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Different effects of immune stimulation on chronic unpredictable mild stress-induced anxiety- and depression-like behaviors depending on timing of stimulation

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ABSTRACT

Stressful life events are thought to be triggering factors of numerous neuropsychiatric disorders, including anxiety and depression. However, the interactions between chronic unpredictable mild stress (CUMS) and immune stimulation have not been thoroughly investigated. In the present study, we evaluated the effects of lipopolysaccharide (LPS) challenge at different time points on CUMS-induced anxiety- and depression-like behaviors. At 1 day before, 18 or 35 days following the initial of CUMS, mice were intraperitoneally given a single LPS (0.1 mg/kg). Neurobehavioral and biochemical studies were performed at the indicated time points. LPS challenge had different effects on CUMS-induced anxiety- and depression-like behaviors depending on the timing of stimulation. When given 1 day before CUMS, LPS restored brain-derived neurotrophic factor level and reversed anxiety- and depression-like behaviors. When given at 18 days after the initial of CUMS, LPS seemed to promote the immune response and even induce a slight exacerbation of neurobehavioral performance, although the difference did not reach statistical significance. Intriguingly, when given at the end of CUMS, LPS reversed some of the anxiety- and depression-like behavior. Taken together, our study highlights the complex interaction between stress and immune challenge, suggesting therapies that modulate immune responses should be tailored to the immune status of the individual.

1. Introduction

Chronic stress exposure has broad effects on health, ranging from dysregulation of immune responses to increased predisposition for neuropsychiatric disorders [1–3]. Accumulating evidence has demonstrated that chronic stress can lead to permanent changes in the central nervous system (CNS) [4]. Indeed, depressed patients exhibit increased inflammatory cytokines in the peripheral circulation and some brain regions [5–7]. The role of neuroinflammation in depression is further confirmed by findings that central stimulation of IL-1β produces several stress-like effects and pathological changes [8], suggesting an exaggerated inflammatory cytokine is closely linked to the development of depressive-like behaviors. However, recent studies also suggest that immune dysfunction plays a pathological role in neuropsychiatric disorders such as depression. This notion is supported by the findings that glial ablation in the prefrontal cortex (PFC) is sufficient to induce depressive-like behaviors and glial loss and neuronal atrophy, which contributes to cognitive dysfunction, a core symptom of depression [9–12]. The emerging links between neuroinflammation and affective disorders highly suggest that modulation of the dysregulated immune response might provide a novel strategy to treat some forms of psychiatric disorders.

There is accumulating evidence suggesting that chronic stress-induced immune dysfunction can be modulated by exogenous immune challenge, which can produce complex results. Depending on its nature and duration, stress can either promote or decrease immune function. For instance, chronic stress sensitizes microglia in the hippocampus to subsequent peripheral and central inflammatory challenges, resulting in an exaggerated neuroinflammatory response [13], whereas chronic or more severe stress is immunosuppressive [14]. On the other hand, pretreatment with low-dose LPS induces hyporesponsiveness to subsequent immune challenge and adverse insult, leading to robust neuroprotection [15,16]. However, the interactions between chronic unpredictable mild stress (CUMS) and immune challenge have not been

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thoroughly investigated. In the present study, we assessed the effects of LPS challenge at different time points on CUMS-induced anxiety- and depression-like behaviors and explored the possible mechanism.

2. Materials and methods

2.1. Animals

All experiments were carried out on male C57BL/6 mice (12–14 weeks old), which were purchased from the Animal Center of Nanjing Medical University. All studies were approved by the Institutional Animal Care and Use Committee of Zhongda Hospital (Number of ethical permit, 32,120,170,426), Medical School, Southeast University, Nanjing, China, and followed the Guide for the Care and Use of Laboratory Animals from the National Institutes of Health. The mice were housed in a colony room maintained at 24 ± 1 °C with a 12-h light–dark cycle (lights on at 07:00). Mouse chow and water were available *ad libitum*.

2.2. CUMS paradigm

The CUMS paradigm was conducted as previously described with some modifications [17]. Briefly, animals for CUMS were housed singly and exposed to four of the following stressors daily in a random order for 35 days: overnight illumination (12 h), mild restraint for 2 h, cage tilt for 2 h, lights-off for 3 h during the daylight phase, wet bedding for 6 h, flashing light for 6 h, noise in the room for 12 h, and food and water deprivation for 12 h during the dark period (Table 1).

2.3. LPS stimulation

LPS (from *Escherichia coli* 0111:B4, Sigma, St. Louis, MO, Shanghai, China) was dissolved in 0.9% NaCl. All injections were prepared fresh on the treatment day and given intraperitoneally (i.p.) in a final injection volume of 10 ml/kg body weight between 8 AM and 9 AM. The LPS dosage of 0.1 mg/kg was selected because it elicits a low-grade pro-inflammatory cytokine response in the brain [18]. In addition, this dose of LPS had minimal effects on control mice, with no effect on locomotor activity or neuroinflammation within the brain after several days of injection [18]. To minimize the animals used, mice without any intervention served as the control group, while only CUMS exposed mice served as the CUMS group. The experimental protocol in the present study is summarized in Fig. 1.

2.4. Open field test

Open field test was performed to evaluate the exploratory behavior and anxiety behavior. Mice were placed individually in the center of a clear Plexiglas box (50 cm × 50 cm × 40 cm). We recorded total distance traveled and time spent in the center in the open field during a 5-min period. The behavior of mice was recorded using a video camera (Shanghai Softmaze Information Technology Co. Ltd., Shanghai, China). The apparatus was cleaned with 70% ethanol before testing each mouse to avoid the presence of olfactory cues.

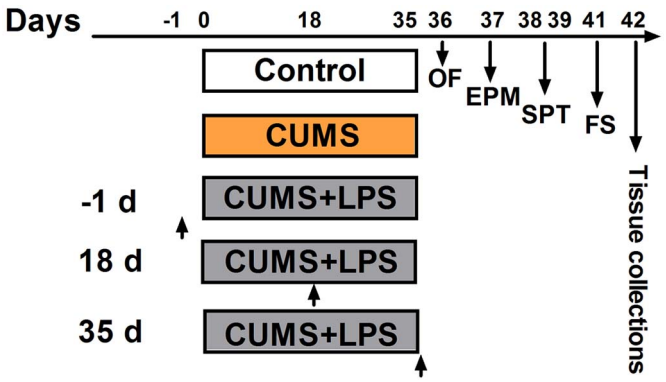

Fig. 1. Experimental protocols in the present study. CUMS, chronic unpredictable mild stress; LPS, lipopolysaccharide; OF, open field; EPM, elevated plus maze; SPT, sucrose preference test; FST, forced swim test.

2.5. Elevated plus maze

The elevated plus maze was conducted as we described previously [19]. Two arms were open without walls, while the other two were enclosed by high walls. Entrance to an arm was counted when all four of an animals' paws were within the arm. Each animal underwent one five-minute testing session. This test assesses anxiety-like behavior by measuring the number of entries to and the time spent in the open arms, with animals exhibiting anxious behaviors preferring closed to open arms.

2.6. Sucrose preference test

Anhedonia was measured by preference for a sucrose solution over water, using a two-bottle free choice method as previously described [20,21]. Briefly, each mouse was presented simultaneously with two bottles (50 ml), one with 1% sucrose solution and the other containing tap water. Mice were then given a free choice between either tap water or 1% sucrose in tap water solution for 24 h. After 12 h, the positions of the two bottles were switched to control for a side preference in drinking behavior. Twenty-four hours later, the bottles were then weighed to measure how much liquid was consumed. Sucrose preference was calculated as sucrose consumption/(sucrose consumption + water consumption) × 100%.

2.7. Forced swim test

Mice were placed singly in a plastic cylinder (15 cm diameter, 30 cm height) filled with water (20–24 °C) for 6 min, with the immobility scored in the final four minutes only. Time spent immobile (absence of movement except leg kicks to stay afloat) was then used as a measure of behavioral despair and helplessness, a rodent analogue of depressive-like behavior.

Table 1
Unpredictable chronic mild stress paradigm.

	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Week 1	A, C, D, F	A, C, F, G	B, D, E, G	A, D, G, H	B, D, E, H	B, D, F, H	B, C, E, G
Week 2	B, E, G, H	A, D, F, G	A, C, F, G	D, E, F, H	E, F, G, H	A, E, G, H	A, B, F, G
Week 3	A, C, F, G	B, E, F, G	B, C, D, F	A, C, D, G	B, D, E, G	B, D, E, H	B, F, G, H
Week 4	A, C, E, F	B, C, E, H	A, B, C, E	B, D, G, H	A, C, D, E	A, D, F, H	A, C, D, E
Week 5	B, C, E, F	A, C, D, F	B, C, D, F	B, C, G, H	D, E, F, H	A, C, G, H	B, C, E, F

A, overnight illumination (12 h); B, mild restraint for 2 h; C, cage tilt for 2 h; D, lights-off for 3 h during the daylight phase; E, wet bedding for 6 h; F, flashing light for 6 h; G, noise in the room for 12 h; H, food and water deprivation for 12 h during the dark period.