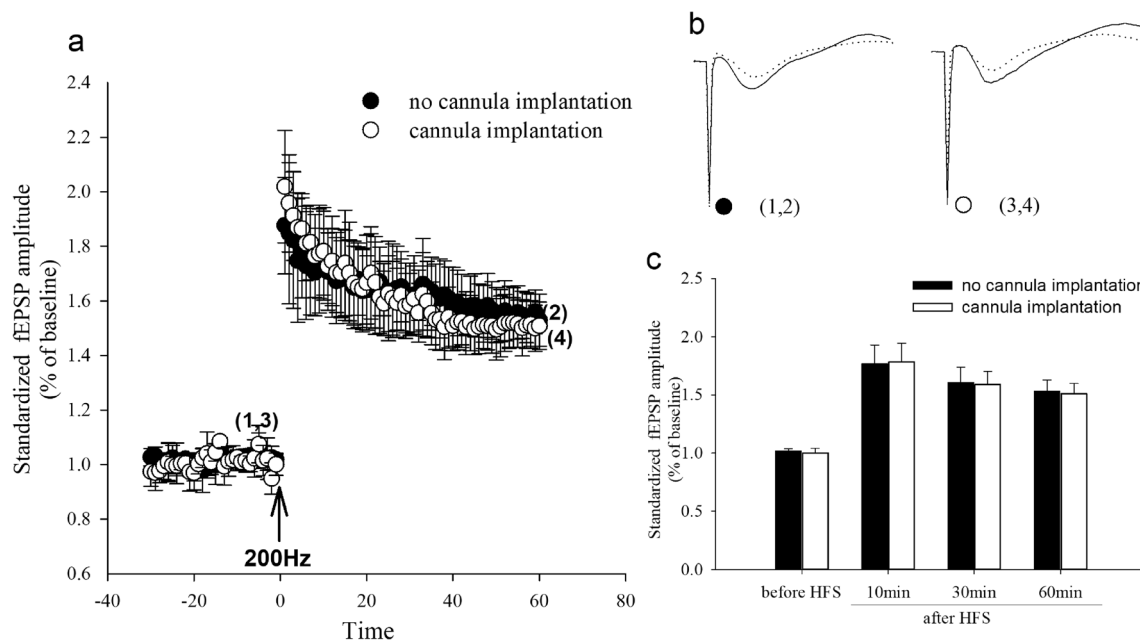


Fig. 1 Effects of cannula implantation on typical LTP in the hippocampal CA1 region in vivo in rats in response to high-frequency stimulation (HFS, 200 Hz). HFS induced stable LTP after cannula implantation. **a** The effect of cannula implantation on LTP in the CA1 region. Each point represents the mean \pm SD of the excitatory postsynaptic potential (fEPSP) amplitude. fEPSP (% baseline) was calculated as $(\text{fEPSP}/\text{fEPSP}_{\text{baseline}})^*$

100. **b** Representative original data traces, with the dotted line representing the trace before HFS and the solid line representing the trace 60 min after HFS. **c** Average fEPSP amplitudes at different time points before and following HFS. Each column shows the mean \pm SD of the fEPSP amplitude, $n=6$. * $P<0.05$ compared with the no cannula implantation group

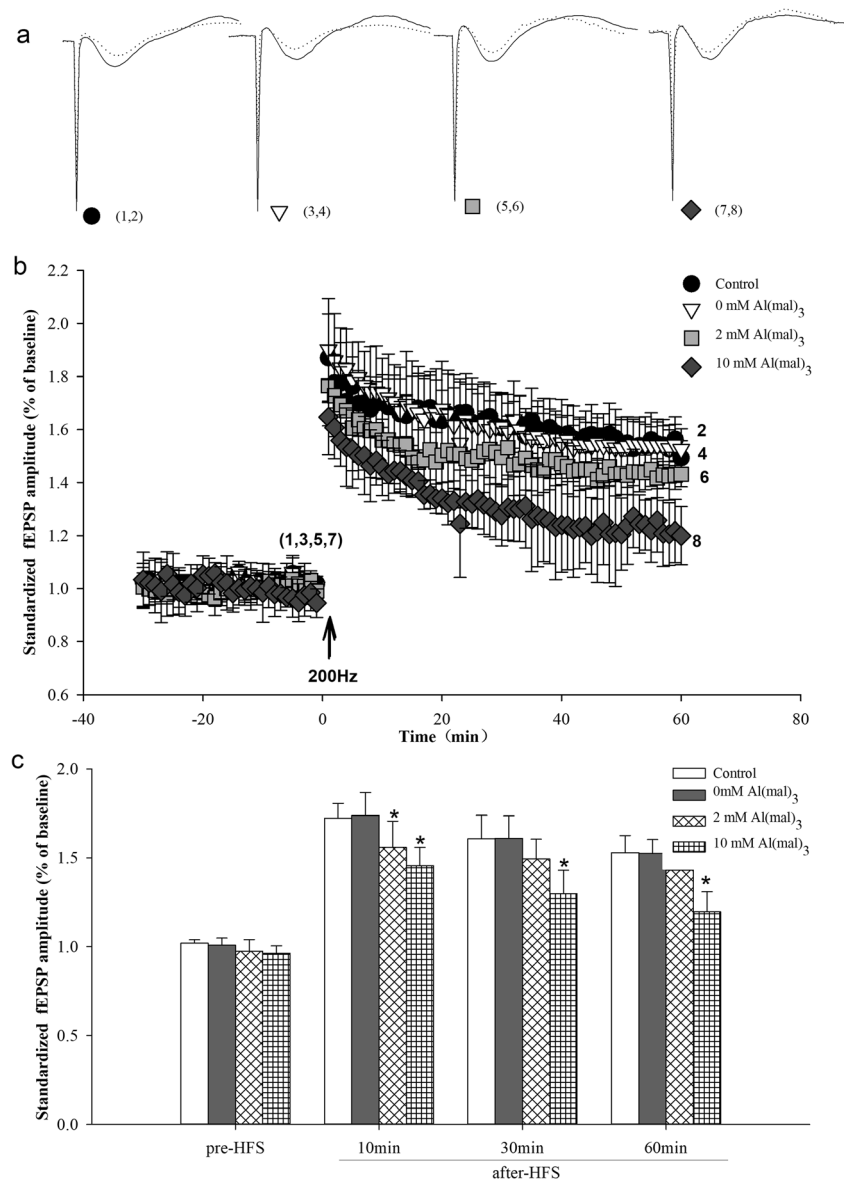
induction of LTP after cannula implantation. The average standardized fEPSP amplitudes were $178\% \pm 16.0\%$, $159\% \pm 11.4\%$, and $151\% \pm 8.7\%$ at 10 min, 30 min, and 60 min after HFS, respectively, in the cannula implantation group. There was no difference in the amplitude of fEPSPs between the cannula implantation group and the no cannula implantation group ($P>0.05$) (Fig. 1c).

No significant change in the mortality rate was observed in any of the control or treatment groups. To determine whether AI affects synaptic plasticity, the effects of AI on LTP in the CA1 region in vivo in rats were further investigated. As shown in Fig. 2a, there was no difference in the amplitude of fEPSPs between the 0 mM Al(mal)₃ group and the saline control group after HFS. However, administration of 2 mM or 10 mM Al(mal)₃ significantly impaired LTP in a concentration-dependent manner. As shown in Fig. 2b and c, the amplitude of fEPSPs in rats from the 2 mM group ($156\% \pm 14.7\%$) and 10 mM group (146 ± 10.4) at 10 min after HFS was significantly lower than that in the control group ($177\% \pm 16.2\%$) in the first minute after HFS. After LTP induction for 60 min, the amplitude of fEPSPs in rats from the control group remained above $153\% \pm 9.6\%$, while repression of LTP was obviously induced in the 10 mM group, and the amplitude of fEPSPs was significantly decreased in the 10 mM group ($120\% \pm 10.9\%$) compared with the control group (Fig. 2c) ($F(3, 20) = 19.517$, $P<0.05$).

AI-Induced Alterations in Hippocampal BDNF, GSK-3 β and β -Catenin Levels

Because of the important role of the GSK-3 β / β -catenin signaling pathway in axonal growth and memory consolidation [34, 35], we assumed that AI can inhibit LTP, which can be reliably used to study learning and memory, by inhibiting GSK-3 β / β -catenin signaling. To verify our hypothesis, we first investigated the phosphorylation status of GSK-3 β in the hippocampus after LTP induction. As shown in Fig. 3a–f, 10 mM AI reduced the inhibitory phosphorylation of GSK-3 β at Ser9, and decreased the ratio of p-GSK-3 β /GSK-3 β to $37.7 \pm 10.1\%$ ($n=6$ for each group, $P<0.05$ vs the control). No obvious change was seen in the total level of GSK-3 β in any of the treatment groups. To further explore the role of the GSK-3 β pathway in the modulatory effect of AI on LTP, we performed western blot analysis to quantify β -catenin and BDNF expression in the hippocampus. As shown in Fig. 3a–c, treatment with AI at various concentrations resulted in dose-dependent decreases in the expression of β -catenin ($F[3, 20] = 31.857$, $P<0.05$) and BDNF ($F[3, 20] = 23.3764$, $P<0.05$). Compared with that in the control group, the expression of β -catenin and BDNF in the 10 mM AI group was obviously decreased ($n=6$ for each group, $P<0.05$ vs the control).

Fig. 2 Effects of Al(mal)₃ exposure on LTP in the hippocampal CA1 region in rats. **a** Representative original data traces, with the dotted line representing the trace before HFS and the solid line representing the trace at 60 min after HFS. **b** The suppressive effect of Al(mal)₃ on LTP. Each point represents the mean \pm SD of the fEPSP amplitude. **c** Summary histogram comparing the effects of different doses of Al(mal)₃ on fEPSPs at 10 min, 30 min, and 60 min after HFS. The data are shown as the mean \pm SE. $n = 6$. * $P < 0.05$ compared with the control group



GSK-3 β Inhibitors Prevented the Effects of Al on Hippocampal LTP In Vivo

To address the question of whether GSK-3 β is involved in the effect of Al on LTP, we first evaluated the effects of i.c.v. injection of SB216763 (2 μ M), an inhibitor of GSK-3 β , combined with 10 mM Al on LTP. As shown in Fig. 4, the average standardized fEPSP amplitudes in the SB216763-treated group were $170 \pm 16.7\%$, $155 \pm 11.3\%$, and $146 \pm 10.0\%$ at 10, 30, and 60-min postHFS, respectively. We did not find any significant change in HFS-induced LTP in rats following SB216763 injection ($n = 6$ for each group, $P > 0.05$ vs the control group). However, pretreatment with inhibitors of GSK-3 β antagonized the effects of 10 mM Al on the induction of LTP in vivo. SB216763 reversed the 10 mM Al-induced impairment of LTP, restoring fEPSP amplitudes to

$149.2 \pm 15.7\%$, $143.5 \pm 10.4\%$, and $134 \pm 9.4\%$ of baseline levels at 10-, 30-, and 60-min postHFS, respectively ($n = 6$ for each group, $P < 0.05$ vs 10 mM Al alone).

SB216763 Reversed the Alterations in the Levels of GSK-3 β -Related Signaling Molecules in the Hippocampi of Rats Treated with Al

SB216763 is a selective inhibitor that can inactivate GSK-3 β by dephosphorylating it at the inhibitory Ser9 site [41]. We observed that SB216763 reversed the effects of Al on LTP in the electrophysiological experiment, and we then further investigated the phosphorylation status of GSK-3 β in the hippocampus after HFS. As shown in Fig. 5 a and b, Al reduced the inhibitory phosphorylation of GSK-3 β at Ser9 and decreased the ratio p-GSK-3 β /GSK-3 β to $27.5 \pm 10.8\%$ ($n = 6$

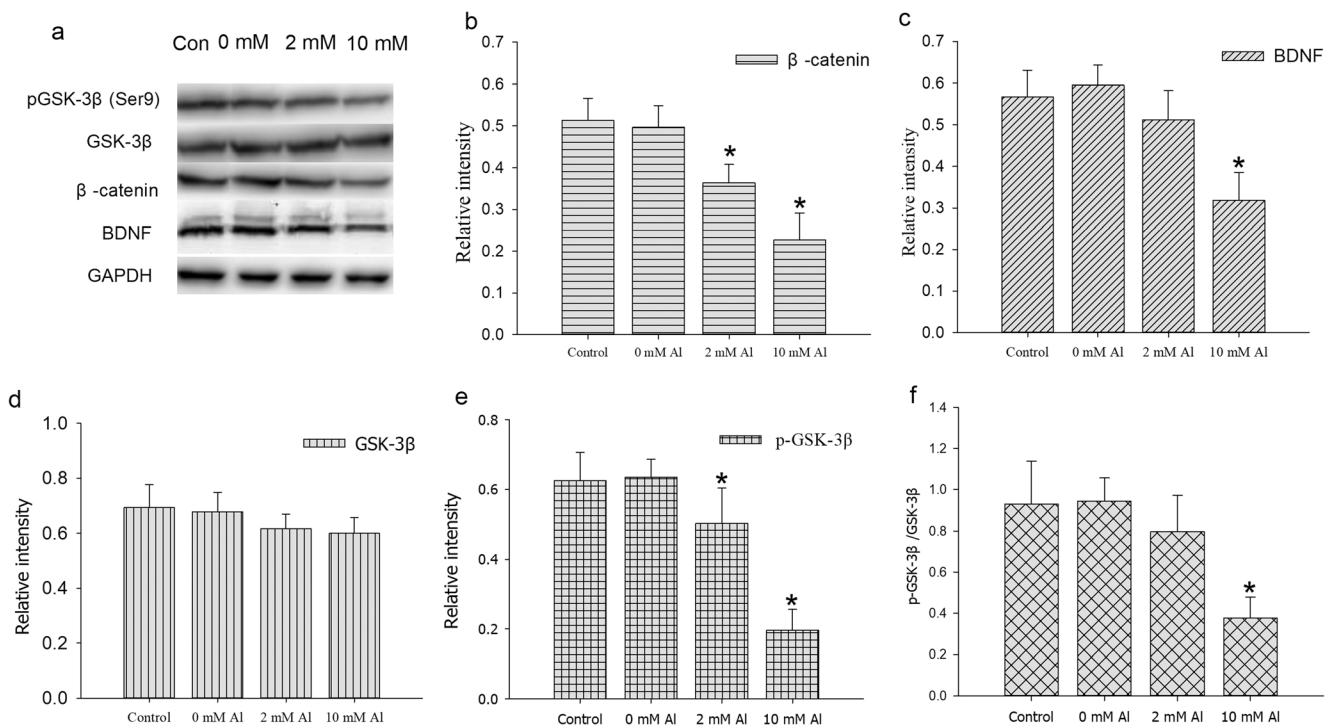


Fig. 3 Al(mal)₃ induced alterations in BDNF, GSK-3β and β-catenin levels in the hippocampus. **a** Protein levels of BDNF, β-catenin and p-GSK3β after exposure to Al(mal)₃ were measured using western blotting. **b, c, d, and e** The ratio of the expression level of the corresponding protein to the expression level of GAPDH. GAPDH was used as a gel

loading control. **f** The level of p-GSK-3β, which is expressed as a ratio of p-GSK-3 β expression to total GSK-3 β expression. The data are shown as the mean ± SE. *n* = 6 and were analyzed by one-way ANOVA followed by the LSD test. **P* < 0.05 compared with the control group

for each group, *P* < 0.05 vs the control). SB216763 could slightly prevent the decrease in the ratio of p-GSK-3β to GSK-3β ($51.0 \pm 9.1\%$) (*n* = 6 for each group, *p* < 0.05 vs the 10 mM Al group), suggesting that SB216763 may inhibit GSK-3β partially through modulating its phosphorylation. We tested whether i.c.v. administration of SB216763 has effects on GSK-3β-related signaling activity in the brain. As shown in Fig. 5a–d, the hippocampal protein levels of β-catenin and BDNF were lower in the Al-exposed rats than in the control rats. However, these differences were ameliorated by SB216763 ($F[3, 20] = 8.677$, *P* = 0.001; $F[3, 20] = 12.085$, *P* < 0.05). These results indicate that SB216763 effectively reversed the Al-induced decrease in β-Catenin and BDNF expression.

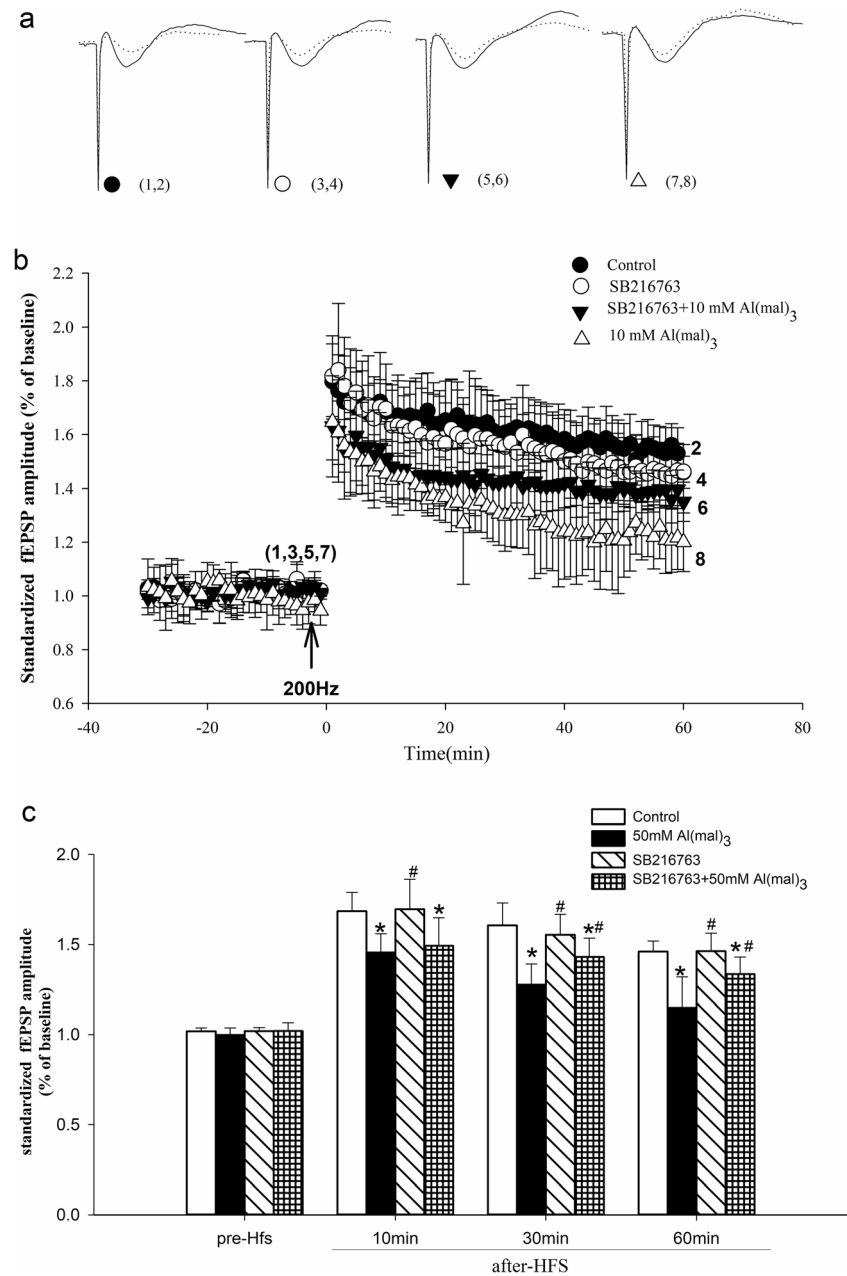
Discussion

The effect of Al on cognitive impairment has been studied for decades. Occupational epidemiologists have reported that workers exposed to Al show worse performance on various neurobehavioral tests than nonexposed individuals [48, 49]. Our group previously reported that Al exposure causes spatial memory deficits in rats [50], and the same conclusion was reached in a previous epidemiological study [7]. In the present study, an animal model of Al-induced neurotoxicity was

established by i.c.v. injection of Al(mal)₃, and changes in LTP, which is well recognized to reflect synaptic plasticity, a crucial neurobiological basis for learning and memory, were detected in vivo. As previously reported by our group [15], we implanted a hollow cannula into the lateral ventricle and fixed it to the skull of the rat for i.c.v. injection. After 2 weeks of adaptation, the agent was injected through the cannula with a microinjector. As shown in the present study, after a stable baseline was established for 30 min, HFS resulted in successful induction of LTP after cannula implantation, and there was no difference in the amplitude of fEPSPs between the cannula implantation group and the no cannula implantation group. The method of i.c.v. injection applied in our study has been widely used in neuropharmaceutical, neurobiological, and neurotoxicological studies, as it is a convenient method for facilitating the precise entry of the injected substance into brain tissue and avoids the influences of gastrointestinal absorption and metabolism [51–53]. The current study indicated that i.c.v. injection of Al(mal)₃ significantly suppressed the amplitude of fEPSPs in a dose-dependent manner and that LTP was significantly inhibited in rats in the 10 mM group, indicating that LTP is a sensitive target of Al. Electrophysiological evidence confirmed that Al caused impairments in cognition, learning, and memory [54, 55].

This study shows that the impairment of LTP may be related to the GSK-3β/β-catenin signaling pathway, which

Fig. 4 Effects of SB216763 on LTP in the hippocampal CA1 region in rats. **a** Representative original data traces, with the dotted line representing the traces before HFS and the solid line representing the traces at 60 min after HFS. **b** The inhibitory effect of AI on LTP magnitude was reversed by SB216763. **c** Histograms showing the normalized fEPSP amplitude at 10 min, 30 min, and 60 min after HFS relative to that of the control, AI-, SB216763-, and SB216763 plus AI-treated rats. The data are shown as the mean \pm SE ($n = 6$) and were analyzed by one-way ANOVA followed by the LSD test. * $P < 0.05$ compared with the control group, # $P < 0.05$ compared with the 10 mM AI(mal)₃ group



provides a molecular basis of AI-induced neurotoxicity. GSK-3 β , a kinase that is inactivated by phosphorylation at Ser9 and is specifically expressed in the central nervous system [56], plays a very important role in axonal growth, polarity formation, and memory processes and is related to nervous system dysfunction [29, 30, 57]. One of the main downstream targets of GSK3 β is β -catenin. Inhibition of GSK3 β allows β -catenin to be stabilized, accumulate in the cytoplasm, and translocate to the nucleus, activating downstream target genes responsible for synaptogenesis [25]. It has also been proven that GSK3 β / β -catenin signaling plays an essential role in learning, memory and psychological status, the dysfunction of which is involved in several neuropsychiatric disorders,

such as Alzheimer's disease and depression [58]. The main functions of the brain are information storage, learning, and memory, which involve changes in synaptic transmission efficiency; thus, abnormalities in GSK-3 β also affect synaptic plasticity, such as LTP and LTD. Several studies have noted that inhibition of GSK-3 β activity by phosphorylation promotes LTP induction, overexpression of GSK-3 β inhibits LTP, and activation of GSK-3 β activity promotes induction of LTD [31–33]. GSK-3 β mutant mice show LTP inhibition because GSK-3 β activity cannot be inhibited through phosphorylation at Ser9 [59]. This phenomenon provides some context for the findings of the present study, in which we confirmed that increased exposure to AI decreased the protein

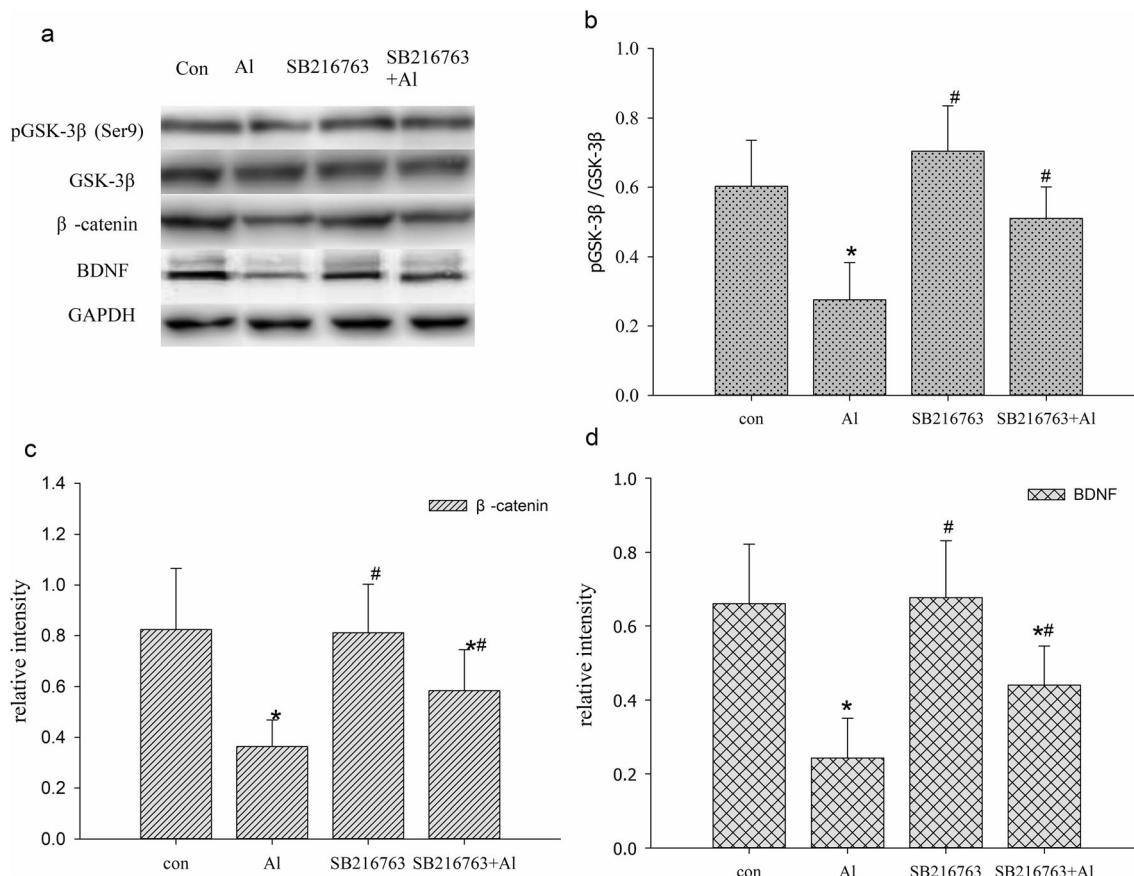


Fig. 5 SB216763 upregulated the hippocampal protein expression of BDNF and β -catenin while increasing the ratio of p-GSK-3 β /GSK-3 β . **a** Protein levels of BDNF, β -catenin, and p-GSK3 β after application of SB216763 or Al(mal)₃. **b** The level of p-GSK-3 β , which is expressed as a ratio of p-GSK-3 β expression to total GSK-3 β expression. **c**, **d** The

expression level of the corresponding protein relative to the expression level of GAPDH. The data are shown as the mean \pm SE ($n = 6$) and were analyzed by one-way ANOVA followed by the LSD test. * $P < 0.05$ compared with the control group, # $P < 0.05$ compared with the 10 mM Al(mal)₃ group

level of β -catenin and the phosphorylation of GSK-3 β at Ser9, causing increased accumulation of active GSK-3 β . Given that LTP deficits can be attenuated/rescued by chronic treatment with a GSK-3 β inhibitor [42], we applied SB216763, a potent irreversible and cell-permeable pharmacological inhibitor of GSK-3 that is structurally distinct from other inhibitors, is highly selective for GSK-3 β and has no significant influence on the activity of other kinases [41], in the present study to determine whether GSK-3 β is involved in the Al-induced impairment of LTP. In the present study, Al-induced suppression of LTP was partly but significantly reversed by SB216763. Furthermore, SB216763 significantly reversed the decrease in β -catenin protein levels in the hippocampus. These results suggest that the GSK-3 β / β -catenin signaling pathway participates in the Al-induced inhibition of LTP. However, the downstream mechanisms that this pathway regulates require further investigation.

BDNF, which is mainly synthesized in brain tissue and is found at abundant levels in the cerebral cortex and hippocampus, is a key factor in the maintenance of synaptic plasticity [60]. BDNF can enhance synaptic transmission by acting on

receptors in the hippocampus and other brain regions and by enhancing the activity of neurotransmitters and receptors involved in learning and memory [19–21]. In the present study, BDNF expression was also decreased by Al treatment, especially 10 mM Al. These findings are in line with previous studies showing decreased BDNF expression [24] and pathological lesions of synaptic structure [53]. This study provides new evidence that BDNF expression is decreased, indicating that the fEPSP amplitudes of functional synapses involved in neurotransmission are reduced.

A recent study further confirmed this direct relationship; similar to BDNF expression, β -catenin signaling is inhibited in neurodegenerative diseases [61]. Similar to β -catenin signaling inhibition, BDNF dysregulation has been implicated in neurodegenerative disorders [62, 63]. BDNF mRNA and protein levels are decreased in various brain regions in patients with Alzheimer's disease, and administration of exogenous BDNF or stimulation of its receptor can attenuate pathology in animal models [60]. Recent evidence has indicated that BDNF is a direct target of the β -catenin transduction pathway. Seven binding motifs for Wnt-dependent TCF/LEF

transcription factors that associate with β -catenin interact with specific DNA sequence motifs were identified in the BDNF gene promoter [39]. Thus, downregulation of β -catenin expression via deletion of one copy of the gene in neurons abolishes capsaicin-induced BDNF expression [38]. Thus, the regulation of β -catenin/BDNF is important for Al-induced impairment of LTP. The results of the current study indicated that i.c.v. injection of 10 mM Al decreased BDNF protein expression, which is consistent with a previous study in a rat model of chronic aluminum exposure [24], and that this decrease was accompanied by reductions in β -catenin protein expression and the phosphorylation of GSK-3 β at Ser9. We applied SB216763 to the same animal model, and the data suggested that the change in BDNF protein expression was significantly reversed. According to the findings of other researchers showing that BDNF or Wnt signaling inhibition reduces BDNF levels [64] and therefore reduces dendritic arbor size in cultured cortical neurons [65], BDNF levels may correlate with GSK-3 β / β -catenin signaling.

Conclusion

The present study was designed to investigate the effects of Al on the induction of LTP in the CA1 region of the rat hippocampus and to further elucidate whether GSK-3 β , a cognition-related serine/threonine kinase, is involved in these effects. However, in this study, an inhibitor of GSK-3 β partially reversed the impairment of LTP induced by Al because synaptic plasticity is regulated by many signaling pathways, and these temporal profiles may not be directly comparable. Nonetheless, our results provide new insight into the effect of Al on synaptic plasticity and its neurotoxicity. From the perspective of the identified β -catenin–BDNF axis, we propose that the GSK-3 β / β -catenin signaling–mediated BDNF pathway may be a critical pathogenic pathway in Al-induced impairment of synaptic plasticity.

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Data Availability Not applicable.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethics Approval All experiments complied with the rulings of the Institute of Laboratory Animal Research, Division on Earth and Life Sciences, National Institute of Health, USA (Guide for the Care and Use of Laboratory Animals). The research protocol was performed in

accordance with the regulations of the Institutional Animal Care and Use Committee of Shanxi Medical University.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

Code Availability Not applicable.

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