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# Optogenetic stimulation of basal forebrain cholinergic neurons prevents neuroinflammation and neuropsychiatric manifestations in pristane induced lupus mice

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## Abstract

**Background** Neuroinflammation has been identified as one of the primary pathogenic factors of neuropsychiatric systemic lupus erythematosus (NPSLE). However, there are no dedicated treatments available in clinics to alleviate neuroinflammation in NPSLE. It has been proposed that stimulating basal forebrain (BF) cholinergic neurons may provide potent anti-inflammatory effects in several inflammatory diseases, but its potential role in NPSLE remains unexplored. This study aims to investigate whether and how stimulating BF cholinergic neurons has a protective effect on NPSLE.

**Results** Optogenetic stimulation of BF cholinergic neurons significantly ameliorated olfactory dysfunction and anxiety- and depression-like phenotype in pristane induced lupus (PIL) mice. The increased expression of adhesion molecules (P-selectin and vascular cell adhesion molecule-1 (VCAM-1)), leukocyte recruitment, blood-brain barrier (BBB) leakage were significantly decreased. Notably, the brain histopathological changes, including the elevated levels of pro-inflammatory cytokines (TNF- $\alpha$ , IL-6 and IL-1 $\beta$ ), IgG deposition in the choroid plexus and lateral ventricle wall and lipofuscin accumulation in the cortical and hippocampal neurons, were also significantly attenuated. Furthermore, we confirmed the colocalization between the BF cholinergic projections and the cerebral vessels, and the expression of  $\alpha 7$ -nicotinic acetylcholine receptor ( $\alpha 7$ nAChR) on the cerebral vessels.

**Conclusion** Our data indicate that stimulation of BF cholinergic neurons could play a neuroprotective role in the brain through its cholinergic anti-inflammatory effects on cerebral vessels. Therefore, this may be a promising preventive target for NPSLE.

**Keywords** Basal forebrain, Cholinergic anti-inflammatory effect, Neuroinflammation, Neuropsychiatric lupus, Behavioral deficits, Optogenetics,  $\alpha 7$ nAChR

## Background

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by the loss of immune tolerance to self-antigens, resulting in inflammation and severe end-organ damage. As one of the most potentially fatal manifestations of SLE, neuropsychiatric SLE (NPSLE) is a series of neuropsychiatric symptoms, manifesting as anxiety, depression, sociability deficits, cognitive impairment and seizures. As for the

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pathogenesis of NPSLE, it is generally believed that numerous inflammatory mediators from the peripheral circulation can disrupt the blood-brain barrier (BBB) and promote an inflammatory process in the central nervous system (CNS) causing glial activation, neuronal dysfunction and behavioral deficits [1, 2]. Therefore, targeting neuroinflammation may be a viable approach for improving the outcomes of NPSLE.

To date, the existing clinical drugs, such as corticosteroids and biologics, have not been demonstrated to have specific therapeutic effects for NPSLE patients. Glucocorticoid therapy provides immunosuppression, but comes with several adverse side effects like hyperlipidemia, hypertension, accelerated circulatory system diseases, diabetes, heightened susceptibility to infection and osteoporosis [3]. Additionally, psychiatric disorders can also be a consequence of corticosteroid administration in SLE patients [4]. The biologics, which typically target a single cytokine, have not been shown effectively in clinical trials, possibly due to the involvement of multiple immune cells and cytokines in the pathogenesis of NPSLE [5]. Thus, exploring new intervention strategies is essential.

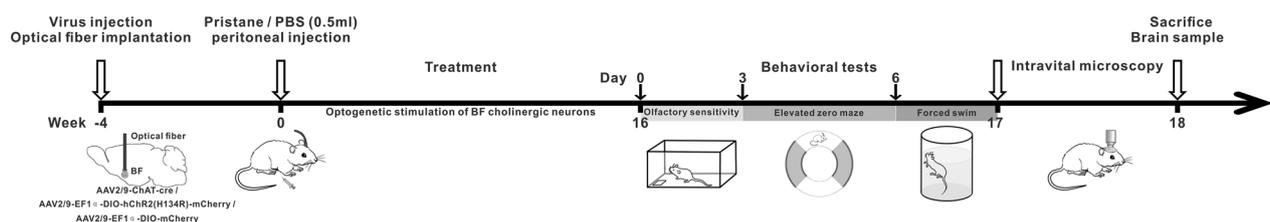
The cholinergic system mainly originates from groups of cholinergic neurons within the basal forebrain (BF), that constitutes a major neuromodulatory system [6, 7]. Studies have demonstrated the significance of cholinergic anti-inflammatory mechanism in the regulation of pro-inflammatory mediators in sepsis, rheumatoid arthritis, multiple sclerosis and SLE [6, 8–10]. It has been noted that the dysfunction of the cholinergic anti-inflammatory system exists in SLE [11] and stimulation of cholinergic signals ultimately results in a decreased release of pro-inflammatory cytokines, consequently controlling inflammation and protecting against tissue injury [10, 12]. Thus, promptly and precisely regulating BF cholinergic neurons may be pivotal to strategies for combating NPSLE.

The optogenetic technique, which controls neural activities through light stimulation, has been proven to be a valuable approach for investigating the mechanisms and therapeutic targets of neuropsychiatric diseases [13]. The pristane induced lupus (PIL) mouse model, despite its primary focus on replicating peripheral manifestations of SLE, has demonstrated some neurological and behavioral abnormalities resembling NPSLE [14]. Studies utilizing PIL mice have provided evidence of neuroinflammation in the CNS including overproduction of cytokines and chemokines, downregulation of hippocampal N-methyl-D-aspartate (NMDA) receptor subunits NR2A/2B, BBB leakage, immunoglobulin G (IgG) deposition, activation of glial cells (such as microglia and astrocytes) and lipofuscin accumulation within the brain [15–17]. Thus, the PIL mice are considered a valuable tool for studying NPSLE. Here, we aim to investigate whether optogenetically stimulating BF cholinergic neurons can potentially contribute to preventing neuroinflammation and subsequent brain pathology and behavioral deficits in PIL mice (Fig. 1). Our findings highlight that stimulation of BF cholinergic neurons exerts anti-inflammatory and neuroprotective effects on NPSLE, making it a potential intervention strategy for NPSLE in clinical settings.

## Results

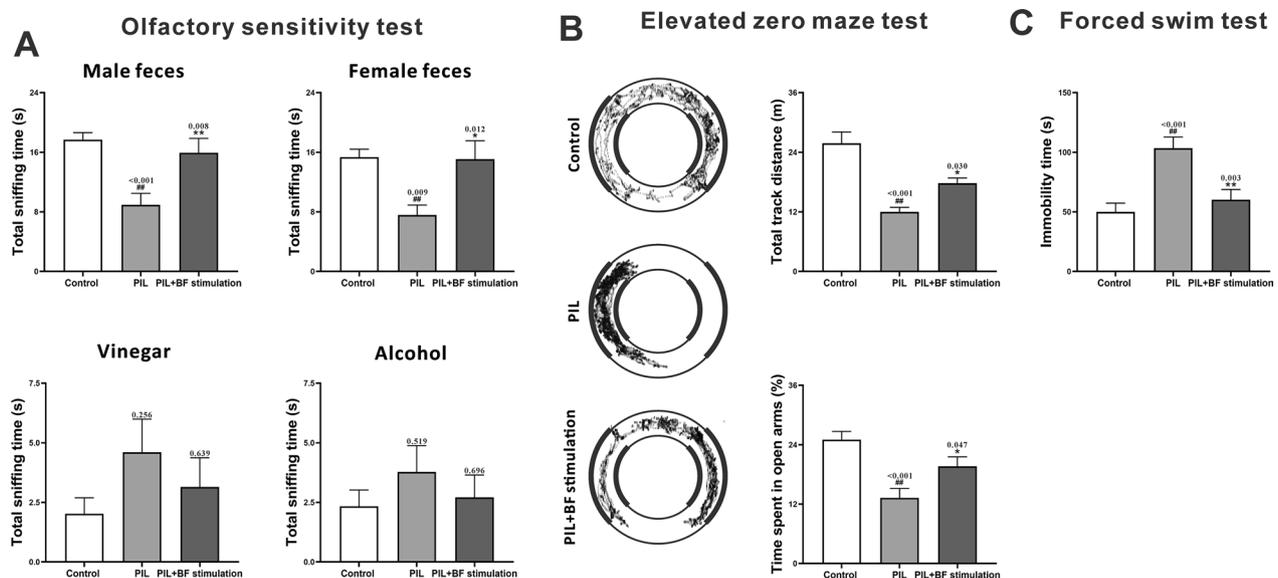
### Optogenetic stimulation of BF cholinergic neurons ameliorated olfactory dysfunction and anxiety- and depression-like behaviors in PIL mice

To test the effect of optogenetic stimulation of BF cholinergic neurons on behavioral deficits, a series of behavioral tests were conducted in all tested groups. As shown in Fig. 2A, PIL mice spent less time sniffing male and female fecal odors (preferred odors) than controls in the olfactory sensitivity test. Following optogenetic stimulation of BF cholinergic neurons, the exploration time of the preferred odors in PIL mice improved significantly (Fig. 2A). Although not statistically significant, PIL mice spent a longer time sniffing vinegar and alcohol (aversive



**Fig. 1** Experimental schedule

Mice received microinjection of viruses and surgical implantation of optical fibers in the BF. Pristane or PBS was administered to mice via intraperitoneal injection. Optogenetic stimulation of BF cholinergic neurons was applied to mice for 4 months. Following a series of behavioral tests and intravital microscopy, mice were sacrificed and brain tissues were collected for further assays



**Fig. 2** Effects of optogenetic stimulation of BF cholinergic neurons on behavioral deficits in PIL mice **A** Olfactory sensitivity test. Quantitative analysis of total time spent sniffing male feces, female feces, vinegar or alcohol. **B** Elevated zero maze test. Left panel showing representative track plots of the path. Right panel showing quantitative analysis of total track distance and percentage of time spent in the open arms (%). **C** Forced swim test. Quantitative analysis of immobility time. The data are expressed as the mean  $\pm$  SEM ( $n = 12$  in each group). One-way ANOVA followed by Tukey's post hoc test: <sup>#</sup>  $p < 0.01$  compared to the control group, <sup>\*</sup>  $p < 0.05$  or <sup>\*\*</sup>  $p < 0.01$  compared to the PIL group

odors) compared to controls, and stimulation of BF showed a decreasing trend in the time spent on the aversive odors (Fig. 2A). The elevated zero maze test showed that PIL mice traversed significantly shorter total track distances and spent lower percentages of time in open arms than control mice, and stimulation of BF markedly reversed the anxiety-like behavior (Fig. 2B). Additionally, PIL mice exhibited longer immobility time than controls in the forced swim test, and stimulation of BF showed a remarkable reduction in immobility time (Fig. 2C). Collectively, these results indicate that stimulation of BF cholinergic neurons alleviated behavioral deficits in PIL mice, including olfactory dysfunction and anxiety- and depression-like behaviors.

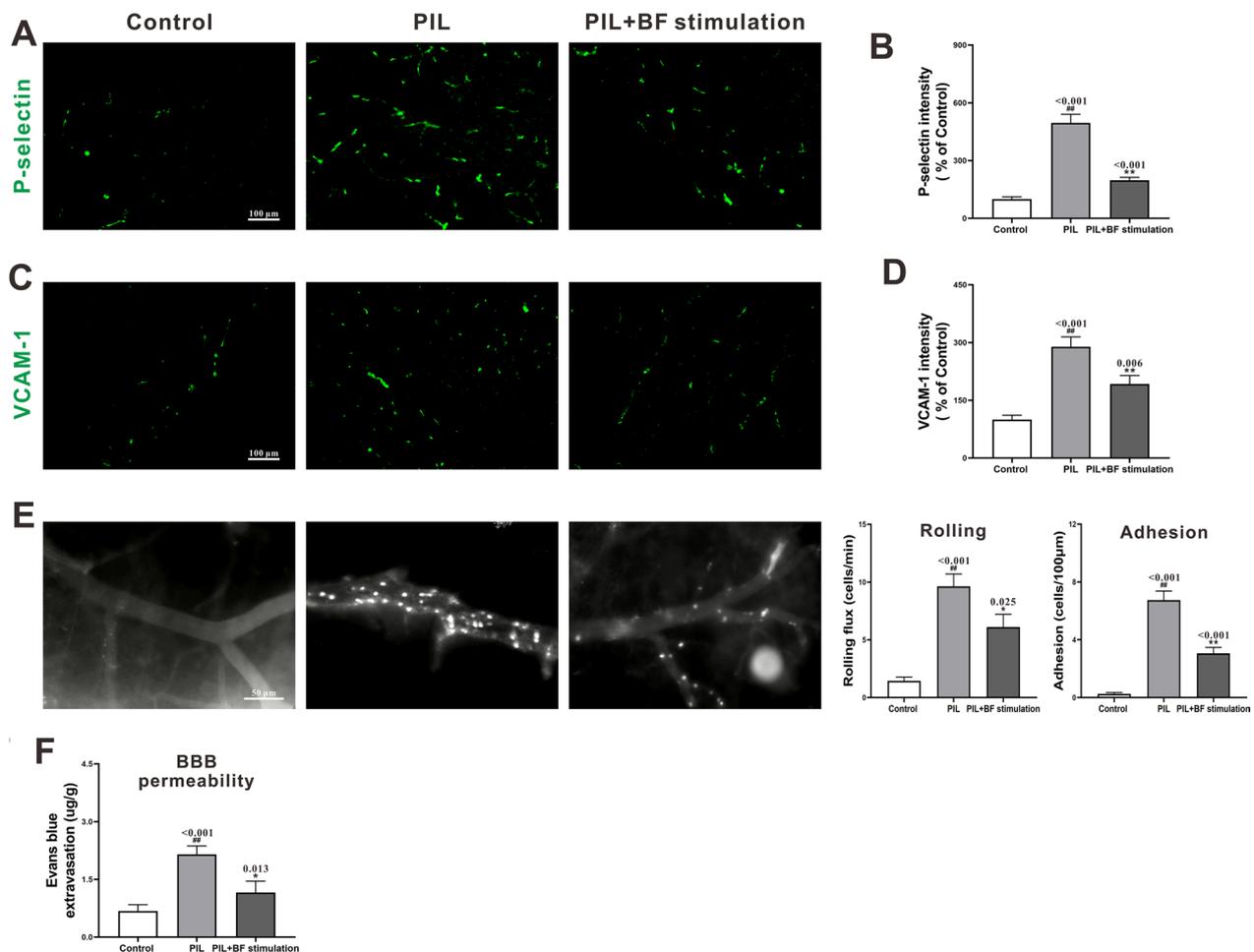
#### Optogenetic stimulation of BF cholinergic neurons attenuated endothelium activation, leukocyte recruitment and BBB leakage in PIL mice

Cerebral endothelium activation, characterized by increased adhesion molecule expression, is essential for circulating leukocyte recruitment and BBB impairment during neuroinflammation. To test the effect of optogenetic stimulation of BF cholinergic neurons on endothelium activation, we assessed P-selectin and vascular cell adhesion molecule-1 (VCAM-1) expression by immunofluorescence staining. As illustrated in Fig. 3A–D, compared to control mice with little expression of P-selectin and VCAM-1 in the cerebral vessels,

PIL mice presented a significant elevation of these markers. Optogenetic stimulation of BF cholinergic neurons markedly decreased the elevated expression of P-selectin and VCAM-1 (Fig. 3A–D). We then utilized intravital microscopy to directly visualize leukocyte-endothelial cell interaction in cerebral vessels. Few rolling and adherent leukocytes were detected in control mice, while PIL mice had a considerable number of such cells (Fig. 3E). Stimulation of BF showed a significant reduction in leukocyte rolling and adhesion, which was concurrent with the alteration in adhesion molecule expression (Fig. 3A–E). Furthermore, significant BBB leakage was observed, as indicated by an increase in Evans blue content in brain tissues of PIL mice, but not in controls (Fig. 3F). After stimulation of BF, the extravasation of Evans blue decreased markedly (Fig. 3F).

#### Optogenetic stimulation of BF cholinergic neurons decreased cytokine expression, IgG deposition and lipofuscin accumulation in the brain of PIL mice

To evaluate the effect of optogenetic stimulation of BF cholinergic neurons on brain pathophysiological changes, we examined cytokine expression, IgG deposition and lipofuscin accumulation in the brain by ELISA and immunofluorescence staining. Consistent with our previous results [15], PIL mice presented significantly increased expression of brain cytokines, including tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-6 (IL-6),

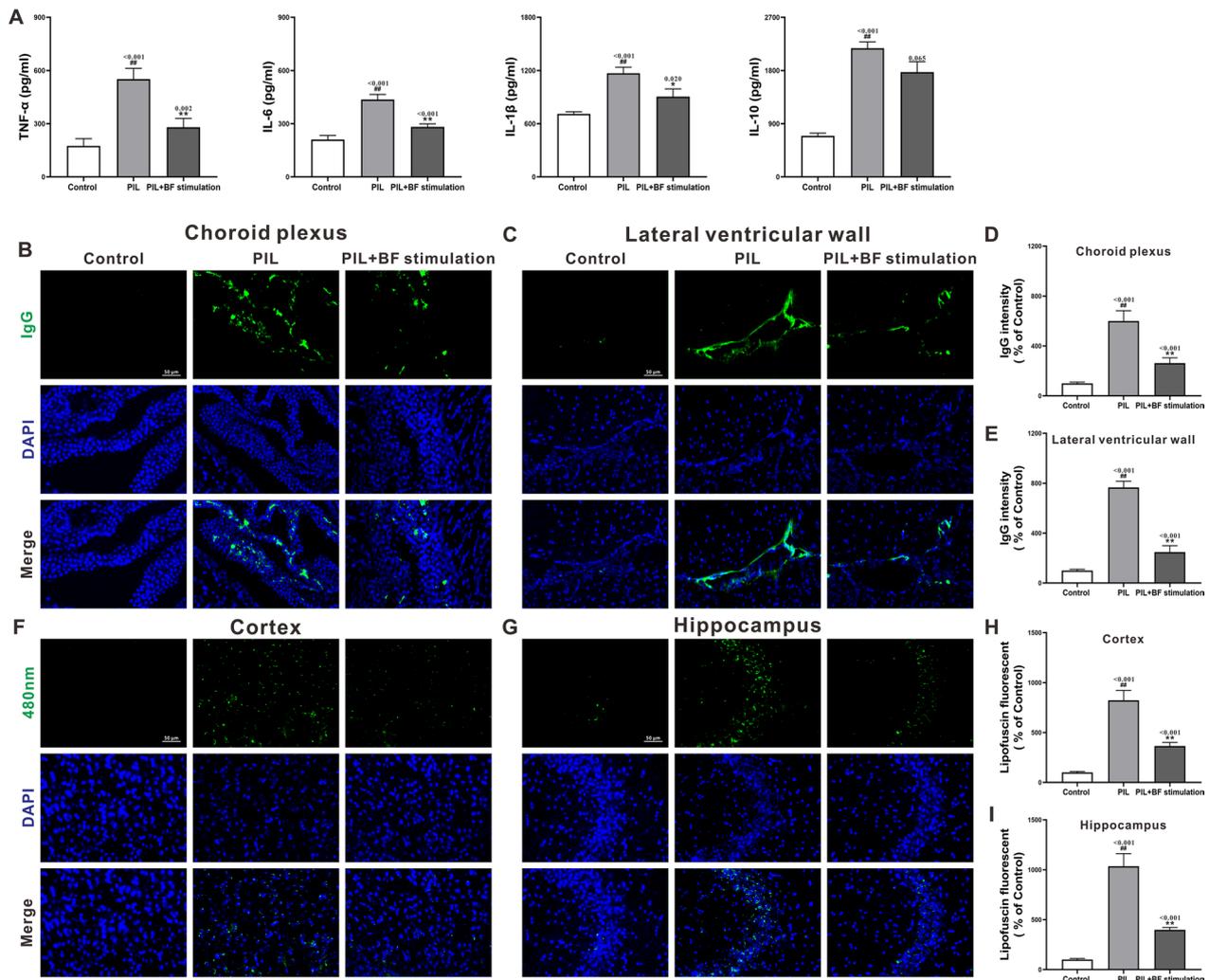


**Fig. 3** Effects of optogenetic stimulation of BF cholinergic neurons on endothelium activation, leukocyte recruitment and BBB leakage in PIL mice (A) and (C) Representative images of P-selectin and VCAM-1 expression. (B) and (D) Quantitative analysis of P-selectin and VCAM-1 expression by MFI and normalized to the control group. (E) Representative images and quantitative analysis of leukocyte rolling and adhesion. (F) Quantitative analysis of Evans blue dye extravasation. The data are expressed as the mean  $\pm$  SEM ( $n = 12$  in each group). One-way ANOVA followed by Tukey's post hoc test:  $\# \# p < 0.01$  compared to the control group,  $* p < 0.05$  or  $** p < 0.01$  compared to the PIL group

IL-1 $\beta$  and IL-10 (Fig. 4A). Optogenetic stimulation of BF cholinergic neurons markedly suppressed the elevated expression of TNF- $\alpha$ , IL-6 and IL-1 $\beta$ , but not IL-10 (Fig. 4A). Through immunofluorescence staining, we observed a pronounced increase in IgG deposition in the choroid plexus and lateral ventricular wall of PIL mice compared to controls, and stimulation of BF dramatically rescued the heightened IgG deposition (Fig. 4B–E). Additionally, there was a notable rise in lipofuscin accumulation in the cortex and hippocampus of PIL mice compared to controls (Fig. 4F–I). Following stimulation of BF, lipofuscin accumulation decreased drastically (Fig. 4F–I). Thus, these results indicate that optogenetic stimulation of BF cholinergic neurons significantly alleviated inflammatory responses and tissue injuries in the brain of PIL mice.

### BF cholinergic projections colocalized with cerebral vessels and cerebral vessels expressed $\alpha 7$ -nicotinic acetylcholine receptor ( $\alpha 7$ nAChR)

To clarify the functional connection between BF cholinergic neurons and central inflammatory responses, central cholinergic circuits were investigated by immunofluorescence staining. Consistent with previous reports [18], we confirmed that some cholinergic projections colocalized with cerebral vessels, indicating that BF cholinergic neurons could modulate the function of cerebral vessels via cholinergic projections (Fig. 5A). Since  $\alpha 7$ nAChR has been reported to mediate the anti-inflammatory effects of cholinergic stimulation both in vitro and vivo [19], we evaluated whether  $\alpha 7$ nAChR was expressed on the cerebral vessels. Using immunofluorescence staining, we verified the expression of  $\alpha 7$ nAChR on the vessels in the cerebral cortex (Fig. 5B). No statistically



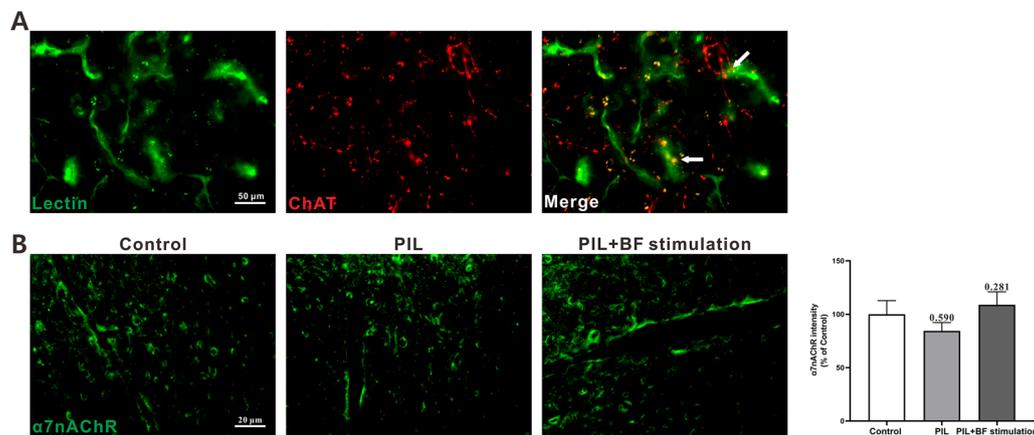
**Fig. 4** Effects of optogenetic stimulation of BF cholinergic neurons on cytokine expression, IgG deposition and lipofuscin accumulation in the brain of PIL mice **A** Quantitative analysis of the levels of brain cytokines (TNF- $\alpha$ , IL-6, IL-1 $\beta$  and IL-10). **B** and **C** Representative images of IgG deposition in the choroid plexus and lateral ventricular wall by MFI and normalized to the control group. **D** and **E** Quantitative analysis of IgG deposition in the choroid plexus and lateral ventricular wall by MFI and normalized to the control group. **F** and **G** Representative images of autofluorescent lipofuscin at 480 nm exciting light (green) in the cortex and hippocampus. DAPI staining for nuclei (blue). **H** and **I** Quantification analysis of lipofuscin foci in the cortex and hippocampus and normalized to the control group. The data are expressed as the mean  $\pm$  SEM ( $n = 12$  in each group). One-way ANOVA followed by Tukey's post hoc test: <sup>##</sup>  $p < 0.01$  compared to the control group, <sup>\*</sup>  $p < 0.05$  or <sup>\*\*</sup>  $p < 0.01$  compared to the PIL group

significant differences were found in the expression of  $\alpha 7nAChR$  among all sampled groups (Fig. 5B). These results indicate that BF cholinergic neurons project to the cerebral vessels, which could provide a neuroanatomical basis for the anti-inflammatory effect of cholinergic signals on the cerebral vessels via  $\alpha 7nAChR$ .

### Discussion

The optogenetic technique utilizes light to control the activity of neurons which have been modified to express light-sensitive proteins, thus avoiding the nonspecific effects of electrical stimulation or pharmacologic

manipulation [20]. Even without direct clinical use of optogenetics, optical control of neurons in animal models of human diseases enables a far more precise exploration of the pathogenesis and helps to address some clinically relevant problems. For example, first, via optogenetic intervention in mouse models of epilepsy, granule cells in the brain's dentate gyrus were found to be implicated in the induction of seizures [21], and manipulation of neurons in regions distal to the seizure focus could serve as seizure-propagation "chokepoints" [22]. Second, optogenetic stimulation of cortical interneurons induced gamma oscillations, which are critical in attention and



**Fig. 5** Examination for the relationship between BF cholinergic projection and cerebral vessels, and  $\alpha 7nAChR$  expression on cerebral vessels **A** Representative images of BF cholinergic projection (red) and lectin immunoreactive cerebral vessels (green). White arrow showing the co-localization between cholinergic projection (red) and cerebral vessels (green). **B** Representative images and quantitative analysis of  $\alpha 7nAChR$  expression in the cerebral cortex and normalized to the control group. The data are expressed as the mean  $\pm$  SEM ( $n=12$  in each group). One-way ANOVA followed by Tukey's post hoc test

focusing, may provide the foundation for treatments of attention deficit hyperactivity disorder [23]. Third, optogenetic manipulation of phasic, but not tonic, firing in ventral tegmental area dopamine neurons of mice rapidly induced a depressive phenotype as measured by social avoidance and decreased sucrose preference [24]. Thus, optogenetic stimulation of BF cholinergic neurons in this study has potential clinical implications through revealing the pathogenesis of NPSLE and providing a new preventive target for NPSLE.

Interactions between the nervous and immune systems are central to the field of neuroimmunology. A major catalyst for growth in this field was the discovery that cholinergic signaling regulates immune functions and inflammatory responses [25]. The cholinergic system, through releasing acetylcholine (ACh), dominates systemic inflammation and inhibits pro-inflammatory cytokines production, depending on the  $\alpha 7nAChR$  [6]. The cholinergic anti-inflammatory mechanism is also under intensive investigations for autoimmune arthritis and SLE. Administration of  $\alpha 7nAChR$  agonists reduced clinical and pathologic signs of collagen-induced arthritis, and lowered serum levels of pro-inflammatory cytokines (i.e., TNF- $\alpha$  and IL-6) [26]. The severity of arthritis was exacerbated by deletion of  $\alpha 7nAChR$  [27]. Activation of the cholinergic anti-inflammatory system, either pharmacologically at the level of cholinergic receptors or upstream at the level of the vagus nerve, can protect the kidney by dampening inflammation and prevent the progression of hypertension in the setting of SLE [10–12, 28]. Additionally, recent findings have also characterized a role for central cholinergic signaling in controlling the immune responses and inflammation in

the CNS [7]. The central cholinergic signaling mainly originates from the cholinergic neurons within the BF and that densely innervate the majority of cerebral vessels [29]. By immunofluorescence staining, we confirmed that the cholinergic projections from BF cholinergic neurons co-localized with the cerebral vessels (immunoreactive for lectin), providing the neuroanatomical basis for the regulatory role of cholinergic signals on the cerebral vessels (Fig. 5A). This result is consistent with previous evidence showing the expression of  $\alpha 7nAChR$  by vascular endothelial cells [30–33]. Several *in vitro* studies reported that ACh and cholinergic agonists exert direct inhibitory effects on TNF- $\alpha$ -induced endothelial cell activation by blocking extracellular signal regulated kinase 1/2 (ERK1/2) and c-Jun N-terminal kinase (JNK) or nuclear factor- $\kappa B$  (NF- $\kappa B$ ) signaling pathways via  $\alpha 7nAChR$  [19, 34]. In this report, we also confirmed the expression of  $\alpha 7nAChR$  on the cerebral vessels (Fig. 5B). Thus, optogenetic stimulation of BF cholinergic neurons may exert the anti-inflammatory effects on the cerebral vessels via  $\alpha 7nAChR$ .

The endothelium of cerebral vessels plays a pivotal role in maintaining BBB integrity and brain homeostasis. Exposure of endothelial cells to inflammatory stimuli activates intracellular signaling pathways resulting in the formation of multiple adhesion molecules, such as P-selectin and VCAM-1, and additional pro-inflammatory mediators [35]. These adhesion molecules facilitate the recruitment of circulating leukocytes and eventually lead to BBB leakage [30]. In this study, we found that optogenetic stimulation of BF cholinergic neurons decreased P-selectin and VCAM-1 elevation, leukocyte rolling and adhesion, and BBB leakage in PIL

mice (Fig. 3). These ameliorations may be attributable to the anti-inflammatory effect of the cholinergic signals which inhibits the activation of endothelial cells, and subsequently decreases leukocyte recruitment and BBB impairment.

Another important finding of our study was the downregulated expression of elevated brain cytokines, decreased IgG deposition in the choroid plexus and lateral ventricle wall, and reduced lipofuscin deposits within neurons in the cortex and hippocampus in PIL mice (Fig. 4). The reduction of cytokines may be related to the lessened cytokine entries from peripheral circulation due to the improved BBB leakage, and the reduced local cytokine production following a decreased invasion of inflammatory stimulators. IgG deposition has been observed in the hippocampus of PIL mice [17], which may result from the impairments of BBB, blood-ventricular barrier and choroid plexus-vascular barrier [15]. Thus, the reduction of IgG deposition in the choroid plexus and lateral ventricular wall may be attributed to the improved vascular barrier function by BF stimulation. Lipofuscin, a product of metabolic imbalance of lipids and metals generally caused by neural oxidative stress, increases with age and pathological processes in CNS [36]. It has been suggested that lipofuscin participates in the pathogenesis of NPSLE in lupus-prone mice with depression [15, 37]. The decrease of lipofuscin deposits may be related to the downregulated expression of brain cytokines, which can induce a reduction in the synthesis of oxidative stress products within neurons. Stimulation of BF may decrease the cytokine-induced synthesis of oxidative stress products, consequently attenuating the generation and accumulation of lipofuscin.

Olfactory dysfunction, characterized by a decreased response to an olfactory stimulus, has been found in animal models of NPSLE such as anti-ribosomal P-injected mice, MRL/lpr mice and PIL mice [15, 38, 39]. Human patients with NPSLE were also reported to have a higher probability of olfactory abnormalities than healthy controls, associated with disease activity, age and positivity for anti-ribosomal P protein antibody [40]. The BF cholinergic system has been demonstrated to innervate the olfactory bulb and play a pivotal role in olfaction-mediated behaviors [41]. Stimulation of cholinergic system can elicit a wide-ranging enhancement of neural responsiveness to odorants and enhance animal performance in odor discrimination [42]. The strong modulation of the central cholinergic system on olfactory function may explain our present results showing that optogenetic stimulation of BF cholinergic neurons could ameliorate olfactory dysfunction in PIL mice (Fig. 2A). Affective deficits such as depression and anxiety are among the most common neuropsychiatric disturbances impacting

quality of life within the NPSLE population. Our previous research has confirmed that PIL mice display anxiety- and depression-like phenotype [15]. Here, for the first time, we found that stimulation of BF could effectively rescue anxiety- and depression-like behaviors in PIL mice (Fig. 2B–C). Several studies have suggested the involvement of the central cholinergic system in anxiety- and depressive-like behaviors [43]. First, lesions of BF cholinergic neurons resulted in anxiety- and depressive-like behaviors [44]. Second, increasing the level of ACh in the brain could reduce anxiety [45]. Third, administering an acetylcholinesterase (the enzyme that degrades ACh) inhibitor into the brain could improve the anxiety-like behavior, as indicated by increased open arm exploration in the elevated plus maze test [46]. Notably, a recent study revealed that stimulation of cholinergic signals by systemic administration of  $\alpha 7nAChR$  ligands did not improve behavioral deficits in mice with advanced SLE [5]. This difference may be related to the different routes of treatment (direct stimulation of the CNS versus systemic drug administration).

In our study, the application of optogenetics in animal research effectively demonstrated that stimulating BF cholinergic neurons produces an anti-inflammatory effect within the CNS. However, a major obstacle to the application of this technology in humans is the concern of biological safety, specifically associated with the expression of artificially manipulated photosensitive opsins within neurons [47]. Therefore, addressing the priority of developing opsins that are safe, effective and compatible with human neural circuits is crucial. Nevertheless, a promising alternative approach is deep brain stimulation (DBS), which has been extensively explored in clinical treatments for neurodegenerative disorders such as Parkinson's disease (PD) and Alzheimer's disease (AD) with promising therapeutic outcomes [48, 49]. Currently, stimulating the BF region through DBS is an alternative approach for treating neuroinflammatory diseases in humans. In the future, the feasibility of optogenetic stimulation of BF in humans will increase with advancements in the development of safe photosensitive opsins.

## Conclusions

In summary, we present the first evidence showing that activation of BF cholinergic neurons may play a neuroprotective role in the brain through its anti-inflammatory effects on cerebral vessels. Our results highlight the significance of manipulating central cholinergic signals in attenuating behavioral deficits and reversing neuroinflammation in NPSLE, which may therefore be a potential preventive target for NPSLE. Further systemic experiments are warranted to examine whether and how

stimulating BF cholinergic neurons can rescue the behavioral deficits and neural damage in NPSLE patients.

## Methods

### Animals

Specific pathogen-free BALB/c mice were purchased from Vital River Laboratory (Beijing, CHN). Female mice were used for the experiment at the age of 8 weeks. Animals were reared in standard animal cages under environmentally controlled laboratory conditions (12/12 h light/dark cycle,  $22 \pm 2$  °C, 40–80% humidity) with *ad libitum* access to food and water. All efforts were made to minimize animal suffering. The animals were maintained and treated in compliance with the policies and procedures detailed in the “*Guide for the Care and Use of Laboratory Animals*” of the National Institutes of Health. The Animal Care and Use Committee of China Medical University reviewed and approved the animal experimental protocols and the treatment procedures (No. KT2018060).

### Experiment procedure

As shown in Fig. 1, mice were randomly divided into three groups ( $n = 12$  per group): control, PIL and PIL + BF stimulation. At the initiation of the experiment, all mice were administered AAV2/9-ChAT-cre. Mice in the control and PIL groups were then given AAV2/9-EF1 $\alpha$ -DIO-mCherry, while those in the PIL + BF stimulation group received AAV2/9-EF1 $\alpha$ -DIO-hChR2(H134R)-mCherry. After recovery from microinjection of viruses and surgical implantation of optical fibers in right BF, each mouse received a single intraperitoneal injection of 0.5 ml phosphate buffer saline (PBS) or pristane (Sigma-Aldrich, St. Louis, MO, USA), respectively. Optogenetic stimulation of BF cholinergic neurons was administered to each mouse for 4 months following pristane or PBS injection. After behavioral tests and intravital microscopy recording, mice were sacrificed and brain tissues were harvested for further examinations.

### Stereotactic viral microinjection and optical fiber implantation to BF

Viruses (AAV2/9-ChAT-cre, AAV2/9-EF1 $\alpha$ -DIO-hChR2 (H134R)-mCherry and AAV2/9-EF1 $\alpha$ -DIO-mCherry) used in this study were obtained from Brain VTA (Wuhan, CHN). Cre-mediated recombination and channelrhodopsin2 (ChR2) expression were confined to choline acetyl transferase (ChAT) immunoreactive neurons located within the BF cholinergic neurons. The BF cholinergic neurons and their projections can be visualized by the fluorescence of mCherry. The activation of BF cholinergic neurons was selectively controlled by ChR2 with the presence of blue light stimulation. The

surgical procedures of viral microinjection were as previously described [50]. Briefly, mice were anaesthetized and mounted onto a stereotaxic apparatus to adjust their skulls parallel to the reference panel. Then, AAV viruses were slowly injected into the right BF (anteroposterior, 0.1 mm; mediolateral, 1.5 mm; dorsoventral, 4.5 mm). An optical fiber was implanted directly above the viral injection site.

### Optogenetic stimulation

The optical fiber was coupled to a diode-pumped solid-state 473 nm laser and controlled by a laser driver. Light pulse trains (30 ms per pulse at 20 Hz for 15 s once per minute for 30 min) were controlled by a Master-8 pulse stimulator (Newdoon Inc., Hangzhou, CHN). Optogenetic stimulation of BF cholinergic neurons was conducted daily throughout the 4-month treatment period.

### Olfactory sensitivity test

The test used to assess olfactory sensitivity was conducted as previously described [51]. Each mouse was exposed to a piece of filter paper scented with an odorant for 2 min. Then, the scented filter paper was removed and the mouse was allowed to rest for 1 min. This procedure was repeated three times. Active investigation was defined as directed sniffing within 0.5 cm of the odorant source and the sniffing time was recorded. The total sniffing time of an odorant was obtained by summing the sniffing time for each trail.

### Elevated zero maze test

The elevated zero maze test was conducted as previously described [52]. Briefly, each mouse was placed at the open arm and allowed to conduct a 10-min free exploration. Total track distance and time spent in the open arms were digitally recorded, and then analyzed by custom-built programs. The percentage of time spent in the open arms was calculated using the following formula: [(time spent in the open arms)/(time spent in all arms)  $\times 100$ ].

### Forced swim test

Each mouse was placed into a glass beaker containing 3,000 ml of water ( $24 \pm 1$  °C) and allowed to habituate to swimming in it for 2 min. A 4-min test session was digitally recorded afterwards. With no way to escape, the mice began to struggle and swim, eventually displaying signs of behavioral despair, assessed as immobility [53]. Depression-like behavior was measured according to time spent immobile.

### Intravital microscopy in mouse brain

Intravital microscopy of the mouse cerebral vessels was performed as previously described [54]. Mice were

mounted onto the stereotaxic apparatus and the optical fiber was carefully removed from the right skull. To create an optical clearing skull window for in vivo imaging, the left skull was thinned and treated with 10% Ethylene Diamine Tetraacetic Acid (EDTA) disodium (Sigma-Aldrich) for 5–10 min. Leukocytes were fluorescently labeled with rhodamine 6G (0.5 mg/kg body weight, Sigma-Aldrich). Rolling leukocytes were defined as leukocytes moving at a lower speed than erythrocytes. Adherent leukocytes were defined as cells that remained stationary for at least 30 s.

#### Measurement of BBB permeability

The assessment of BBB permeability was performed by measuring Evans blue extravasation, as described previously [55]. Briefly, mice were administered 2% Evans blue dye solution (4 ml/kg, Beyotime biotechnology, Shanghai, CHN) intravenously 30 min before sacrifice. After the whole brains were harvested, brain tissues used for BBB permeability assessment were homogenized and incubated in formamide (24 h, 55 °C). The quantification of BBB permeability was performed by measuring the absorbance at 620 nm in the supernatant from each sample.

#### ELISA for brain cytokine detection

Brain tissues, used for cytokine detection, were homogenized on ice in 0.01 M PBS (pH 7.4) with a protease inhibitor cocktail (diluted 1:100 (v/v), MedChemExpress, Shanghai, CHN), using a FastPrep-96 high-throughput homogenizer (MP Biomedicals, CA, USA) at 281.7 ×g for 45 s (2 cycles). After homogenization, each sample was visually examined to ensure thorough sample disruption. Then, the samples were centrifuged at 12,000 rpm for 15 min at 4 °C to remove cell debris. The supernatants were collected and stored at – 80 °C until the measurement. The protein concentration was determined using a BCA protein concentration assay kit (Beyotime biotechnology). The cytokine levels in brain tissues were examined using TNF- $\alpha$ , IL-6, IL-1 $\beta$  and IL-10 ELISA kits (Boster & Biological Technology, Wuhan, CHN). Experimental procedures were strictly followed according to the manufacturer's instructions. Briefly, the protein samples were added to a 96-well microplate coated with antibodies against mouse TNF- $\alpha$ , IL-6, IL-1 $\beta$ , or IL-10. After 2 h incubation at room temperature, the microplate was incubated with horseradish peroxidase-conjugated streptavidin for an additional 1 h. The plates were then developed with tetramethylbenzidine substrate solution, and the optical densities were measured at 450 nm. Mouse cytokine standards with known concentrations were used to establish standard curves, and cytokine levels were expressed as relative titers.

#### Immunofluorescence staining

Brain tissues used for immunofluorescence staining were fixed in paraformaldehyde, followed by immersion in sucrose solution. The tissues were cut into 10  $\mu$ m-thick frozen sections and blocked with 10% goat serum. Then, sections were incubated with primary antibodies, including anti-P-selectin (1:80; Santa Cruz, CA, USA), VCAM-1 (1:200; Abcam, Cambridge, UK) and  $\alpha$ 7nAChR (1:100; ABclonal, Wuhan, CHN). On the following day, the primary antibodies were removed and sections were incubated with secondary antibody Alexa Fluor 488-conjugated goat anti-rabbit IgG (1:200, Proteintech, Wuhan, CHN). For IgG and lectin staining, sections were incubated with Alexa Fluor 488-conjugated goat anti-mouse IgG (1:200, Proteintech) and lectin solution (Thermo Fisher, Massachusetts, USA). DAPI staining (Beyotime biotechnology) was used to locate cell nuclei. To examine autofluorescent lipofuscin, regions of interest were captured at 480 nm exciting light. The mean fluorescence intensity of P-selectin, VCAM-1 and IgG and the mean gray value of autofluorescent lipofuscin were calculated using Image J software.

#### Statistical analysis

GraphPad Prism V8 software (La Jolla, CA, USA) was used for statistical analysis. Before applying parametric statistics, all data were assessed for normality using the *D'Agostino-Pearson omnibus normality* test. Data were expressed as the mean  $\pm$  SEM. Differences in this study were analyzed using one-way analysis of variance (ANOVA) followed by *Tukey's post hoc* test for multiple comparison.  $P < 0.05$  was considered a statistically significant difference in all tested groups.

#### Abbreviations

NPSLE	Neuropsychiatric systemic lupus erythematosus
BF	Basal forebrain
PIL	Pristane induced lupus
VCAM-1	Vascular cell adhesion molecule-1
BBB	Blood-brain barrier
$\alpha$ 7nAChR	$\alpha$ 7-nicotinic acetylcholine receptor
SLE	Systemic lupus erythematosus
NMDA	N-methyl-D-aspartate
IgG	Immunoglobulin G
CNS	Central nervous system
ACh	Acetylcholine
ERK1/2	Extracellular signal regulated kinase 1/2
JNK	c-Jun N-terminal kinase
NF- $\kappa$ B	Nuclear factor- $\kappa$ B
DBS	Deep brain stimulation
PD	Parkinson's disease
AD	Alzheimer's disease
ChR2	Channelrhodopsin2
ChAT	Choline acetyl transferase
PBS	Phosphate buffer saline
EDTA	Ethylene diamine tetraacetic acid
TNF- $\alpha$	Tumor necrosis factor- $\alpha$

IL-6 Interleukin-6  
ANOVA Analysis of variance

#### Author contributions

YY, XJW and JYX wrote the main manuscript text and YY, JYC and XRW prepared Figs. 1, 2, 3, 4 and 5. All authors reviewed the manuscript.

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#### Availability of data and materials

The data are available for any scientific use with kind permission.

#### Declarations

##### Ethics approval and consent to participate

The animals were maintained and treated in compliance with the policies and procedures detailed in the "Guide for the Care and Use of Laboratory Animals" of the National Institutes of Health. The Animal Care and Use Committee of China Medical University reviewed and approved the animal experimental protocols and the treatment procedures (No. KT2018060).

##### Consent for publication

The authors declare no competing interests.

##### Competing interests

Not applicable.

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#### References

- Yoshio T, Okamoto H, Hirohata S, Minota S. IgG anti-NR2 glutamate receptor autoantibodies from patients with systemic lupus erythematosus activate endothelial cells. *Arthritis Rheum*. 2013;65(2):457–63.
- Ballok DA, Millward JM, Sakic B. Neurodegeneration in autoimmune MRL-lpr mice as revealed by Fluoro Jade B staining. *Brain Res*. 2003;964(2):200–10.
- Popescu A, Kao AH. Neuropsychiatric systemic lupus erythematosus. *Curr Neuropharmacol*. 2011;9(3):449–57.
- Nishimura K, Harigai M, Omori M, Sato E, Hara M. Blood-brain barrier damage as a risk factor for corticosteroid-induced psychiatric disorders in systemic lupus erythematosus. *Psychoneuroendocrinology*. 2008;33(3):395–403.
- Morales JY, Young-Stubbs CM, Shimoura CG, Kem WR, Uteshev VV, Mathis KW. Systemic administration of  $\alpha 7$ -nicotinic acetylcholine receptor ligands does not improve renal injury or behavior in mice with advanced systemic lupus erythematosus. *Front Med*. 2021;8:642960.
- Zhai Q, Lai D, Cui P, Zhou R, Chen Q, Hou J, et al. Selective activation of basal forebrain cholinergic neurons attenuates polymicrobial sepsis-Induced inflammation via the cholinergic anti-inflammatory pathway. *Crit Care Med*. 2017;45(10):e1075–82.
- Zaghloul N, Addorisio ME, Silverman HA, Patel HL, Valdés-Ferrer SI, Ayasolla KR, et al. Forebrain Cholinergic dysfunction and systemic and brain inflammation in murine Sepsis survivors. *Front Immunol*. 2017;8:1673.
- Nizri E, Irony-Tur-Sinai M, Lory O, Orr-Urtreger A, Lavi E, Brenner T. Activation of the cholinergic anti-inflammatory system by nicotine attenuates neuroinflammation via suppression of Th1 and Th17 responses. *J Immunol*. 2009;183(10):6681–8.
- Goldstein RS, Bruchfeld A, Yang L, Qureshi AR, Gallowitsch-Puerta M, Patel NB, et al. Cholinergic anti-inflammatory pathway activity and high mobility group box-1 (HMGB1) serum levels in patients with rheumatoid arthritis. *Mol Med*. 2007;13(3–4):210–5.
- Pham GS, Shimoura CG, Chaudhari S, Kulp DV, Mathis KW. Chronic unilateral cervical vagotomy reduces renal inflammation, blood pressure, and renal injury in a mouse model of Lupus. *Am J Physiol Ren Physiol*. 2020;319(2):F155–f61.
- Mathis KW. An impaired neuroimmune pathway promotes the development of hypertension in systemic lupus erythematosus. *Am J Physiol Regul Integr Comp Physiol*. 2015;309(9):R1074–7.
- Fairley AS, Mathis KW. Cholinergic agonists reduce blood pressure in a mouse model of systemic lupus erythematosus. *Physiological Rep*. 2017;5(7):e13213.
- Huang F, Tang B, Jiang H. Optogenetic investigation of neuropsychiatric diseases. *Int J Neurosci*. 2013;123(1):7–16.
- Karnopp TE, Chapacais GF, Freitas EC, Monticelio OA. Lupus animal models and neuropsychiatric implications. *Clin Rheumatol*. 2021;40(7):2535–45.
- Yun Y, Wang X, Xu J, Jin C, Chen J, Wang X, et al. Pristane induced lupus mice as a model for neuropsychiatric lupus (NPSLE). *Behav brain functions: BBF*. 2023;19(1):3.
- Luciano-Jaramillo J, Sandoval-García F, Vázquez-Del Mercado M, Gutiérrez-Mercado YK, Navarro-Hernández RE, Martínez-García EA, et al. Downregulation of hippocampal NR2A/2B subunits related to cognitive impairment in a pristane-induced lupus BALB/c mice. *PLoS ONE*. 2019;14(9):e0217190.
- Karnopp TE, Freitas EC, Rieger A, Chapacais GF, Monticelio OA. Higher IgG level correlated with vitamin D receptor in the hippocampus of a pristane-induced lupus model. *Clin Rheumatol*. 2022;41(6):1859–66.
- Sato A, Sato Y, Uchida S. Activation of the intracerebral cholinergic nerve fibers originating in the basal forebrain increases regional cerebral blood flow in the rat's cortex and hippocampus. *Neurosci Lett*. 2004;361(1–3):90–3.
- Saeed RW, Varma S, Peng-Nemeroff T, Sherry B, Balakhaneh D, Huston J, et al. Cholinergic stimulation blocks endothelial cell activation and leukocyte recruitment during inflammation. *J Exp Med*. 2005;201(7):1113–23.
- LaLumiere RT. A new technique for controlling the brain: optogenetics and its potential for use in research and the clinic. *Brain Stimul*. 2011;4(1):1–6.
- Krook-Magnuson E, Armstrong C, Bui A, Lew S, Ojala M, Soltesz I. In vivo evaluation of the dentate gate theory in epilepsy. *J Physiol*. 2015;593(10):2379–88.
- Soper C, Wicker E, Kulick CV, N'Gouemo P, Forcelli PA. Optogenetic activation of superior colliculus neurons suppresses seizures originating in diverse brain networks. *Neurobiol Dis*. 2016;87:102–15.
- Sohal VS, Zhang F, Yizhar O, Deisseroth K. Parvalbumin neurons and gamma rhythms enhance cortical circuit performance. *Nature*. 2009;459(7247):698–702.
- Chaudhury D, Walsh JJ, Friedman AK, Juarez B, Ku SM, Koo JW, et al. Rapid regulation of depression-related behaviours by control of midbrain dopamine neurons. *Nature*. 2013;493(7433):532–6.
- Pavlov VA, Ochani M, Gallowitsch-Puerta M, Ochani K, Huston JM, Czura CJ, et al. Central muscarinic cholinergic regulation of the systemic inflammatory response during endotoxemia. *Proc Natl Acad Sci USA*. 2006;103(13):5219–23.
- van Maanen MA, Lebre MC, van der Poll T, LaRosa GJ, Elbaum D, Vervoordeldonk MJ, et al. Stimulation of nicotinic acetylcholine receptors attenuates collagen-induced arthritis in mice. *Arthritis Rheum*. 2009;60(1):114–22.
- van Maanen MA, Stoof SP, Larosa GJ, Vervoordeldonk MJ, Tak PP. Role of the cholinergic nervous system in rheumatoid arthritis: aggravation of arthritis in nicotinic acetylcholine receptor  $\alpha 7$  subunit gene knockout mice. *Ann Rheum Dis*. 2010;69(9):1717–23.
- Pham GS, Wang LA, Mathis KW. Pharmacological potentiation of the efferent vagus nerve attenuates blood pressure and renal injury in a murine model of systemic lupus erythematosus. *Am J Physiol Regul Integr Comp Physiol*. 2018;315(6):R1261–r71.
- Sato A, Sato Y, Uchida S. Regulation of cerebral cortical blood flow by the basal forebrain cholinergic fibers and aging. *Auton neuroscience: basic Clin*. 2002;96(1):13–9.

30. Abbruscato TJ, Lopez SP, Mark KS, Hawkins BT, Davis TP. Nicotine and cotinine modulate cerebral microvascular permeability and protein expression of ZO-1 through nicotinic acetylcholine receptors expressed on brain endothelial cells. *J Pharm Sci*. 2002;91(12):2525–38.
31. Hawkins BT, Egleton RD, Davis TP. Modulation of cerebral microvascular permeability by endothelial nicotinic acetylcholine receptors. *Am J Physiol Heart Circ Physiol*. 2005;289(1):H212–9.
32. Heeschen C, Weis M, Aicher A, Dimmeler S, Cooke JP. A novel angiogenic pathway mediated by non-neuronal nicotinic acetylcholine receptors. *J Clin Invest*. 2002;110(4):527–36.
33. Wang Y, Pereira EF, Maus AD, Ostlie NS, Navaneetham D, Lei S, et al. Human bronchial epithelial and endothelial cells express alpha7 nicotinic acetylcholine receptors. *Mol Pharmacol*. 2001;60(6):1201–9.
34. Li YZ, Liu XH, Rong F, Hu S, Sheng ZY. Carbachol inhibits TNF- $\alpha$ -induced endothelial barrier dysfunction through alpha 7 nicotinic receptors. *Acta Pharmacol Sin*. 2010;31(10):1389–94.
35. Huang H, Lavoie-Lamoureux A, Lavoie JP. Cholinergic stimulation attenuates the IL-4 induced expression of E-selectin and vascular endothelial growth factor by equine pulmonary artery endothelial cells. *Vet Immunol Immunopathol*. 2009;132(2–4):116–21.
36. Riga D, Riga S, Halalau F, Schneider F. Brain lipopigment accumulation in normal and pathological aging. *Ann N Y Acad Sci*. 2006;1067:158–63.
37. Saito Y, Miyajima M, Yamamoto S, Sato T, Miura N, Fujimiya M, et al. Accumulation of senescent neural cells in murine lupus with depression-like behavior. *Front Immunol*. 2021;12:692321.
38. Katzav A, Ben-Ziv T, Chapman J, Blank M, Reichlin M, Shoenfeld Y. Anti-P ribosomal antibodies induce defect in smell capability in a model of CNS -SLE (depression). *J Autoimmun*. 2008;31(4):393–8.
39. Kapadia M, Stanojic M, Earls AM, Pulapaka S, Lee J, Sakic B. Altered olfactory function in the MRL model of CNS lupus. *Behav Brain Res*. 2012;234(2):303–11.
40. Bombini MF, Peres FA, Lapa AT, Sinicato NA, Quental BR, Pincelli ASM, et al. Olfactory function in systemic lupus erythematosus and systemic sclerosis. a longitudinal study and review of the literature. *Autoimmun Rev*. 2018;17(4):405–12.
41. Chaudhury D, Escanilla O, Linster C. Bulbar Acetylcholine enhances neural and perceptual odor discrimination. *J Neurosci*. 2009;29(1):52–60.
42. Ma M, Luo M. Optogenetic activation of basal forebrain cholinergic neurons modulates neuronal excitability and sensory responses in the main olfactory bulb. *J Neurosci*. 2012;32(30):10105–16.
43. Dulawa SC, Janowsky DS. Cholinergic regulation of mood: from basic and clinical studies to emerging therapeutics. *Mol Psychiatry*. 2019;24(5):694–709.
44. Chen L, Ke Y, Ma H, Gao L, Zhou Y, Zhu H, et al. Fluoxetine and ketamine reverse the depressive but not anxiety behavior induced by lesion of cholinergic neurons in the horizontal limb of the diagonal Band of Broca in male rat. *Front Behav Neurosci*. 2021;15:602708.
45. Degroot A, Treit D. Dorsal and ventral hippocampal cholinergic systems modulate anxiety in the plus-maze and shock-probe tests. *Brain Res*. 2002;949(1–2):60–70.
46. Degroot A, Kashluba S, Treit D. Septal GABAergic and hippocampal cholinergic systems modulate anxiety in the plus-maze and shock-probe tests. *Pharmacol Biochem Behav*. 2001;69(3–4):391–9.
47. Bansal A, Shikha S, Zhang Y. Towards translational optogenetics. *Nat Biomed Eng*. 2023;7(4):349–69.
48. Beudel M, Brown P. Adaptive deep brain stimulation in Parkinson's disease. *Parkinsonism Relat Disord*. 2016;22(Suppl 1):123–6.
49. Hardenacke K, Hashemiyooun R, Visser-Vandewalle V, Zapf A, Freund HJ, Sturm V, et al. Deep brain stimulation of the Nucleus Basalis of Meynert in Alzheimer's dementia: potential predictors of cognitive change and results of a long-term Follow-Up in eight patients. *Brain Stimul*. 2016;9(5):799–800.
50. Zhang X, Lei B, Yuan Y, Zhang L, Hu L, Jin S, et al. Brain control of humoral immune responses amenable to behavioural modulation. *Nature*. 2020;581(7807):204–8.
51. Kapadia M, Zhao H, Ma D, Sakic B. Sustained immunosuppression alters olfactory function in the MRL model of CNS lupus. *J Neuroimmune Pharmacol*. 2017;12(3):555–64.
52. Zhu Y, Ye Y, Zhou C, Sun S, Zhang J, Zhao Z, et al. Effect of sensory deprivation of nasal respiratory on behavior of C57BL/6J mice. *Brain Sci*. 2021;11(12):1426.
53. Katzav A, Solodееv I, Brodsky O, Chapman J, Pick CG, Blank M, et al. Induction of autoimmune depression in mice by anti-ribosomal P antibodies via the limbic system. *Arthritis Rheum*. 2007;56(3):938–48.
54. Zhao YJ, Yu TT, Zhang C, Li Z, Luo QM, Xu TH, et al. Skull optical clearing window for in vivo imaging of the mouse cortex at synaptic resolution. *Light Sci Appl*. 2018;7:17153.
55. Kikuchi DS, Campos ACP, Qu H, Forrester SJ, Pagano RL, Lassègue B, et al. Poldip2 mediates blood-brain barrier disruption in a model of sepsis-associated encephalopathy. *J Neuroinflamm*. 2019;16(1):241.

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