Minocycline Activates the Nucleus of the Solitary Tract-Associated Network to Alleviate Lipopolysaccharide-Induced Neuroinflammation

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ABSTRACT

Objective To examine the neuroanatomical substrates underlying the effects of minocycline in alleviating lipopolysaccharide (LPS)-induced neuroinflammation.

Methods Forty C57BL/6 male mice were randomly and equally divided into eight groups. Over three consecutive days, saline was administered to four groups of mice and minocycline to the other four groups. Immediately after the administration of saline or minocycline on the third day, two groups of mice were additionally injected with saline and the other two groups were injected with LPS. Six or 24 hours after the last injection, mice were sacrificed and the brains were removed. Immunohistochemical staining across the whole brain was performed to detect microglia activation via Iba1 and neuronal activation via c-Fos. Morphology of microglia and the number of c-Fo-positive neurons were analyzed by Image-Pro Premier 3D. One-way ANOVA and Fisher's least-significant differences were employed for statistical analyses.

Results Minocycline alleviated LPS-induced neuroinflammation as evidenced by reduced activation of microglia in multiple brain regions, including the shell part of the nucleus accumbens (Acbs), paraventricular nucleus (PVN) of the hypothalamus, central nucleus of the amygdala (CeA), locus coeruleus (LC), and nucleus tractus solitarius (NTS). Minocycline significantly increased the number of c-Fo-positive neurons in NTS and area postrema (AP) after LPS treatment. Furthermore, in NTS-associated brain areas, including LC, lateral parabrachial nucleus (LPB), periaqueductal gray (PAG), dorsal raphe nucleus (DR), amygdala, PVN, and bed nucleus of the stria terminali (BNST), minocycline also significantly increased the number of c-Fo-positive neurons after LPS administration.

Conclusion Minocycline alleviates LPS-induced neuroinflammation in multiple brain regions, possibly due to increased activation of neurons in the NTS-associated network.

Key words: neuroinflammation; lipopolysaccharide; depression; nucleus tractus solitaries; microglia

INTRODUCTION

Inflammation is a key factor that contributes to the development of depression in both humans and animal models^[1-5]. Peripheral inflammatory challenges may activate microglia in brain and trigger central neuroinflammation, ultimately leading to symptoms of sickness and depressive-like behaviors. Microglia, as

the primary brain-resident immune cells, exhibit ramified morphology with highly motile processes and distribute in a stable tiling pattern throughout the whole brain^[6,7]. Orchestrating neuroinflammation is one of the major roles that microglia exert in response to local environmental changes^[8]. Mounting evidence demonstrates microglia as a critical player in the pathophysiology of depression^[9-11].

Lipopolysaccharide (LPS), the prototypical endotoxin released by bacteria, can activate the peripheral immune system. LPS first induces sickness behavior at approximately 6 hours after administration, while one day later, animals show depressive-like behaviors^[12]. Sickness behavior during acute inflammation is an adaptive response to infection or tissue damage. Once the pathogen is cleared, the sickness can be fully recovered, while depressive behaviors persist for a relatively long period of time. The chronic responses are thought to be mediated through pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs), even after inflammation has subsided. Once molecules with characteristic PAMPs and/or DAMPs enter the brain, the activation of microglia is directly stimulated by specific receptors, which induces the secretion of inflammatory mediators and neurotransmitters, further promotes the development of depressive symptoms^[13]. Microglia participate in LPS-induced sickness and delay depressive-like behaviors^[11]. LPS-induced sickness and depressive-like symptoms can be alleviated by treatment with minocycline, a microglia inhibitor^[14]. However, which regions at the global brain level where microglia are activated after LPS administration remains to be clarified.

Preclinical and preliminary clinical studies have shown that minocycline has antidepressant effects^[14-28]. Minocycline is a tetracycline derivative with good permeability through blood-brain barrier and exerts a variety of neuroprotective effects by inhibiting microglia activation^[29-31]. Minocycline attenuates LPS-stimulated mRNA expression of inflammatory genes including IL-6, IL-1 β , MHC II, and TLR2, in BV-2 cells, a microglial-derived cell line^[14]. Minocycline also improves depressive-like behaviors with a reduction in microglial activation in the prefrontal cortex^[28]. Twenty-four hours after LPS treatment, minocycline improves depressive-like behaviors^[14,32]. At the molecular level, minocycline reduces mRNA expression of pro-inflammatory genes including IL-1 β , IL-6, and indoleamine 2,3-dioxygenase (IDO) in cortex and hippocampus^[14]. Previous work mainly focuses on limited brain regions, such as hippocampus; however, how minocycline exerts its effects across the whole brain remains unclear.

Here, we examined the LPS-induced activation of microglia and expression of c-Fos by immunohistochemistry throughout the mouse brain at 6 hours and 24 hours after treatment. We also investigated with minocycline pretreatment, which brain regions can suppress

activation of microglia, as well as which brain network that may be involved in the antidepressant effects of minocycline. Previous studies have shown that a number of nuclei may be involved in LPS-induced neuropathological responses, including nucleus tractus solitarius (NTS), area postrema (AP), lateral parabrachial nucleus (LPB), paraventricular nucleus (PVN), and central nucleus of the amygdala (CeA)^[33]. Our results reveal that minocycline may attenuate LPS-induced neuroinflammation by activating the NTS-associated network.

MATERIALS AND METHODS

Ethics and animals

This animal study was approved by the institutional review board of the Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences. All mice were treated according to the guidelines on the care and use of laboratory animals by the Beijing Laboratory Animal Management Office. Efforts were made to ensure minimal suffering of the mice used in this study. Eight-week-old C57BL/6 male mice were purchased from Vital River Laboratories (Vital River Laboratories, Beijing, China). Mice were acclimated to the environment for two weeks prior to the formal experiment. Food and water were available without restriction in a room with a 12-hour light/dark cycle. The temperature and humidity were maintained at 23 °C $\pm$ 2 °C and 60% $\pm$ 5%, respectively.

Experimental design

LPS (Sigma, USA) was administered intraperitoneally (i.p.) at a dose of 0.83 mg/kg. At this dose, it was reported that LPS can induce sickness and depressive-like behaviors in mice^[32]. Minocycline (Sigma, USA) was administered via i.p. at a dose of 50 mg/kg. At this dose, it was reported that minocycline can alleviate the neuroinflammation caused by LPS and block sickness and depressive-like behaviors^[14,32].

Forty mice were randomly and equally divided into eight groups, each group representing different combinations of treatments with saline (S), minocycline (M), or LPS (L). As illustrated in **Figure 1**, for three consecutive days, we administered saline to four groups (designated as S3) and minocycline to the other four groups (designated as M3). Immediately after the saline or minocycline administration on the third day, half of the mice in each of the saline/minocycline-treated groups were additionally injected with

saline and the other half were injected with LPS. Six or 24 hours after the last injection, mice were sacrificed to remove the brains. Therefore, we obtained eight treatment combinations, designated as S3S6, S3S24, S3L6, S3L24, M3S6, M3S24, M3L6, and M3L24. For example, the S3S6 represents the group of mice receiving three days of saline administration and additional saline treatment and was sacrificed 6 hours after the last treatment.

Immunohistochemical examinations

For immunostaining, mice were deeply anesthetized with 10% (wt/vol) chloral hydrate (Sangon, China), followed by cardiac perfusion with ice-cold 0.01M phosphate buffered saline (PBS, pH 7.4) and paraformaldehyde (formulated in 0.01M PBS, 4%, wt/vol, Sigma, USA). Brains were postfixed for 24 hours in the same fixative and stored at 4 °C. After dehydration for 24 hours in 30% sucrose (wt/vol), serial 40- μ m-thick coronal sections were cut on a cryostat (CM1950, Leica, Germany) and immediately subjected to immunohistochemistry staining.

The following primary antibodies were used: anti-c-Fos antibody (1:2000, Cell Signaling Technology, USA) and anti-Iba1 antibody (1:800, Abcam, USA). First, brain sections were incubated in the solution containing the corresponding primary antibody overnight at 4 °C. After washing with 0.01 M PBS (pH 7.4, 3 x 10 mins), the slices were incubated with the biotinylated secondary antibody against c-Fos primary antibody (1:300, MXB, China) or Iba-1 primary antibody (1:800, Solarbio, China) overnight at 4 °C. On the third day, after washing with 0.01 M PBS (pH 7.4, 3 x 10 mins), the slices were treated with PBS containing 1% hydrogen peroxide for 10 mins to block the activity of endogenous peroxidase. Next, after washing with 0.01 M

PBS (pH 7.4, 3 x 10 mins), the slices were incubated with streptavidin-conjugated HRP (1:300, MXB, China) for two hours followed by a DAB reaction to label the signal. After dehydration with gradient ethanol and clearance with xylene, slices were mounted and sealed with neutral resin.

Image acquisition and analysis

All images were acquired by a full-slice linear scanning pathology device (Aperio Pathology, Leica, Germany). All slides were scanned in a bright field. Among them, images of c-Fos staining were collected at 20 $\times$ magnification, and images of microglia staining were collected at 40 $\times$ magnification. The brain regions investigated in this study were as follows: core part of the nucleus accumbens (Acbc), +1.10 mm; shell part of the nucleus accumbens (Acbs), +1.10 mm; dorsal part of bed nucleus of the stria terminali (BNSTd), +0.26 mm; ventral division of BNST (BNSTv), +0.26 mm; PVN, -0.82 mm; CeA, -0.94 mm; basolateral nucleus of amygdala (BLA), -0.94 mm; dentate gyrus (DG), -1.46mm; ventral tegmental area (VTA), -3.28 mm; periaqueductal gray (PAG), -3.28 mm; dorsal raphe nucleus (DR), -4.48 mm; LPB, -5.34 mm; locus coeruleus (LC), -5.56 mm; area postrema (AP), -7.48 mm; and nucleus tractus solitarius (NTS), -7.48 mm. The numbers indicate distances of each brain region from the Bregma, plus and minus means before and after the Bregma, respectively, along the anteroposterior axis of the brain.

The number of c-Fos positive cells was analyzed by the image analysis software (Image-Pro Premier 3D, Media Cybernetics, Inc. Rockville, USA). To avoid false positives or false negatives, we used the manual counting function of Image-Pro Premier 3D to count the number of c-Fos positive cells. Morphological analysis of microglia was also performed with Image-Pro

Figure 1. Experimental design and grouping of mice treated with saline, lipopolysaccharide(LPS), and minocycline at different time point.

Premier 3D. Firstly, the measurement area of 1436 pixels in width and 871 pixels in height was randomly selected from the interested brain region in each sample. Secondly, the measurement of the cell body area was based on the two-dimensional automatic selection function of the Image-Pro Premier 3D. We manually identified the background area in the software. Multiple background areas were marked manually so that the background recognition of the software can be more accurate. Then, we identified the positive cell area manually and the software can automatically identify the positive areas in the entire picture. Thirdly, the scale correction was performed. As a result, the software can convert pixel units into area units (μm^2). Fourthly, the screening area from 20 μm^2 to 90 μm^2 was set to exclude non-cell positive particles. Fifthly, by selecting "Smooth" in the options, the software automatically recognized the cell body more accurately. Finally, each sample data was exported, and we pooled data from five samples of the same group for further analysis.

As for determining the activation of microglia, we are concerned with the change in the average area of microglia cell bodies in each group. It has been consistently reported that once microglia are activated, the size of their cell bodies increases^[34,35]. The size of each microglia cell indicated by Iba1 immunoreactivity can be used as a reliable indicator for its activation. Throughout the process of data acquisition, the analyzer did not know the experimental conditions.

Statistical analysis

Statistical significance was calculated with Prism software (GraphPad Prism 7). All data were represented as mean $\pm$ standard error of means (SEM). One-way ANOVA and Fisher's least-significant difference were employed for statistical analyses. $P < 0.05$ was used as a criterion for statistical significance. All animals were analyzed, and no data points were excluded.

RESULTS

Minocycline blocks the activation of microglia induced by LPS across the brain

Microglia are the resident immune cells in the brain and have highly specialized morphologies. Under quiescent conditions, microglia are characterized by small cell bodies and many long-branched filaments, which constantly scan the environment for signs of danger

associated with pathogens or damage^[36]. Upon neuroinflammatory stimuli, the morphology and function of activated microglia change rapidly. Usually, their protrusions become contracted and thickened. The cell body size increases, and the cells begin to secrete cytokines and free radicals. Finally, microglia may become amoeba-like cells that can phagocytose other cells, such as neurons^[36,37]. Here, we conducted morphological analysis of microglia among different groups in multiple brain regions involved in emotion regulation, including Acbs, BNSTd, PVN, CeA, DG, LPB, LC, NTS, and AP. We found that 6 hours after LPS treatment, the cell body size of the microglia in group S3L6 was significantly increased in Acbs, BNSTd, PVN, CeA, DG, LPB, LC, and NTS, compared with that in group S3S6, indicating that at this time point LPS had already resulted in neuroinflammation in most observed brain regions (**Table 1** and **Fig. 2**).

Next, we found that in group M3L6, 6 hours after LPS administration, the cell body size of the microglia in Acbs, PVN, CeA, LC, and NTS decreased significantly compared with that in group S3L6, revealing that treatment with minocycline for three days markedly blocked the activation of microglia induced by LPS (**Table 1** and **Fig. 2**). Although the effects of LPS on the activation of microglia were mitigated from 6 hours to 24 hours in some brain regions, the cell body size of the microglia in group S3L24 was still significantly larger than that in group S3S24 in most brain regions, including PVN, CeA, DG, LPB, and LC, suggesting that neuroinflammation still existed 24 hours after LPS treatment. It is interesting that the cell body size of microglia significantly increased in Acbs, BNSTd, and CeA at 24 hours after LPS treatment in group M3L24 compared with group S3L24. The underlying mechanisms remain to be clarified.

Minocycline increased the number of neurons activated by LPS in NTS and AP

NTS is the central target of the sensory afferents of the vagus nerve, which acts to integrate visceral signals. AP is located close and dorsally to NTS. Through vagus-mediated neural immune signals and humoral signals transduced by the AP region, NTS and AP play a critical role in the early integration of pathogen-associated signals^[38]. We investigated the role of NTS and AP in the minocycline-mediated alleviation of neuroinflammation induced by LPS. As previously reported, we used c-Fos as a marker of neuronal activation to map activated

Table 1. Statistical summary of the cell body area of microglia (μm^2) in various regions across the brains of mice treated with saline(S)/minocycline(M) and saline/LPS(L)[§]

Brain Region	number of microglia cells	cell body areas of microglia (μm^2) of the group							
		S3S6	S3L6	M3S6	M3L6	S3S24	S3L24	M3S24	M3L24
Acbs	850	29.5±1.4	48.3±2.8 ^{†††}	33.8±1.7	34.7±1.8 ^{***}	31.0±1.7	31.2±1.7	34.2±1.6	41.2±1.9 ^{###}
BNSTd	1077	31.3±1.6	41.2±2.2 ^{††}	37.3±2.0	35.7±2.3	31.9±1.9	40.0±2.3	35.7±2.0	49.7±2.8 ^{##}
PVN	698	28.8±1.4	43.0±4.1 ^{†††}	29.7±1.4	32.6±3.1 [*]	30.5±1.3	38.5±2.4 [‡]	32.9±1.5	40.8±2.4
CeA	1461	32.9±1.8	49.4±2.2 ^{†††}	38.0±1.2	39.8±2.7 ^{***}	32.4±1.2	39.5±1.7 ^{‡‡}	37.6±1.3	47.5±2.0 ^{###}
DG	1405	35.7±1.4	49.9±2.5 ^{†††}	38.1±2.0	44.7±2.2	34.7±1.4	49.4±2.3 ^{‡‡}	39.9±1.5	45.6±2.0
LPB	846	33.9±1.9	44.9±3.1 ^{††}	35.4±1.6	41.0±2.6	30.0±1.5	43±2.2 ^{†††}	35.4±1.6	38.3±2.2
LC	590	31.7±1.5	43.8±3.2 ^{†††}	36.6±1.8	37.2±2.0 [*]	32.8±1.6	38.7±1.9 [‡]	35.6±3.9	36.8±2.9
NTS	927	30.8±1.7	50.7±3.1 ^{†††}	37.2±2.2	35.9±2.1 ^{***}	33.2±1.9	38.9±2.1	35.1±1.9	40.8±2.4
AP	1061	46.2±4.5	54.8±3.2	55.3±3.3	58.5±4.8	48.9±3.2	57.9±2.5	47.0±3.5	54.2±3.2

[§]Plus and minus represent mean $\pm$ SEM. ^{†††} $P < 0.01$ and ^{†††} $P < 0.001$ for S3L6 vs. S3S6; ^{*} $P < 0.05$ and ^{***} $P < 0.001$ for M3L6 vs. S3L6; [‡] $P < 0.05$, ^{‡‡} $P < 0.01$, and ^{†††} $P < 0.001$ for S3L24 vs. S3S24. ^{##} $P < 0.01$ and ^{###} $P < 0.001$ for M3L24 vs. S3L24. ANOVA and Fisher's least-significant differences were employed for statistical analysis. Acbs: shell part of the nucleus accumbens; AP: area postrema; BNSTd: dorsal part of the bed nucleus of the stria terminali; CeA: central nucleus of the amygdala; DG: dentate gyrus; LC: locus coeruleus; LPB: lateral parabrachial nucleus; NTS: nucleus tractus solitarius; PVN: paraventricular nucleus of the hypothalamus.

A

B

C

Figure 2. Microglial activation in nucleus tractus solitarius (NTS) in response to lipopolysaccharide (LPS) and minocycline treatment.

(A) Microglia morphology detected by Iba-1 immunohistochemistry in NTS of mice of the eight groups. Scale bars: 60 μm ; scale bars of the insert: 30 μm . (B) Schematic illustration of the area of NTS analyzed in this study. (C) Statistical summary of the cell body area of microglia (μm^2) in NTS of different groups of animals. Values were expressed as the mean $\pm$ SEM. S3S6: $n = 97$; S3L6: $n = 123$; M3S6: $n = 133$; M3L6: $n = 75$; S3S24: $n = 107$; S3L24: $n = 124$; M3S24: $n = 118$; M3L24: $n = 150$. ^{†††} $P < 0.001$; ^{***} $P < 0.001$.

neurons under specific stimulation conditions.

The expression levels of c-Fos protein in groups S3S6 and S3S24 that were treated with saline were very low in most of the analyzed brain structures (**Table 2**, and **A, C, E, G** in **Fig. 3**, **Fig. 4**, and **Fig. 5**). Consistent with other studies^[33], we found that significantly more neurons in NTS and AP showed c-Fos immunoreactivity in group S3L6 than in group S3S6 (**Table 2**, and **A, B, I, J** in **Fig. 3**). When mice were treated with minocycline, the number of c-Fos positive neurons in group M3L6 significantly increased to 195% in NTS and to 237% in AP of those in group S3L6, indicating that minocycline activated more neurons in these two regions after LPS administration (**Table 2**, and **B, D, I, J** in **Fig. 3**). Twenty-four hours later, the number of LPS-induced activated neurons in NTS remained nearly the same in group S3L24 compared to group S3L6, while there were almost no c-Fos positive neurons 24 hours later in AP (**Table 2**, and **B, F, I, J** in **Fig. 3**). For the minocycline-treated groups, the number of LPS-induced activated neurons in NTS was reduced by 52% in group M3L24 compared to group

M3L6, while in AP there were almost no c-Fos positive neurons 24 hours later (**D, H, I, J** in **Fig. 3**). There was no statistically significant difference in the number of activated neurons between group S3L24 and M3L24 in both NTS and AP (**Table 2**, and **F, H, I, J** in **Fig. 3**). Taken together, these results indicate that minocycline recruited more neurons in NTS and AP to cope with the peripheral immune inflammation evoked by LPS.

Minocycline increased the number of neurons activated by LPS in LPB, CEA, and BNST

We further investigated the responses to LPS in other brain regions, including LPB, CeA, and BNST, which receive direct projections from NTS^[39,40]. Six hours after LPS administration, there were more neurons in LPB with c-Fos immunoreactivity in group S3L6 than in group S3S6 (**Table 2**, and **A, B, I, J** in **Fig. 4**). When mice were treated with minocycline, the number of c-Fos positive neurons in group M3L6 significantly increased to 281% of that in group S3L6 in LPB, indicating that minocycline activated more neurons in these regions after LPS treatment (**Table 2**, and **B**,

Table 2. Statistical summary of the number of c-Fos immunoreactive neurons in various regions across the brains of mice treated with saline(s)/minocycline(M) and saline/LPS(L)[§]

Brain Region	number of c-Fos immunoreactive neurons of the group (n)							
	S3S6	S3L6	M3S6	M3L6	S3S24	S3L24	M3S24	M3L24
Acbc	0	0	0	0	0	35.4±6.8 ^{†††}	0	30.2±2.5
Acbs	0	37.8±2.5 [†]	8.6±2.2	23.0±5.7	0	59.0±11.9	41.8±15.7	87.6±18.1
BNSTd	0	29.0±4.9 ^{†††}	4.4±2.9	68.3±11.6 ^{***}	0	7.2±2.8 ^{††}	4.4±2.77	12.4±3.9
BNSTv	5.0±3.5	46.0±7.3 ^{†††}	2±2	123.0±17.2 ^{***}	0	16.8±2.4 ^{††}	5.8±2.7	27.4±8.3 [#]
PVN	0.8±0.8	174±19.8 ^{†††}	6±1.1	240.0±3.6 ^{***}	15.4±7.4	36.4±6.2 ^{†††}	17.2±4.3	90.8±21.9 ^{##}
CeA	0	28.0±3 ^{†††}	6.8±2	52.6±7.8 ^{***}	2.0±2.0	13.6±4.2 [†]	11.6±3.2	45.0±5.8 ^{###}
BLA	0	25.3±2 ^{†††}	6±0.8	9.4±3.0 [†]	3.2±3.2	7.8±3.4 [†]	9.8±4.7	47.2±9.3 ^{###}
DG	0	0	0	0	5.0±1.4	39.4±6.8 ^{†††}	16.2±4.3	36.2±2.5
VTA	0	0	0	0	0	0	0	0
DR	1.0±1.0	65.0±7.9 ^{††}	12.4±3.5	121.6±21.0 ^{**}	11.8±8.9	60.4±19.6	29.2±4.1	83.4±10.6
PAG	7.0±1.2	14.0±1.8	9.0±1.9	51.0±8.3 ^{***}	8.0±5.8	18±5.7	25.4±6.7	103.0±9.5 ^{###}
LPB	3.0±1.8	36.0±12 ^{†††}	5.8±2.9	101.0±8.5 ^{***}	2.0±2.0	21.0±4.3	1.8±1.1	38.0±5.6 [#]
LC	4.3±3.3	34.0±2.1 [†]	6.4±4.7	70.4±8.1 ^{**}	14.6±9.0	24.6±5.9	52.8±7.4	58.2±10.8 ^{##}
NTS	0	104.8±8.2 ^{†††}	0	204.8±13.5 ^{***}	13.8±7.8	106.8±35.9	11.6±1.3	97.4±9.4
AP	0	72.0±8.6 ^{†††}	0	170.6±22.1 ^{***}	0	4.3±4.3 ^{†††}	0	3.0±2.1

[§] Plus and minus represent mean ± SEM. The number of sections analyzed for each brain region is 36-38. [†]*P* < 0.05, ^{††}*P* < 0.01, and ^{†††}*P* < 0.001 for S3L6 vs. S3S6; ^{*}*P* < 0.05, ^{**}*P* < 0.01, and ^{***}*P* < 0.001 for M3L6 vs. S3L6; [†]*P* < 0.05, ^{**}*P* < 0.01, and ^{***}*P* < 0.001 for S3L24 vs. S3L6; [#]*P* < 0.05, ^{##}*P* < 0.01, and ^{###}*P* < 0.001 for M3L24 vs. S3L24. ANOVA and Fisher's least-significant differences were employed for statistical analysis. BNSTv: ventral part of the bed nucleus of the stria terminali; CeA: central nucleus of the amygdala; DG: dentate gyrus; BLA: asolateral nuceus of amygdala ; DR: dorsal raphe; PAG: periaqueductal gray; VTA: ventral tegmental area.

Figure 3. Neuronal activation in nucleus tractus solitarius (NTS) and area postrema (AP) in response to LPS and minocycline treatment.

(A-H) Representative images showing c-Fos immunostaining. Scale bars: 100 μ m. scale bar of the insert: 25 μ m (I, J) Statistical summary of the number of c-fos positive neurons in NTS and AP. Values were expressed as mean $\pm$ SEM. $n = 4-5$ per group, $n = 37$ per brain region. ††† $P < 0.001$, *** $P < 0.001$, ### $P < 0.001$.

D, I, J in **Fig. 4**). Twenty-four hours after LPS administration, the number of activated neurons in LPB was reduced by 42% in group S3L24 compared with group S3L6 (**Table 2**, and **B, F, I, J** in **Fig. 4**). In the minocycline-treated groups, the number of c-Fos positive neurons in LPB was reduced by 62% in group M3L24

compared with group M3L6 (**Table 2**, and **D, H, I, J** in **Fig. 4**). Notably, 24 hours after LPS treatment, mice administered with minocycline still showed a larger number of c-Fos positive neurons in LPB in group M3L24 than in group S3L24 (**Table 2**, and **F, H, I, J** in **Fig. 4**). Similar results were observed in CEA and BNST.

Figure 4. Neuronal activation in lateral parabrachial nucleus (LPB) in response to LPS and minocycline treatment.

(A-H) Representative images showing c-Fos immunostaining. Scale bars: 100 μ m. (I) Schematic illustration of the area of LPB analyzed in this study. (J) Statistical summary of the number of c-Fos positive neurons. Values are expressed as mean $\pm$ SEM. $n = 4-5$ per group, $n = 38$ per brain region. $^{+++}P < 0.001$; $^{***}P < 0.001$; $^{\#}P < 0.05$.

Minocycline increased the number of neurons activated by LPS in PVN

PVN is another projecting target of NTS, which is the initial hub to constitute the hypothalamic-pituitary-adrenal (HPA) axis^[39,40]. The activity of the HPA axis is initiated by corticotropin-releasing hormone (CRH) neurons in PVN. In the anterior pituitary, CRH causes systemic release of adrenocorticotrophic hormone, a major stimulatory factor for glucocorticoid release from the adrenal cortex. Glucocorticoids then signal to regulate many systemic and neurological functions in response to peripheral immune inflammation^[41]. Therefore, we were concerned about the role of PVN in the attenuation of LPS-induced neuroinflammation by minocycline.

As previously reported^[33], six hours after LPS administration, a significantly larger number of neurons in PVN showed c-Fos immunoreactivity in group S3L6 than in group S3S6 (Table 2, and A, B, I, J in Fig. 5). When mice were treated with minocycline, the number of c-Fos positive neurons in group M3L6 significantly

increased to 138% of that in group S3L6, indicating that minocycline activated more neurons in PVN after LPS administration (Table 2, and B, D, I, J in Fig. 5). Twenty-four hours after LPS administration, the number of c-Fos positive neurons in PVN was reduced by 79% in group S3L6 compared with that in group S3L24 (Table 2, and B, F, I, J in Fig. 5). In minocycline-treated groups, the number of c-Fos positive neurons in PVN was reduced by 62% in group M3L24 compared with that in group M3L6 (D, H, I, J in Fig. 5). Notably, 24 hours after LPS treatment, mice administered minocycline still showed a significantly larger number of c-Fos positive neurons in PVN in group M3L24 than in group S3L24 (Table 2, and F, H, I, J in Fig. 5). These results indicate that much more neurons in PVN are activated and involved in the effects of minocycline on alleviating neuroinflammation induced by LPS.

Contributions of other nuclei to the effects of minocycline

We further examined several other nuclei anatomically

Figure 5. Neuronal activation in paraventricular nucleus (PVN) in response to LPS and minocycline treatment.

(A-H) Representative images showing c-Fos immunostaining. Scale bars: 100 μm. (I) Schematic illustration of the area of PVN analyzed in this study. (J) Statistical summary of the number of c-Fos positive neurons in PVN. Values are expressed as mean ± SEM. $n = 4-5$ per group, $n = 38$ per brain region. $^{+++}P < 0.001$; $^{***}P < 0.001$; $^{##}P < 0.01$.

associated with NTS and functionally related to emotion regulation, including Acbc/Acbs, DG, BLA, VTA, DR, PAG, and LC. VTA and Acbc/Acbs are key components of the brain's reward system, which has long been associated with depression and addiction^[42]. DR is mainly beneath the periaqueductal gray matter of the midbrain and plays an important role in regulating moods^[43]. PAG is the midbrain region surrounding the cerebral aqueduct, which processes negative emotions with autonomic, neuroendocrine, and immune signals to facilitate adaptation to threat^[44]. LC is a discrete pontine nucleus adjacent to the fourth ventricle, which houses noradrenergic neurons regulating behaviors associated with arousal, attention, cognitive flexibility, and stress response^[45].

As shown in **Table 2**, no positive signal for c-Fos was detected in VTA in any of the eight groups. At 6

hours, minocycline significantly increased the number of c-Fos positive neurons induced by LPS in Acbs, BLA, DR, and LC in group S3L6 compared with that in group S3S6. In Acbc, DG, and PAG, LPS induced little c-Fos immunoreactivity at 6 hours, which is consistent with previous report^[33]. When mice were treated with minocycline, the number of c-Fos positive neurons significantly increased in DR, PAG, and LC in group M3L6 compared with that in group S3L6. Interestingly, a delayed response was observed in Acbc and DG, as revealed by the increased number of activated neurons in group S3L24 compared with that in group S3L6, while there was no change in Acbs, DR, PAG, and LC. Notably, 24 hours after LPS treatment, mice treated with minocycline showed significantly larger number of c-Fos positive neurons in BLA, PAG, and LC in group M3L24 than in group S3L24, while there was no

significant change in Acbs, Acbs, DG, or DR.

DISCUSSION

As Prof. Daniel S. Zahm has noted, "it seems unlikely that a particular behavioral state should correlate specifically with activity in a particular structure or its outputs, but rather that behavior will instead correspond with more global profiles of activity distributed throughout the continuum"^[46]. Here, we studied the global profiles of LPS-induced neuroinflammation across the brain as revealed by microglial activation. Furthermore, we examined the global profiles of the effects of minocycline on alleviating LPS-induced neuroinflammation across the brain as revealed by neuronal activation. These profiles have not been investigated before.

We found that at six and 24 hours after LPS treatment, the cell body size of microglia significantly increased in Acbs, BNSTd, PVN, CeA, DG, LPB, LC, and NTS. Minocycline inhibited the increase of the cell body size of microglia induced by LPS in multiple brain regions, including Acbs, PVN, CeA, LC, and NTS at six hours. Consistent with previous reports^[33], LPS treatment induced neuronal activation in NTS, AP, LC, LPB, DR, BLA, CeA, PVN, BNSTv, BNSTd, and Acbs at six hours. Interestingly, minocycline treatment resulted in an increase in the number of activated neurons in NTS, AP, LC, LPB, PAG, DR, CeA, PVN, BNSTv, and BNSTd at 6 hours after LPS administration (**Fig. 6**). Although the effects of minocycline attenuated in most brain regions at 24 hours after LPS administration, there were still more activated neurons in LC, LPB, PAG, BLA, CeA, PVN, and BNSTv in group M3L24 compared with group S3L24. The spatiotemporal selectivity of the effects of minocycline may underlie its effects on ameliorating LPS-induced sickness and depressive-like behaviors.

One of the main functions of NTS is to transmit visceral reflex information to forebrain region to coordinate neuroendocrine and autonomic responses to internal challenges. Subphrenic vagotomy and whole-brain c-Fos mapping have revealed that the vagus sensory pathway and its primary and secondary projection regions are strongly involved in cytokine responses^[47]. One of the main targets of NTS projection neurons is LPB, which further can transmit the LPS-induced immune response to BNST and CeA^[48]. Reversibly inactivating NTS completely blocks depressive symptoms of LPS-induced social withdrawal and significantly reduces LPS-induced c-Fos expression in LPB, CeA, BNST,

Figure 6. Schematic summary of neuronal activation in the NTS-associated network in response to lipopolysaccharide (LPS) and minocycline.

(A) Neuronal activation in the brains of animals treated with saline for three days and once more 6 hours before sacrifice (group S3S6). (B) Neuronal activation in the brains of animals treated with saline for three days and LPS for 6 hours before sacrifice (group S3L6). (C) Neuronal activation in the brains of animals treated with minocycline for three days and LPS for 6 hours before sacrifice (group M3L6). Brain regions with red dots show significantly increased number of c-Fos-positive neurons in group M3L6 compared with group S3L6. Each dot in every brain region represents one-tenth of the actual number of c-Fos positive neurons.

and PVN^[49,50]. Medullary catecholaminergic neurons in NTS play a key role in mediating the effects of interleukin-1 on stress-related neuroendocrine neurons.^[51] Therefore, NTS may be a nexus in mediating central responses to peripheral inflammatory challenges. Our

data show that neurotransmission through NTS may play an important role in LPS-induced neuroinflammation, sickness, and depressive behaviors. By activating nearly double neurons, minocycline alleviates neuroinflammation induced by LPS in NTS, although the types of activated neurons are unclear and require further investigation. Minocycline significantly increased the number of activated neurons in LPB, CeA, and BNSTd to 281%, 188% and 236% respectively, suggesting that minocycline treatment recruits more neurons to inhibit the neuroinflammation induced by LPS in these nuclei.

LC directly receives projections from NTS^[39,40]. Previous studies demonstrate that barosensitive NTS neurons may directly modulate the activity of neurons in LC through efferent projections of NTS to peri-LC dendrites in rat brain^[52]. A single i.p. injection of LPS causes long-lasting increase in the activity of LC neurons^[53]. What's more, selective transection of dorsal subdiaphragmatic vagus nerve trunk fails to excite LC neurons in response to i.p. LPS, suggesting the important role of NTS in transmitting peripheral inflammatory signals.^[54] PAG also directly receive innervations from NTS^[39,40]. Visceral nociceptive information can be transmitted to NTS via the vagus nerve and further passed down to PAG by the catecholaminergic neurons in NTS^[55]. PAG plays an important role in behavioral responses to uncontrollable stress, threats, anxiety and pain^[44]. Peripheral administration of LPS in the cat significantly elevates response latencies from 60 mins to 300 mins for defensive rage induced by electrical stimulation from PAG, which was demonstrated to act through peripheral TNF- α , suggesting that NTS may be involved in transmitting peripheral inflammatory signal to PAG^[56]. Fibers from the ventrolateral PAG strongly target the medial, dorsal, and anterior parvocellular divisions, as well as the lateral and medial posterior magnocellular divisions of PVN, providing an anatomical basis for HPA axis regulation^[57]. PVN is the key hub in brain to integrate autonomic, neuroendocrine, and cardiovascular responses to external or internal challenges^[41]. It receives substantial noradrenergic input from NTS A2 and C2 neurons^[58]. Noradrenergic inputs to PVN contribute to LPS-induced activation of the HPA axis and c-Fos expression^[59], indicating that NTS plays a critical role in regulating the HPA response to stressors.^[60] Our results showed that minocycline pretreatment for three days significantly increased the number of activated neurons in LC, PAG, and PVN to 207%, 364%, and 138%, respectively, indicating that

minocycline treatment recruits more neurons in these NTS-associated nuclei to inhibit the neuroinflammation induced by LPS.

The brain can sense peripheral inflammation through two main pathways: the neural and humoral pathways^[61]. The neural pathway relies on the stimulation of the afferent sensory fibers of the vagus nerve by specific pathogens or cytokines released from immune cells. The main vagal sensory input is received by neurons in NTS, which coordinates autonomic function and interaction with the endocrine system. Ascending projections from NTS reach most brain regions regulating emotions and stress responses, including LC, LPB, PAG, DR, VTA, CEA, PVN, BNST, and Acb^[39,40]. Our results show that minocycline significantly increases the number of activated neurons in NTS, AP, LC, LPB, PAG, DR, CeA, PVN, BNSTv, and BNSTd. Therefore, we propose that the activated NTS-associated network may contribute to the effects of minocycline on reducing the neuroinflammation induced by LPS and improving sickness and depressive-like behaviors. As to the humoral pathway, cytokine-to-brain communication may also occur via circumventricular organs that lack normal blood-brain barrier function^[62]. AP is located in the floor of the caudal 4th ventricle and has close proximity to NTS, which is an important circumventricular organ and site for humoral immune-to-brain communication^[63]. Minocycline has good permeability through the blood-brain barrier and exerts anti-inflammatory, antioxidant, anti-apoptotic and neuroprotective effects^[15]. Our results showed that minocycline significantly increases the number of activated neurons in AP. Therefore, minocycline may directly act on this region through the humoral pathway. The antidepressant effects of minocycline have been well reported in many studies^[14-28]; our work is the first to investigate the neural substrates across the brain that may contribute to the effects of minocycline. Although we used well-referred doses of minocycline/LPS^[14,32], one limitation of the work is that only a single dose of minocycline/LPS was employed in our study. Different concentrations of minocycline/LPS might elicit different reactions in brain, which needs to be investigated in the future.

The present study shows that minocycline alleviates LPS-induced neuroinflammation in multiple brain regions. The effects may result from increased activation of neurons in the NTS-associated network. Although the specific types of neurons activated by

minocycline and whether each specific component within the activated network is directly or indirectly connected to NTS remain to be determined, these findings reveal an alternative mechanism at the neural circuitry level underlying the effects of minocycline on reducing the neuroinflammation induced by LPS. Our work may open new avenues to combat depression, especially related to inflammation.

Conflict of interests

The authors have no conflicts of interest disclosed.

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Author contributions

Qi Xu and Jianbo Xiu conceived and designed the study. Lanlan Li and Jianbo Xiu performed the main experiments and analysed the data. Qi Xu, Jianbo Xiu and Lanlan Li wrote and revised the manuscript. Qi Xu supervised the project.

REFERENCES

1. Wohleb ES, Franklin T, Iwata M, et al. Integrating neuroimmune systems in the neurobiology of depression. *Nat Rev Neurosci* 2016; 17(8): 497-511. doi:10.1038/nrn.2016.69.
2. Miller AH, Raison CL. The role of inflammation in depression: from evolutionary imperative to modern treatment target. *Nat Rev Immunol* 2016(1): 16, 22-34. doi:10.1038/nri.2015.5.
3. Dantzer R, O'Connor JC, Freund GG, et al. From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat Rev Neurosci* 2008; 9(1): 46-56. doi:10.1038/nrn2297.
4. Hodes GE, Kana V, Menard C, et al. Neuroimmune mechanisms of depression. *Nat neurosci* 2015; 18(10): 1386-93. doi:10.1038/nn.4113.
5. Beurel E, Toups M, Nemeroff CB. The Bidirectional Relationship of Depression and Inflammation: Double Trouble. *Neuron* 2020; 107(2): 234-56. doi:10.1016/j.neuron.2020.06.002.
6. Zhan L, Krabbe G, Du F, et al. Proximal recolonization by self-renewing microglia re-establishes microglial homeostasis in the adult mouse brain. *PLoS biology* 2019; 17(2): e3000134. doi:10.1371/journal.pbio.3000134.
7. Lawson LJ, Perry VH, Dri P, et al. Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. *Neurosci* 1990; 39(1): 151-70. doi:10.1016/0306-4522(90)90229-w.
8. Prinz M, Jung S, Priller J. Microglia Biology: One Century of Evolving Concepts. *Cell* 2019; 179(2): 292-311. doi:10.1016/j.cell.2019.08.053.
9. Deng SL, Chen JG, Wang F. Microglia: A central player in depression. *Curr Med Sci* 2020; 40(3): 391-400. doi:10.1007/s11596-020-2193-1.
10. Jia X, Gao Z, Hu H. Microglia in depression: current perspectives. *Sci China Life Sci* 2021; 64(6):911-25. doi:10.1007/s11427-020-1815-6.
11. Yirmiya R, Rimmerman N, Reshef R. Depression as a microglial disease. *Trends Neurosci* 2015; 38(10):637-58. doi:10.1016/j.tins.2015.08.001.
12. Dantzer R. Cytokine-induced sickness behavior: Mechanisms and implications. *Ann NY Acad Sci* 2001; 933, 222-34. doi:10.1111/j.1749-6632.2001.tb05827.x.
13. Quan N, Banks WA. Brain-immune communication pathways. *Brain Behav Immun* 2007; 21(6): 727-35. doi:10.1016/j.bbi.2007.05.005.
14. Henry CJ, Huang Y, Wynne A, et al. Minocycline attenuates lipopolysaccharide (LPS)-induced neuroinflammation, sickness behavior, and anhedonia. *J Neuroinflammation* 2008; 5: 15. doi:10.1186/1742-2094-5-15.
15. Soczynska JK, Mansur RB, Brietzke E, et al. Novel therapeutic targets in depression: Minocycline as a candidate treatment. *Behav Brain Res* 2012; 235(2): 302-17. doi:10.1016/j.bbr.2012.07.026.
16. Zhu FR, Zheng YJ, Ding YQ, et al. Minocycline and Risperidone Prevent microglia activation and rescue behavioral deficits induced by neonatal intrahippocampal injection of lipopolysaccharide in rats. *Plos One* 2014; 9(4):e93966. doi:10.1371/journal.pone.0093966.
17. Zheng LS, Kaneko N, Sawamoto K. Minocycline treatment ameliorates interferon-alpha-induced neurogenic defects and depression-like behaviors in mice. *Front Cell Neurosci* 2015; 9:5. doi:10.3389/fncel.2015.00005.
18. Majidi J, Kosari-Nasab M, Salari AA. Developmental minocycline treatment reverses the effects of neonatal immune activation on anxiety- and depression-like behaviors, hippocampal inflammation, and HPA axis activity in adult mice. *Brain Res Bull* 2016; 120:1-13. doi:10.1016/j.brainresbull.2015.10.009.
19. Husain MI, Chaudhry IB, Husain N, et al. Minocycline as an adjunct for treatment-resistant depressive symptoms: A pilot randomised placebo-controlled trial. *J Psychopharmacol* 2017; 31(9):1166-75. doi:10.1177/0269881117724352.
20. Han Y, Zhang LJ, Wang QZ, et al. Minocycline inhibits microglial activation and alleviates depressive-like behaviors in male adolescent mice subjected to maternal separation. *Psychoneuroendocrinology* 2019; 107: 37-45. doi:10.1016/j.psychen.2019.04.021.
21. Zhang C, Zhang YP, Li YY, et al. Minocycline ameliorates depressive behaviors and neuro-immune dysfunction induced by chronic unpredictable mild stress in the rat. *Behav Brain Res* 2019; 356: 348-57. doi:10.1016/j.bbr.2018.07.001.

22. Wang B, Huang X, Pan X, et al. Minocycline prevents the depressive-like behavior through inhibiting the release of HMGB1 from microglia and neurons. *Brain Behav Immun* 2020; 88: 132-43. doi:10.1016/j.bbi.2020.06.019.
23. Yang Q, Luo L, Sun T, et al. Chronic minocycline treatment exerts antidepressant effect, inhibits neuroinflammation, and modulates gut microbiota in mice. *Psychopharmacology* 2020; 237: 3201-13. doi:10.1007/s00213-020-05604-x.
24. Nettis MA, Lombardo G, Hastings C, et al. Augmentation therapy with minocycline in treatment-resistant depression patients with low-grade peripheral inflammation: results from a double-blind randomised clinical trial. *Neuropsychopharmacology* 2021; 46(5):939-48. doi:10.1038/s41386-020-00948-6.
25. Bassett B, Subramaniam S, Fan Y, et al. Minocycline alleviates depression-like symptoms by rescuing decrease in neurogenesis in dorsal hippocampus via blocking microglia activation/phagocytosis. *Brain Behav Immun* 2021; 91: 519-30. doi:10.1016/j.bbi.2020.11.009.
26. Rosenblat JD, McIntyre RS. Efficacy and tolerability of minocycline for depression: a systematic review and meta-analysis of clinical trials. *J Affect Disorders* 2018; 227: 219-25. doi:10.1016/j.jad.2017.10.042.
27. Reis DJ, Casteen EJ, Ilardi SS. The antidepressant impact of minocycline in rodents: a systematic review and meta-analysis. *Sci Rep* 2019; 9(1):261. doi:10.1038/s41598-018-36507-9.
28. Burke NN, Kerr DM, Moriarty O, et al. Minocycline modulates neuropathic pain behaviour and cortical M1-M2 microglial gene expression in a rat model of depression. *Brain Behav Immun* 2014; 42: 147-56. doi:10.1016/j.bbi.2014.06.015.
29. Yrjanheikki J, Keinanen R, Pellikka M, et al. Tetracyclines inhibit microglial activation and are neuroprotective in global brain ischemia. *P Natl Acad Sci U S A* 1998; 95(26): 15769-74. doi: 10.1073/pnas.95.26.15769.
30. Tikka T, Fiebich BL, Goldsteins G, et al. Minocycline, a tetracycline derivative, is neuroprotective against excitotoxicity by inhibiting activation and proliferation of microglia. *J Neurosci* 2001; 21(8):2580-8. doi:10.1523/Jneurosci.21-08-02580.2001.
31. Ekdahl CT, Claassen JH, Bonde S, et al. Inflammation is detrimental for neurogenesis in adult brain. *P Natl Acad Sci U S A* 2003; 100(23):13632-7. doi:10.1073/pnas.2234031100.
32. O'Connor JC, Lawson MA, Andre C, et al. Lipopolysaccharide-induced depressive-like behavior is mediated by indoleamine 2,3-dioxygenase activation in mice. *Mol Psychiatry* 2009; 14(5):511-22. doi:10.1038/sj.mp.4002148.
33. Frenois F, Moreau M, O'Connor J, et al. Lipopolysaccharide induces delayed FosB/DeltaFosB immunostaining within the mouse extended amygdala, hippocampus and hypothalamus, that parallel the expression of depressive-like behavior. *Psychoneuroendocrinology* 2007; 32(5):516-31. doi:10.1016/j.psyneuen.2007.03.005.
34. Tynan RJ, Naicker S, Hinwood M, et al. Chronic stress alters the density and morphology of microglia in a subset of stress-responsive brain regions. *Brain Behav Immun* 2010; 24: 1058-68. doi:10.1016/j.bbi.2010.02.001.
35. Han RR, Liu ZY, Sun NN, et al. BDNF alleviates neuroinflammation in the hippocampus of type 1 diabetic mice via blocking the aberrant HMGB1/RAGE/NF-kappa B pathway. *Aging Dis* 2019; 10(3): 611-25. doi:10.14336/Ad.2018.0707.
36. Ransohoff RM, Perry VH. Microglial physiology: unique stimuli, specialized responses. *Annu Rev Immunol* 2009; 27: 119-45. doi:10.1146/annurev.immunol.021908.132528.
37. Kettenmann H, Hanisch UK, Noda M, et al. Physiology of microglia. *Physiol Rev* 2001; 91(2): 461-553. doi:10.1152/physrev.00011.2010.
38. Konsman JP, Kelley K, Dantzer R. Temporal and spatial relationships between lipopolysaccharide-induced expression of fos, interleukin-1 beta and inducible nitric oxide synthase in rat brain. *Neuroscience* 1999; 89(2):535-548. doi:10.1016/S0306-4522(98)00368-6.
39. Rinaman L. Ascending projections from the caudal visceral nucleus of the solitary tract to brain regions involved in food intake and energy expenditure. *Brain Res* 2010; 1350:18-34. doi:10.1016/j.brainres.2010.03.059.
40. Kawai Y. Differential ascending projections from the male rat caudal nucleus of the tractus solitarius: an interface between local microcircuits and global macrocircuits. *Front Neuroanat* 2018; 12: 63. doi:10.3389/Fnana.2018.00063.
41. Ulrich-Lai YM, Herman JP. Neural regulation of endocrine and autonomic stress responses. *Nat Rev Neurosci* 2009; 10(6): 397-409. doi:10.1038/nrn2647.
42. Russo SJ, Nestler EJ. The brain reward circuitry in mood disorders. *Nat Rev Neurosci* 2013; 14(9):609-25. doi:10.1038/nrn3381.
43. Michelsen KA, Schmitz C, Steinbusch HWM. The dorsal raphe nucleus: from silver stainings to a role in depression. *Brain Res Rev* 2007; 55(2):329-342. doi:10.1016/j.brainres-rev.2007.01.002.
44. George DT, Ameli R, Koob GF. Periaqueductal gray sheds light on dark areas of psychopathology. *Trends Neurosci* 2019; 42(5):349-60. doi:10.1016/j.tins.2019.03.004.
45. Schwarz LA, Luo LQ. Organization of the locus coeruleus-noradrenaline system. *Curr Biol* 2015; 25(21):R1051-R1056. doi:10.1016/j.cub.2015.09.039.
46. Zahm DS. An integrative neuroanatomical perspective on some subcortical substrates of adaptive responding with emphasis on the nucleus accumbens. *Neurosci Biobehav Rev* 2000; 24(1):85-105. doi:10.1016/S0149-7634(99)00065-2.
47. Konsman JP, Luheshi GN, Bluthé RM, et al. The vagus nerve mediates behavioural depression, but not fever, in response to peripheral immune signals: a functional anatomical analysis. *Eur J Neurosci* 2000; 12: 4434-46. doi:10.1046/j.0953-816X.2000.01319.x.
48. Sarhan M, Freund-Mercier MJ, Veinante P. Branching patterns of parabrachial neurons projecting to the central extended amygdala: Single axonal reconstructions. *J Comp Neurol* 2005; 491(4): 418-42. doi:10.1002/cne.20697.
49. Gaykema RPA, Chen CC, Goehler LE. Organization of immune-responsive medullary projections to the bed nucleus

- of the stria terminalis, central amygdala, and paraventricular nucleus of the hypothalamus: evidence for parallel viscerosensory pathways in the rat brain. *Brain Res* 2007; 1130(1): 130-45. doi:10.1016/j.brainres.2006.10.084.
50. Marvel FA, Chen CC, Badr N, et al. Reversible inactivation of the dorsal vagal complex blocks lipopolysaccharide-induced social withdrawal and c-Fos expression in central autonomic nuclei. *Brain Behav Immun* 2004; 18:123-34. doi:10.1016/j.bbi.2003.09.004.
 51. Ericsson A, Kovacs KJ, Sawchenko PE. A functional anatomical analysis of central pathways subserving the effects of interleukin-1 on stress-related neuroendocrine neurons. *J Neurosci* 1994; 14(2):897-913. doi:10.1523/JNEUROSCI.14-02-00897.1994.
 52. Van Bockstaele EJ, Peoples J, Telegan P. Efferent projections of the nucleus of the solitary tract to peri-locus coeruleus dendrites in rat brain: evidence for a monosynaptic pathway. *J Comp Neurol* 1999; 412(3):410-28. doi:10.1002/(Sici)1096-9861(19990927)412:3<410::Aid-Cne3>3.0.Co;2-F.
 53. Borsody MK, Weiss JM. The effects of endogenous interleukin-1 bioactivity on locus coeruleus neurons in response to bacterial and viral substances. *Brain Res* 2004; 1007(1-2): 39-56. doi:10.1016/j.brainres.2004.02.011.
 54. Borsody MK, Weiss JM. The subdiaphragmatic vagus nerves mediate activation of locus coeruleus neurons by peripherally administered microbial substances. *Neuroscience* 2005; 131(1):235-45. doi:10.1016/j.neuroscience.2004.09.061.
 55. Chen LW, Rao ZR, Shi JW. Catecholaminergic neurons in the nucleus-tractus-solitarii which send their axons to the midbrain periaqueductal gray express fos protein after noxious-stimulation of the stomach: a triple labeling study in the rat. *Neurosci Lett* 1995; 189(3):179-81. doi:10.1016/0304-3940(95)11475-C.
 56. Bhatt S, Bhatt RS, Zalcman SS, et al. Peripheral and central mediators of lipopolysaccharide induced suppression of defensive rage behavior in the cat. *Neuroscience* 2009; 163(4): 1002-11. doi:10.1016/j.neuroscience.2009.07.050.
 57. Floyd NS, Keay KA, Arias CM, et al. Projections from the ventrolateral periaqueductal gray to endocrine regulatory subdivisions of the paraventricular nucleus of the hypothalamus in the rat. *Neurosci Lett* 1996; 220(2):105-8. doi:10.1016/S0304-3940(96)13240-7.
 58. Sawchenko PE, Swanson LW. The organization of noradrenergic pathways from the brain-stem to the paraventricular and supraoptic nuclei in the rat. *Brain Res Rev* 1982; 257(3): 275-325. doi:10.1016/0165-0173(82)90010-8.
 59. Bienkowski MS, Rinaman L. Noradrenergic inputs to the paraventricular hypothalamus contribute to hypothalamic-pituitary-adrenal axis and central fos activation in rats after acute systemic endotoxin exposure. *Neuroscience* 2008; 156(4): 1093-2. doi:10.1016/j.neuroscience.2008.08.011.
 60. Herman JP. Regulation of hypothalamo-pituitary-adrenocortical responses to stressors by the nucleus of the solitary tract/dorsal vagal complex. *Cell Mol Neurobiol* 2018; 38(1):25-35. doi:10.1007/s10571-017-0543-8.
 61. Watkins LR, Maier SF, Goehler LE. Cytokine-to-brain communication: a review and analysis of alternative mechanisms. *Life Sci* 1995; 57(11):1011-26. doi:10.1016/0024-3205(95)02047-M.
 62. Buller KM. Role of circumventricular organs in pro-inflammatory cytokine-induced activation of the hypothalamic-pituitary-adrenal axis. *Clin Exp Pharmacol Physiol* 2001; 28(7): 581-9. doi:10.1046/j.1440-1681.2001.03490.x.
 63. Shapiro RE, Miselis RR. The central neural connections of the area postrema of the rat. *J Comp Neurol* 1985; 234(3):344-64. doi:10.1002/cne.902340306.

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论著

米诺环素活化孤束核相关网络减轻脂多糖诱发的神经炎症

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摘要

目的 研究米诺环素减轻脂多糖 (LPS) 诱导的神经炎症的神经解剖基础。

方法 将 40 只 C57BL/6 雄性小鼠随机分为 8 组。连续三天, 四组小鼠服用生理盐水, 其他四组服用米诺环素。在第三天服用生理盐水或米诺环素后, 两组小鼠立即额外注射生理盐水, 另外两组小鼠注射 LPS。最后一次注射后 6 或 24 小时, 处死小鼠, 取出大脑。对整个大脑进行免疫组织化学染色, 通过 Iba1 检测小胶质细胞激活情况, 通过 c-Fos 检测神经元的活化。通过 Image Pro Premier 3D 分析小胶质细胞的形态和 c-Fos 阳性神经元的数量。采用单因素方差分析和 Fisher 最小显著性差异进行统计分析。

结果 米诺环素可减轻 LPS 诱导的神经炎症, 其表现为多个脑区小胶质细胞的激活减少, 包括伏隔核的壳亚区、下丘脑室旁核 (PVN)、中央杏仁核、蓝斑 (LC) 和孤束核 (NTS)。米诺环素显著增加 LPS 注射后 NTS 和极后区中 c-Fos 阳性神经元的数量。此外, 在 NTS 相关的脑区, 包括 LC、臂旁外侧核、中脑导水管周围灰质、中缝背核、杏仁核、PVN 和终纹床核, 米诺环素也显著增加了 LPS 注射后 c-Fos 阳性神经元的数量。

结论 米诺环素可减轻脂多糖诱导的多个脑区神经炎症, 这种效应可能是由于孤束核相关网络中神经元的激活增加所致。

关键词: 神经炎症; 脂多糖; 抑郁症; 孤束核; 小胶质细胞

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