

Dexmedetomidine alleviates LPS-induced neuronal dysfunction via AKT/GSK-3 β /CRMP-2 pathway

Wei Zeng

Jinan University First Affiliated Hospital

Chunyuan Zhang

Southern Medical University

Qingshan Long

Guangzhou Medical University

Yalan Li (✉ tyalan@jnu.edu.cn)

the First Affiliated Hospital of Jinan University

Research article

Keywords: Dexmedetomidine, postoperative cognitive dysfunction, neurological impairment, AKT, GSK-3 β , CRMP-2

Posted Date: June 4th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-27596/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background

Dexmedetomidine (Dex), an α_2 -adrenergic receptor agonist, shows intervention effect on cognitive dysfunction in elderly patients after general anesthesia. However, the underlying pathological mechanism remains to be further elucidated. The aim of the study was to investigate effects of Dex on LPS-induced neuronal damages in cultured hippocampal neurons.

Methods

We applied LPS to mimic the post-surgical inflammation in cultured hippocampal neuron. Cultured hippocampal neurons were treated with LPS in a dose- and time- dependent manner, then were administrated with or without Dex. The neuronal morphology including neurite outgrowth and synaptic transmission was observed, and mEPSCs was recorded by electrophysiological patch clamp. Cell lysates were subjected for western blot to assess the explore the potential underlying mechanism.

Results

Compared to control group, administration of LPS significantly impaired the neurite outgrowth in a concentration- and time-dependent manner. Dex treatment markedly reversed LPS-induced impairment of neurite outgrowth in hippocampal neurons. Electrophysiological patch clamp results showed that LPS induced synaptic transmission dysfunction, which could be restored after Dex addition. Furthermore, western blotting assays showed that LPS suppressed the AKT/GSK-3 β /CRMP-2 signaling pathway and Dex administration significantly re-activated this pathway to encounter the inhibitory effect of LPS.

Conclusion

Addition of Dex showed significant protection effect on LPS-induced hippocampal neuron damages, including neurite outgrowth and synaptic transmission. Dex functioned via the activation of GSK-3 β /CRMP-2 signaling pathway to alleviate LPS-induced neurological dysfunctions.

Background

Elderly patients are susceptible to brain dysfunction after surgery, especially after general anesthesia, collectively referred to postoperative cognitive dysfunction (POCD) [1]. POCD is first found in elderly patients after cardiac surgery, however, in recent years, it has been found that patients with other surgical procedures also have a higher incidence [2–4]. The total incidence of POCD after cardiac surgery can reach 51% [5]. POCD is reversible for most patients, but there are still a few patients with long-term or even permanent cognitive impairment. POCD leads to delayed rehabilitation, increased complications and

even loss of self-care ability, prolongs the length of hospital stay, increases medical costs, and causes a series of medical, social and economic problems. POCD is closely related to neurodegenerative diseases in the elderly such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease, multiple sclerosis [6, 7]. Therefore, how to reduce the occurrence of cognitive dysfunction after general anesthesia in the elderly has become a research hotspot in recent years.

The inflammatory response can affect the function of the central nervous system, especially the inflammation of the central nervous system (CNS) can lead to changes in cognitive function [8], which leads to the occurrence of POCD. Current research suggests that both anesthesia and surgery may induce inflammatory responses in the central nervous system [9, 10]. Thus, the occurrence and development of POCD is closely related to inflammatory response. Lipopolysaccharide (LPS; cell wall immunostimulatory component of Gram-negative bacteria) was originally identified as a Toll-like receptor 4 (TLR-4) ligand [11]. Once microglia activated by LPS, it produces pro-inflammatory cytokines such as TNF- α , IL-1 β , prostaglandin E2 (PGE2), and NO [12, 13]. These cytokines are key mediators that mediate the neuroinflammatory process. Inhibition of TLR-4 may abolish LPS induced inflammatory response [14]. The administration of LPS in animals induces cognitive impairment [15, 16] and other complex dysfunctions, including anorexia, decreased exercise, weight loss, exploratory behavior, increased anxiety, lethargy and behavioral depression. These symptoms are very similar to the clinical symptoms of human neurodegenerative diseases. However, the specific mechanism of LPS-induced cognitive impairment remains to be elucidated.

Dexmedetomidine (Dex) is a highly selective α_2 adrenergic receptor agonist, similar to remifentanyl during anesthesia [17]. Dex also shows anti-inflammatory activity [18–20]. In recent years, Dex has been reported to have a certain intervention effect on cognitive dysfunction in elderly patients after general anesthesia. Data show that the administration of Dex extends the survival time and only 6% of patients with general anesthesia have postoperative delirium [21, 22], comparing to 45% higher rate of postoperative delirium with the application of general drugs such as propofol or midazolam [23]. However, the detailed mechanism of Dex in reducing POCD remain unknown.

Here, the main purpose of this study is to determine whether Dex regulates LPS-induced neurological damage and trying to elucidate the underlying mechanisms, trying to provide new approaches and intervention targets for the clinical prevention and treatment of POCD.

Methods

Neuron culture and transfection

Newborn 1-day (male and female) and 7 days (male) of Sprague-Dawley (SD) rats were provided by Zhongshan Animal Medical Center, Zhongshan University. The experiments were performed on 1-day-old newborn Sprague-Dawley rats, and were prepared as previously described (Zhang et al., 2007). Briefly, rats were sacrificed using CO₂ anesthetization, hippocampi from brains were dissected and gently

minced brain tissue in a 60 mm tissue culture dish. The hippocampal tissue was incubated with 0.25% trypsin at 37°C for 5 min. Repeat a total of 3 times. Neurons were counted and cultured in Dulbecco's modified Eagle's medium (Gibco, USA), supplemented with 10% fetal bovine serum (Gibco), and plated in a 24 well plate with coverslips at a density of 6×10^4 cells/well. Neurons were placed in a 37°C, 5% CO₂ incubator overnight and the medium was replaced with neurobasal feeding media (Neurobasal medium containing 2% B27 supplement and 0.5 mM glutamine solution). Half the volume of media was replaced with same volume of fresh neurobasal feeding media every 3 days. Transient transfections of neurons were performed with Lipofectamine[®] 2000 according to the manufacturer's instructions. The protocol was approved by the Institutional Animal Care and Use Committee at Jinan University.

Western blotting

Protein samples were extracted from rat hippocampal neurons, and the protein concentrations were quantified using the BCA assay (Sigma, MO, USA). The extracted protein samples were separated by 10% SDS-PAGE with ~30 µg protein loaded per lane and transferred onto a PVDF membrane (EMD Millipore, MA, USA). The membrane was blocked with 5% non-fat milk in TBS, 0.1% tween-20 (TBST) at room temperature for 1 h and then was incubated with antibodies against p-AKT, AKT, p-GSK-3β, GSK-3β, p-CRMP2 and CRMP2 (all from Abcam, USA) in TBS buffer with 3% BSA at 4°C overnight, respectively. GAPDH was used as a loading control. After incubation with the secondary antibodies at room temperature for 1 h, the blots were visualized using enhanced chemiluminescence reagents. The intensity of the bands was analyzed quantified by densitometric analysis using Image-Pro Plus 7.0 (Media Cybernetics, Inc.). The Western blotting results are representative of three independent experiments.

Immunocytochemistry

Immunofluorescence assay was performed as previously described [24]. Briefly, neurons were fixed in 4% paraformaldehyde supplemented with 4% sucrose for 40 min at 4°C and blocked with blocking buffer (TBS in 3% BSA). Then the sections were incubated with rabbit anti-GFP (1:1000, cat no. Ab290, Abcam, USA) and Mouse anti-Tau-1 antibody (1:500; cat no. MAB3420; Millipore) overnight at 4 °C. After washed three times with TBST (0.1% Triton X-100 in TBS), the neurons were incubated with goat anti-Mouse IgG FITC (1:1000; cat no. ab150115; Abcam) for 1 h at room temperature, then the neurons were mounted on glass slides using Fluoro Gel II with DAPI for confocal microscopy (LSM 700; Zeiss GmbH, Germany).

Neuronal morphology analysis

All images were collected by ordinary fluorescence microscope or confocal microscope. Image-Pro Plus software for neurite length or ImageJ software with the Neuronal Tracer plug-in and Sholl plug-in for the Sholl analysis were used to analysis the morphometric of protrusion as previously described [25]. Briefly, each image was processed with the Neuronal Tracer plug-in, and a mask of all neurite on a confocal image was drawn manually. By transfection GFP and staining GFP and Tau-1 to reveal all the neurite by immunocytochemistry. The total length of neurite and sholl analysis were performed to reveal the neuronal development.

Whole-cell patch-clamp

The whole-cell patch-clamp technique was used [24] to record miniature EPSCs (mEPSCs) were obtained from cultured hippocampal neurons treated with LPS and Dex on DIV 11-12 neurons. The external solution containing in (mM): 1 MgCl₂, 5 KCl, 128 NaCl, 20 HEPES, 2 CaCl₂, 15 glucose, 1 tetrodotoxin, and 100 μM picrotoxin. The pH of external solution was adjusted to 7.2–7.3 with KOH. The internal solution of the recording electrodes (4- to 6-MΩ tip resistance) contained (in mM): 2 MgATP, 5 Na₂-phosphocreatine, 147 KCl, 2 EGTA, 10 HEPES, and 0.3 Na₂GTP. pH was adjusted to 7.2–7.3 with KOH and the osmolarity to 280 mmol/kg with sucrose. The amplifier used was multiclamp 700 B amplifier (Molecular Devices, Sunnyvale, CA, USA) and Clampex 10.5 software (Axon Instruments, Union City, CA, USA).

Statistical analysis

The experimental data were presented as mean ± SEM from at least three experiments, and SPSS 19.0 software (SPSS Software, Chicago, IL, USA) was used to make statistics. Statistics were performed by t-test or One-way ANOVA method, $p < 0.05$ was considered to be significantly different, * or # donates $p < 0.05$, ** donates $p < 0.01$, *** donates $p < 0.001$.

Results

LPS administration impairs hippocampal neurite outgrowth

In order to clarify the effect of LPS on the morphology of hippocampal neurons, neurons were transfected with GFP encoding plasmids and treated with different concentrations of LPS for 12 h (0, 0.1, 0.5, 1.0, 2.0 μg/ml). LPS was found to induce significant changes in the growth of neuronal processes in a concentration-dependent manner (Fig. 1A). The protrusion length of LPS groups was significantly lower than that of the control group ($p < 0.01$) (Fig. 1B). Sholl analysis counted the number of branch points in different radius ranges showed that the complexity of neuronal morphology was also decreased in a concentration-dependent manner (Fig. 1C). We selected the dose of 1.0 μg/ml LPS to further explore the effect. Neurons were treated with LPS for different time range (0, 3, 6, 12 and 24 h; Fig. 1D) and the total length (Fig. 1E) and the sholl analysis (Fig. 1F) of protrusions were calculated. The data showed that LPS induced neuronal morphological changes in a time-dependent manner. In general, these data suggest that LPS impairs neurite outgrowth.

Dex antagonizes LPS-induced impairment of neurite outgrowth

To determine the effect of Dex on the development of hippocampal neuron development, the neuronal development was observed under the treatment of LPS with or without the addition of Dex. As shown in Fig. 2A, LPS significantly induced neuronal morphological changes and these damages were rescued by the addition of Dex. The length of total neurite (Fig. 2B) and number of intersection (Fig. 2C) in each

group were calculated. The data suggest that Dex treatment would antagonize LPS induced damage on neuronal morphology.

Dex rescues synaptic transmission disorder induced by LPS

To verify whether Dex and LPS were related to neuronal function, electrophysiological patch-clamp assay was applied in cultured hippocampal neurons to determine the micro excitatory currents (mEPSCs) (Fig. 1A). As shown in Fig. 3B, the results showed that the synaptic transmission of neurons was impaired by LPS, and the amplitude (Fig. 3C) and frequency (Fig. 3D) of mEPSCs were significantly decreased. However, the suppressed amplitude and frequency of mEPSCs by LPS were restored after the addition of Dex. Dex alone treatment could induce the increase of amplitude and frequency. These data suggest that Dex could resist LPS induced synaptic transmission disorder.

Dex encounters the inhibitory effect of LPS via AKT/GSK-3 β /CRMP-2 pathway

To further explore the molecular mechanism of Dex resistance to LPS-induced neurodevelopmental impairment and synaptic transmission disorder, neuronal lysates were subjected to western blotting. The results showed that the phosphorylation levels of AKT and GSK-3 β were downregulated, while that of CRMP-2 was upregulated under LPS treatment (Fig. 4A). Application of Dex significantly increased the phosphorylation levels of AKT and GSK-3 β and decreased that of CRMP-2 and encountered the inhibitory effect of LPS (Fig. 4B). These results suggest that Dex may antagonize LPS-induced neuronal damage by activating the AKT/GSK-3 β /CRMP-2 pathway.

Inhibition of AKT/GSK-3 abolishes the alternative effect of Dex against LPS

In order to further determine that Dex may interact with LPS-induced neural damage through activation of the AKT/GSK-3 β /CRMP-2 pathway, pharmacological approach was applied. IGF was used to promote neuron development and AKT inhibitor LY294002 and GSK-3 inhibitor SB216763 were used to suppress the AKT/ AKT/GSK-3 axis. As shown in Fig. 5A, IGF function to activate AKT pathway, induced the phosphorylation levels of AKT and GSK-3 β and inhibited that of CRMP-2, consistent with the pattern of Dex. However, the addition of AKT inhibitor and GSK-3 inhibitor significantly abolished the activation effect of Dex on this pathway, the phosphorylation levels of AKT, GSK-3 β and CRMP-2 returned to levels equal to LPS treatment group (Fig. 5B). These data confirm that Dex functions via the AKT/GSK-3 β /CRMP-2 pathway to antagonize LPS treatment in hippocampal neurons.

Discussion

In the current study, we demonstrated that LPS induction impaired hippocampal neuron development and disrupted synaptic transmission. Dex can antagonize LPS induced impairment on hippocampal neuronal developmental and synaptic transmission. In addition, DEX reactivated the AKT/GSK-3 β /CRMP-2 pathway to antagonize LPS-induced nerve damage.

POCD refers to the absence of mental disorders and abnormal brain function in elderly patients after surgery, referring to such as mental disorder, anxiety, personality changes and memory impairment [26]. General anesthesia combined with surgical shock can change the physiological function of elderly patients [27]. Under the stimulation of stress response, a series of adverse cardiovascular and cerebrovascular events can easily cause the occurrence of cognitive dysfunction in elderly patients [28]. It is generally believed that the pathogenesis of POCD is mainly considered from three aspects: (1) the influence of surgical factors on the internal environment, such as the size of the surgical wound, the duration of the operation, stress response, arthritis response, micro thromboembolism, blood loss and fluid loss [29]; (2) The effects of anesthetic factors on patients, such as the effect of general anesthetics on the central nervous system and the effects of anesthetics on the homeostasis of the body, such as hypotension, hypertension, bradycardia and hypothermia [30]; (3) The pathophysiological status and social factors of the patients undergoing surgery, such as the patient's old age, combined diabetes, hypertension, and cognitive impairment before surgery have a certain correlation with the occurrence of POCD [31]. Therefore, in-depth study of the neuropathic mechanism of POCD shows important scientific value and significance.

Surgeries could trigger neuroinflammation, which would induced POCD [32]. Surgeries induced release of inflammatory factors or immune cells from the peripheral blood enter into the brain and affects the central nervous system [33]. These inflammatory factors could activate microglia cells to exaggerate immune response, leading to the release of large number of inflammatory factors [34], such as IL-1 β , TNF- α and *et al.*. In addition, peripheral immune cells that entered the brain could participate to amplify the inflammatory response [35]. Accumulated inflammatory mediators cause reversible or irreversible damages to brain tissue, leading to the degeneration of neurite and cognitive dysfunction. The memory center, the hippocampus is more sensitive to overloaded inflammatory cytokines because of the widely-expressed receptors [36]. Here, we applied LPS to induced inflammatory response in cultured primary hippocampal neurons. LPS significantly induced damages to neurite and synaptic transmission, which is consistent with previous studies [37].

In clinical practice, Dex is widely used as an α 2-agonist [38]. Studies show that Dex performs anti-inflammatory effect [39], where the underlying mechanisms has not been fully described. In a rat model of cerebral ischemia, Dex was found to decrease the blood catecholamine content and to decrease sympathetic nerve activity [40]. In septic shock rats, Dex inhibits systemic inflammatory responses and improves survival [41]. In spinal cord injury mode of rats, Dex also showed anti-inflammatory effects [42]. In clinical patients, Dex application markedly reduced the expression of pro-inflammatory cytokines, such as IL-1 β , TNF- α and IL-6 [43]. In rat model of POCD, Dex protected aged rats from cognitive dysfunction by decreasing hippocampal inflammation [44]. Here, although we did not detect the levels of inflammatory factors, the data showed that Dex significantly alleviated LPS induced neurite outgrowth and synaptic transmission damages.

PI3K/AKT pathway is closely related to cognitive function in POCD [45]. Zhang et al. discovered that ADAM2 could activate PI3K/AKT pathway to attenuate isoflurane-induced POCD [46] and Zhou et al.

showed that gap junctional communication dysfunction during ischemia-reperfusion injury could cause cognitive impairment via PI3K/AKT pathway [47]. Rui et al. reported that miR-410 showed neuroprotective effect against sevoflurane induced cognitive dysfunction in rats via PI3K/AKT pathway [48]. Here in the current study, LPS administration significantly suppressed the phosphorylation activation of AKT/GSK-3 β /CRMP-2 pathway and Dex re-activated this pathway way to encounter the inhibitory effect of LPS in cultured hippocampal neurons, rescuing the neurite development and synaptic transmission disorder.

In summary, the mechanism by which Dex interferes with cognitive dysfunction in elderly patients after general anesthesia remains unclear. The main purpose of this study is to determine whether Dex regulates LPS-induced neurological damage, trying to provide new approaches and intervention targets for clinical prevention and treatment of cognitive dysfunction in POCD.

Conclusions

To summarize, we demonstrated that LPS-induced neuronal outgrowth inhibition and synaptic transmission dysfunction could be retarded by the administration of dexmedetomidine, via the regulation of PI3K/ AKT/GSK-3 β signalling pathway. This study provided basis for the study of dexmedetomidine effect, assisting the clinical application for the treatment of POCD.

Abbreviations

AD: Alzheimer's disease; CNS: the central nervous system; Dex: Dexmedetomidine; LPS: Lipopolysaccharide; mEPSCs: miniature excitatory postsynaptic currents; PD: Parkinson's disease; PGE2: prostaglandin E2; POCD: postoperative cognitive dysfunction; TLR-4: Toll-like receptor 4

Declarations

Ethics approval and consent to participate

The protocol was approved by the Institutional Animal Care and Use Committee at Jinan University. Newborn 1-day (male and female) and 7 days (male) of Sprague-Dawley (SD) rats were provided by Zhongshan Animal Medical Center, Zhongshan University.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare no conflict of interest.

Funding

No funding.

Authors' contributions

Conceived and designed the study and wrote the manuscript: YL, WZ. Analysed and interpreted the data: WZ, CZ, QL. Performed molecular experiments: WZ. All authors have read and approved the manuscript.

Acknowledgment

Not applicable.

References

1. Luyten FP, Tylzanowski P, Lories RJ. Wnt signaling and osteoarthritis. *Bone*. 2009;44(4):522–7.
2. Hudetz JA, Patterson KM, Byrne AJ, Pagel PS, Warltier DC. Postoperative delirium is associated with postoperative cognitive dysfunction at one week after cardiac surgery with cardiopulmonary bypass. *Psychol Rep*. 2009;105(3 Pt 1):921–32.
3. Gao L, Taha R, Gauvin D, Othmen LB, Wang Y, Blaise G. Postoperative Cognitive Dysfunction After Cardiac Surgery. *Chest*, 128(5):3664–3670.
4. Ida M, Kawaguchi M. Postoperative cognitive dysfunction after non-cardiac surgery. *Masui*. 2014;63(11):1228–34.
5. Deiner S, Silverstein JH. Postoperative delirium and cognitive dysfunction. *British Journal of Anaesthesia*, 103(Supplement 1):i41-i46.
6. Allison DJ, Ditor DS. The common inflammatory etiology of depression and cognitive impairment: a therapeutic target. *Journal of Neuroinflammation*, 11(1).
7. Nguyen MD, Julien J-P, Rivest S. INNATE IMMUNITY: THE MISSING LINK IN NEUROPROTECTION AND NEURODEGENERATION?, 3(3):216–227.
8. Combs KC. Inflammation and Microglia Actions in Alzheimer's Disease. *J Neuroimmune Pharmacol*, 4(4):380–388.
9. Hudson AE, Hemmings HC. Are anaesthetics toxic to the brain? *British Journal of Anaesthesia*, 107(1):30–37.
10. Vanderweyde T, Bednar MM, Forman SA, Wolozin B. Iatrogenic Risk Factors for Alzheimer's Disease: Surgery and Anesthesia. *Journal of Alzheimers Disease* *Jad*. 2010;22(Suppl 3(Suppl 3):91–104.
11. BEUTLER B. Tlr4: central component of the sole mammalian LPS sensor. *Current Opinion in Immunology* 2000, 12.

12. Shorena. Janelidze, Niklas, Mattsson, Erik, Stomrud, Olof, Lindberg, Sebastian, Palmqvist: CSF biomarkers of neuroinflammation and cerebrovascular dysfunction in early Alzheimer disease.
13. Mrak RE, Griffin WST. Glia and their cytokines in progression of neurodegeneration. *Neurobiol Aging*. 2005;26(3):349–54.
14. Lysakova-Devine T, Keogh B, Harrington B, Nagpal K, Halle A, Golenbock DT, Monie T, Bowie AG. Viral Inhibitory Peptide of TLR4, a Peptide Derived from Vaccinia Protein A46, Specifically Inhibits TLR4 by Directly Targeting MyD88 Adaptor-Like and TRIF-Related Adaptor Molecule. *Journal of Immunology*, 185(7):4261–4271.
15. Choi DY, Lee JW, Lin G, Yong KL, Lee MS. Obovatol attenuates LPS-induced memory impairments in mice via inhibition of NF- κ B signaling pathway. *Neurochem Int*. 2011;60(1):68–77.
16. Golestaneh AF, Atashi A, Langroudi L, Shafiee A, Ghaemi N, Soleimani M. miRNAs expressed differently in cancer stem cells and cancer cells of human gastric cancer cell line MKN-45. *Cell Biochem Funct*. 2012;30(5):411–8.
17. Schenarts CL, Burton JH, Riker RR. Adrenocortical Dysfunction Following Etomidate Induction in Emergency Department Patients. *Academic Emergency Medicine Official Journal of the Society for Academic Emergency Medicine*, 8(1):1–7.
18. Rohan D, Buggy DJ, Crowley S, Ling FKH, Moriarty DC. Increased incidence of postoperative cognitive dysfunction 24 hr after minor surgery in the elderly. *Can J Anaesth*. 2005;52(2):137–42.
19. Yamanaka D, Kawano T, Nishigaki A, Aoyama B, Yokoyama M. Preventive effects of dexmedetomidine on the development of cognitive dysfunction following systemic inflammation in aged rats. *Journal of Anesthesia*. 2016;31(1):1–11.
20. Iliopoulos D, Malizos KN, Oikonomou P, Tsezou A. Integrative microRNA and proteomic approaches identify novel osteoarthritis genes and their collaborative metabolic and inflammatory networks. *PLoS One*. 2008;3(11):e3740.
21. Han JH, Wilson A, Ely EW. Delirium in the Older Emergency Department Patient: A Quiet Epidemic. *Emergency Medicine Clinics of North America*, 28(3):611–631.
22. Delaney M, Pepin J, Somes J. Emergency Department Delirium Screening Improves Care and Reduces Revisits for the Older Adult Patient. *Journal of Emergency Nursing*, 41(6):S0099176715004092.
23. Jie Y, Yongfang Z, Yan K, Binbin X, Peng W, Yinxia L, Zhen W. Risk Factors of Delirium in Sequential Sedation Patients in Intensive Care Units. *BioMed Research International*, 2017:1–9.
24. Zhang Z, Zhang J, Li J, Zhang J, Chen L, Li Y, Guo G. Ketamine Regulates Phosphorylation of CRMP2 To Mediate Dendritic Spine Plasticity. *J Mol Neurosci*. 2020;70(3):353–64.
25. Rojek KO, Krzemien J, Dolezyczek H, Boguszewski PM, Kaczmarek L, Konopka W, Rylski M, Jaworski J, Holmgren L, Proszynski TJ. Amot and Yap1 regulate neuronal dendritic tree complexity and locomotor coordination in mice. *PLoS Biol*. 2019;17(5):e3000253.
26. Czyz-Szypenbejl K, Medrzycka-Dabrowska W, Kwiecien-Jagus K, Lewandowska K. The Occurrence of Postoperative Cognitive Dysfunction (POCD) - Systematic Review. *Psychiatr Pol*. 2019;53(1):145–60.

27. Ho YS, Zhao FY, Yeung WF, Wong GT, Zhang HQ, Chang RC: Application of Acupuncture to Attenuate Immune Responses and Oxidative Stress in Postoperative Cognitive Dysfunction: What Do We Know So Far? *Oxid Med Cell Longev* 2020, 2020:9641904.
28. Schenarts CL, Burton JH, Riker RR. Adrenocortical dysfunction following etomidate induction in emergency department patients. *Acad Emerg Med*. 2001;8(1):1–7.
29. Bekker AY, Weeks EJ. Cognitive function after anaesthesia in the elderly. 2003, 17(2):259–272.
30. Mason SE, Noel-Storr A, Ritchie CW. The Impact of General and Regional Anesthesia on the Incidence of Post-Operative Cognitive Dysfunction and Post-Operative Delirium: A Systematic Review with Meta-Analysis. *Journal of Alzheimers Disease* *Jad*. 2010;22(Suppl 3(3):67–79.
31. xing L: Clinical study of postoperative cognitive dysfunction. Shanghai Jiaotong University; 2009.
32. Chu JMT, Xiong W, Linghu KG, Liu Y, Zhang Y, Zhao GD, Irwin MG, Wong GTC, Yu H. *Siegesbeckia Orientalis* L. Extract Attenuates Postoperative Cognitive Dysfunction, Systemic Inflammation, and Neuroinflammation. *Exp Neurobiol*. 2018;27(6):564–73.
33. Podjaski C, Alvarez JI, Bourbonniere L, Larouche S, Terouz S, Bin JM, Lecuyer MA, Saint-Laurent O, Larochelle C, Darlington PJ, et al. Netrin 1 regulates blood-brain barrier function and neuroinflammation. *Brain*. 2015;138(Pt 6):1598–612.
34. Hoogland IC, Houbolt C, van Westerloo DJ, van Gool WA, van de Beek D. Systemic inflammation and microglial activation: systematic review of animal experiments. *J Neuroinflammation*. 2015;12:114.
35. Gyoneva S, Davalos D, Biswas D, Swanger SA, Garnier-Amblard E, Loth F, Akassoglou K, Traynelis SF. Systemic inflammation regulates microglial responses to tissue damage in vivo. *Glia*. 2014;62(8):1345–60.
36. Fakhoury M. Role of Immunity and Inflammation in the Pathophysiology of Neurodegenerative Diseases. *Neurodegener Dis*. 2015;15(2):63–9.
37. Chen J, Buchanan JB, Sparkman NL, Godbout JP, Freund GG, Johnson RW. Neuroinflammation and disruption in working memory in aged mice after acute stimulation of the peripheral innate immune system. *Brain Behav Immun*. 2008;22(3):301–11.
38. Keating GM. Dexmedetomidine: A Review of Its Use for Sedation in the Intensive Care Setting. *Drugs*. 2015;75(10):1119–30.
39. Daskalopoulos EP, Malliou F, Rentesi G, Marselos M, Lang MA, Konstandi M. Stress is a critical player in CYP3A, CYP2C, and CYP2D regulation: role of adrenergic receptor signaling pathways. *Am J Physiol Endocrinol Metab*. 2012;303(1):E40–54.
40. Hoffman WE, Kochs E, Werner C, Thomas C, Albrecht RF. Dexmedetomidine improves neurologic outcome from incomplete ischemia in the rat. Reversal by the alpha 2-adrenergic antagonist atipamezole. *Anesthesiology*. 1991;75(2):328–32.
41. Ma Y, Yu XY, Wang Y. Dose-related effects of dexmedetomidine on immunomodulation and mortality to septic shock in rats. *World J Emerg Med*. 2018;9(1):56–63.

42. Fang B, Li XQ, Bi B, Tan WF, Liu G, Zhang Y, Ma H. Dexmedetomidine attenuates blood-spinal cord barrier disruption induced by spinal cord ischemia reperfusion injury in rats. *Cell Physiol Biochem*. 2015;36(1):373–83.
43. Yang D, Hong JH. Dexmedetomidine Modulates Histamine-induced Ca(2+) Signaling and Pro-inflammatory Cytokine Expression. *Korean J Physiol Pharmacol*. 2015;19(5):413–20.
44. Chen N, Chen X, Xie J, Wu C, Qian J. Dexmedetomidine protects aged rats from postoperative cognitive dysfunction by alleviating hippocampal inflammation. *Mol Med Rep*. 2019;20(3):2119–26.
45. Small DH, Mok SS, Bornstein JC. Alzheimer's disease and Abeta toxicity: from top to bottom. *Nat Rev Neurosci*. 2001;2(8):595–8.
46. Zhang BJ, Yuan CX. Effects of ADAM2 silencing on isoflurane-induced cognitive dysfunction via the P13K/Akt signaling pathway in immature rats. *Biomed Pharmacother*. 2019;109:217–25.
47. Zhou S, Fang Z, Wang G, Wu S. Gap junctional intercellular communication dysfunction mediates the cognitive impairment induced by cerebral ischemia-reperfusion injury: PI3K/Akt pathway involved. *Am J Transl Res*. 2017;9(12):5442–51.
48. Su R, Sun P, Zhang D, Xiao W, Feng C, Zhong L. Neuroprotective effect of miR-410-3p against sevoflurane anesthesia-induced cognitive dysfunction in rats through PI3K/Akt signaling pathway via targeting C-X-C motif chemokine receptor 5. *Genes Genomics*. 2019;41(10):1223–31.

Figures

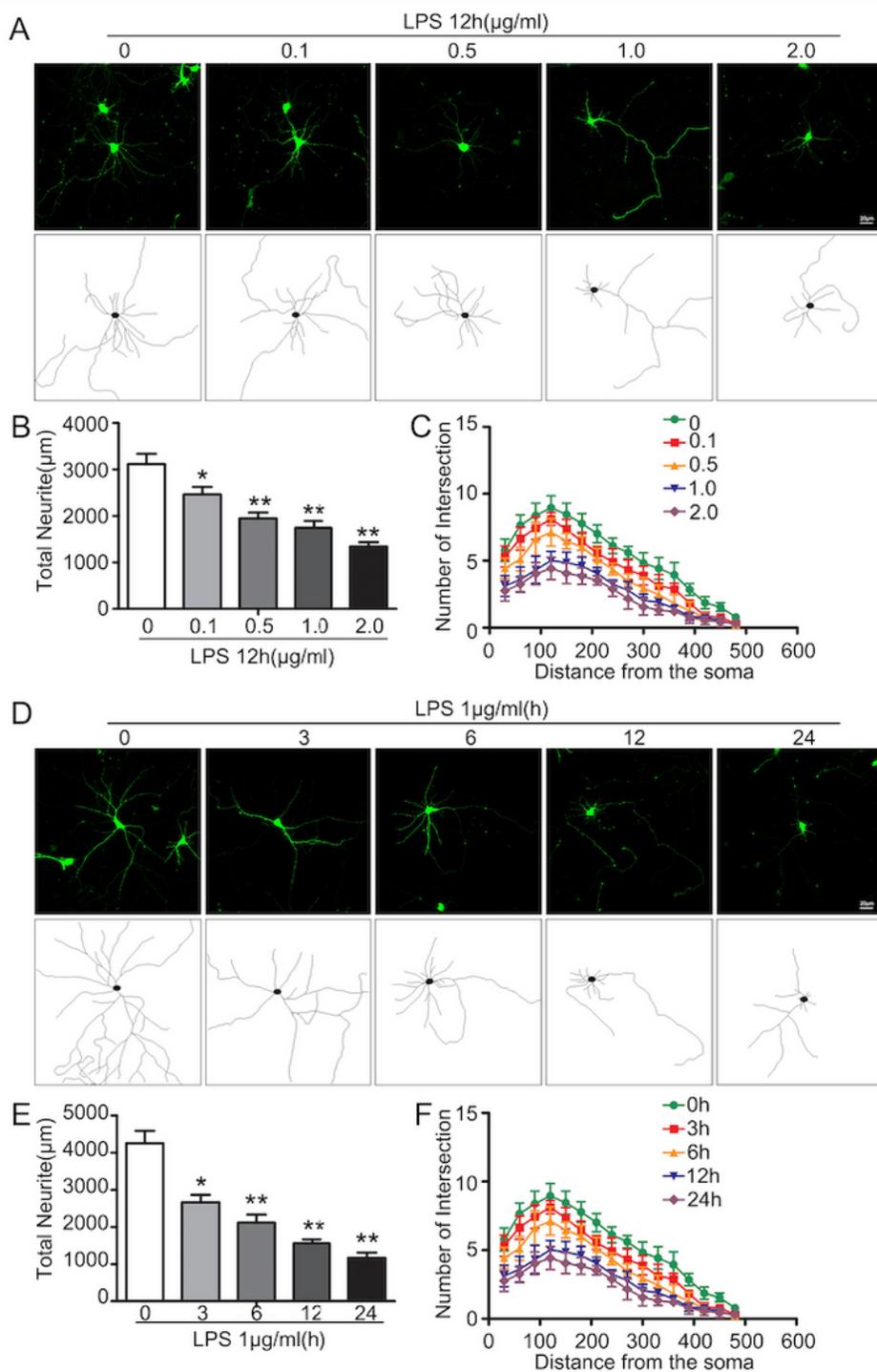


Figure 1

LPS treatment impairs hippocampal neurite development. Hippocampal neurons cultured in vitro were transfected with the GFP expression plasmid and treated with LPS at different concentrations for 12 hours. The growth of the protrusions was observed by GFP staining. (A) Typical neuron morphology; (B) Statistics of the total length of all protrusions in each treatment group; (C) Sholl analysis counts the number of branch points in different radius ranges of each group of neurons. Hippocampal neurons were

treated at different concentrations for 1 $\mu\text{g}/\text{mL}$, and the growth of each time-point was observed. (D) Typical cell morphology; (E) Full-length statistics of protrusions in each group; (F) Sholl analysis of branch points. * represents $p < 0.05$, ** represents $p < 0.01$. Scale bar, 20 μm .

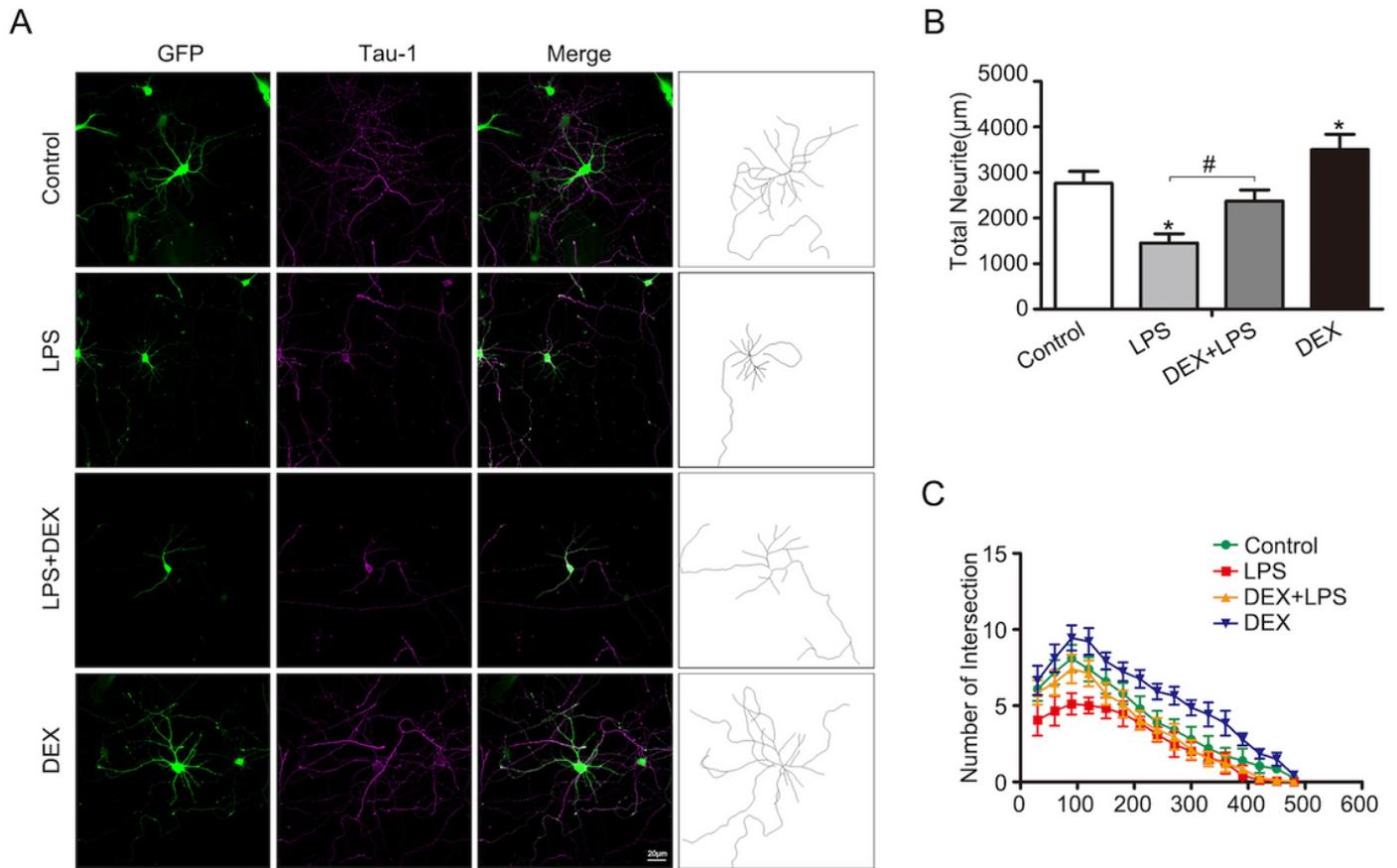


Figure 2

Dexmedetomidine (Dex) antagonizes LPS-induced neurite growth inhibition of hippocampal neurons. GFP-transfected neurons were treated with LPS, DEX, LPS + DEX, the growth of neurons was observed. (A) Typical neuron morphology; (B) The total length of all protrusions in each treatment group; (C) Sholl analysis and statistics on the number of branch points in different radius ranges of each group of neurons. * represents $p < 0.05$, compared to the control group. # represents $p < 0.05$, compared to LPS. Scale bar, 20 μm .

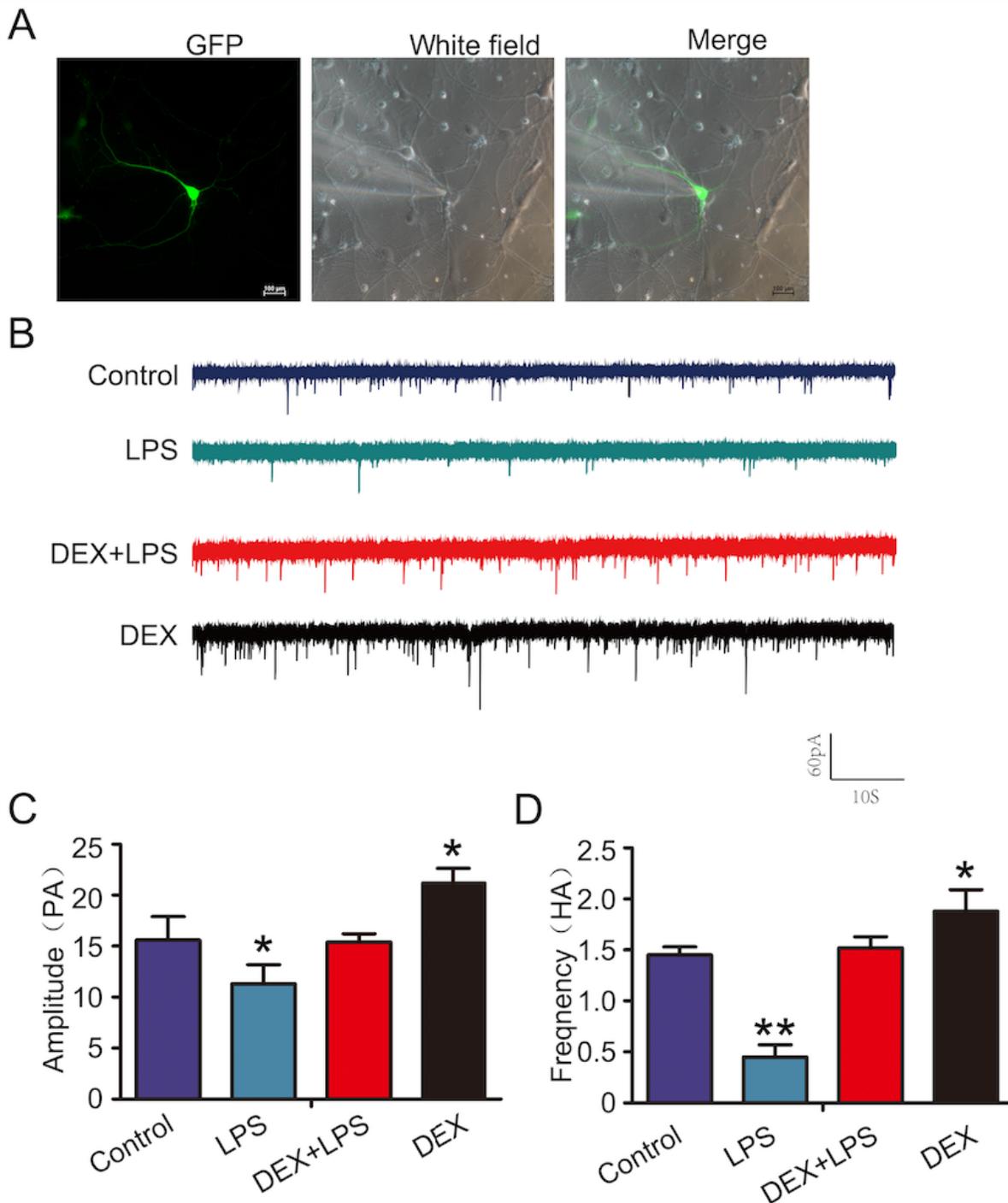


Figure 3

Dex antagonizes LPS-induced synaptic transmission disorder of hippocampal neurons. GFP transfected hippocampal neurons treated with LPS and DEX, cell patch clamp was used to detect their electrophysiology, and mEPSCs were observed to respond to synaptic transmission. (A) Electrophysiological performance of GFP transfected neurons; (B) Typical mEPSCs record under each

treatment; the amplitude (C) and frequency (D) of mEPSCs were counted. * represents $p < 0.05$, compared to the control group.

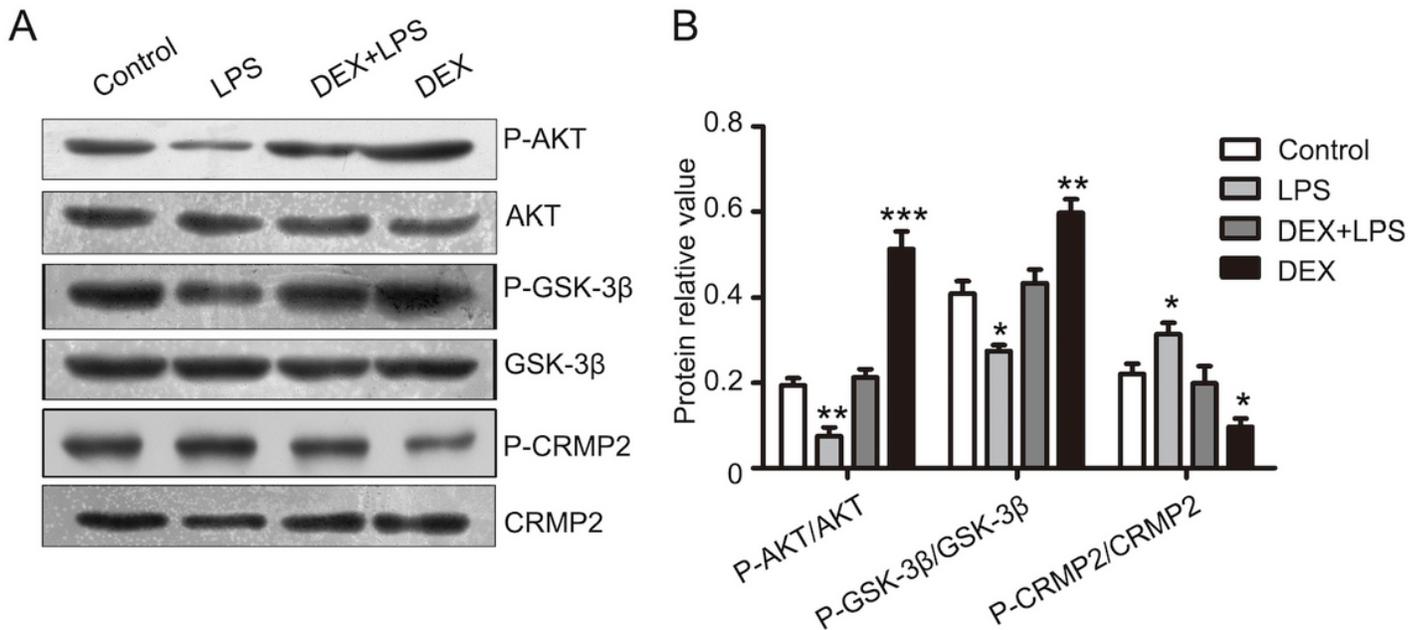


Figure 4

Dex reactivates the inhibited AKT/GSK-3β/CRMP-2 pathway by LPS. The hippocampal neurons were treated with LPS together with or without Dex. Cell lysates were subjected to western blotting and immune-stained with phosphorylated and total AKT, GSK-3β and CRMP2 antibodies to observe the activation of this pathway. The typical blots were shown in (A) and the gray intensity measurement was shown in (B). * represents $p < 0.05$, ** represents $p < 0.01$, and *** represents $p < 0.001$, compared to the control group.

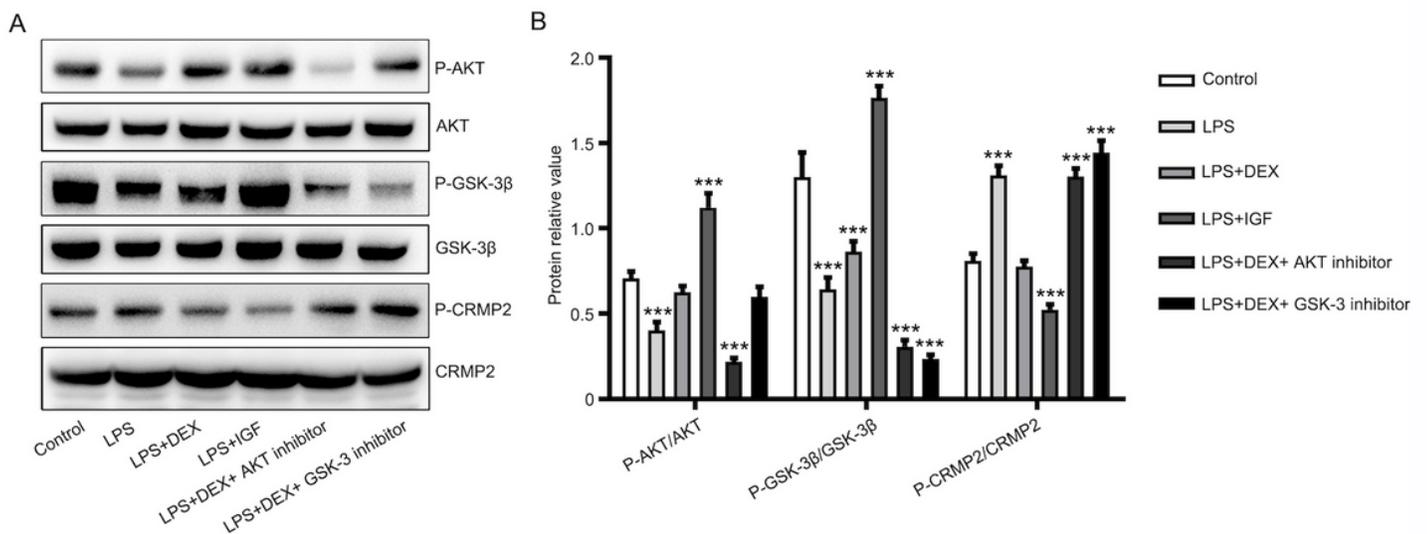


Figure 5

Pharmacological inhibition of AKT/GSK-3 pathway abolishes the antagonize effect of Dex against LPS. The hippocampal neurons were treated as indicated, with or without AKT inhibitor LY294002 or GSK-3 inhibitor SB216763. Then the cell lysates were subjected to western blotting as Figure 4. * represents $p < 0.05$, ** represents $p < 0.01$, and *** represents $p < 0.001$, compared to the control group.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [NC3RsARRIVEGuidelinesChecklistfilled.pdf](#)
- [originalblots.tif](#)