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Neonatal immune challenge exacerbates seizure-induced hippocampus-dependent memory impairment in adult rats

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ABSTRACT

Our aim was to examine whether neonatal lipopolysaccharide (LPS) exposure is associated with changes in microglia and whether these alternations could influence later seizure-induced neurobehavioral outcomes. Male pups were first injected intraperitoneally with either LPS or saline on postnatal day 3 (P3) and postnatal day 5 (P5). Immunohistochemical analysis showed that LPS-treated animals exhibited increased microglia activation that persisted into adolescence. At P45, seizures were induced in rats by intraperitoneal injection of kainic acid (KA). Rats treated with LPS neonatally showed significantly greater proinflammatory responses and performed significantly worse in the Y-maze, Morris water maze, and inhibitory avoidance tasks after KA insult. Treatment with minocycline at the time of neonatal LPS exposure to block LPS-induced microglia alternation attenuated the exaggerated neuroinflammatory responses and alleviated memory impairment associated with the KA insult. Our findings suggest that neonatal immune activation can predispose the brain to exacerbated behavioral deficits following seizures in adulthood, possibly by priming microglia.

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1. Introduction

Status epilepticus (SE) is a common and potentially life-threatening neurologic emergency characterized by prolonged or repetitive seizures lasting more than 30 min. Although a wide range of neuropsychological deficits may follow SE, memory disturbances comprise one of the most disturbing problems [1]. Recently, evidence from animal models has indicated that neonatal inflammation can predispose individuals to exacerbated behavioral dysfunction after subsequent pathological challenges in later life [2,3]. For instance, adult rats exposed to LPS as neonates display worse exploratory ability following stroke [4] and increased anxiety-like behavior following stress [5]. Systemic inflammation during early postnatal development has been proved to increase seizure susceptibility in adult rats [6]. However, few studies have investigated whether inflammation during the neonatal period alters cognitive functions such as learning and memory following repetitive seizures in adulthood.

Central nervous system (CNS) inflammation characterized by excessive microglial activation and the associated increase in proinflammatory cytokine production has long been recognized as a response to seizures and a potential contributor to mechanisms of epileptogenesis as well as seizure-related brain injury [reviewed in 7]. Recently, there is strong evidence that microglia can be primed by an early insult, resulting in greater responses (enhanced microglia activation, increased proinflammatory cytokine synthesis, and exaggerated behavioral changes) or increased pathology following exposure to a subsequent challenge [reviewed in 8]. Stimuli that lead to microglial priming, such as systemic infections, are correlated with exacerbated cognitive impairment and accelerated disease progression in Alzheimer's disease and prion disease models [9]. Microglia are long-lived cells and can become and remain activated chronically [10]. Notably, the most rapid period of brain growth in the rat is during the first week of life, when microglial proliferation, migration, and density reach their maximum [11]. Thus, the neonatal stage may be particularly sensitive to insult, with subsequent changes in microglial function.

Lipopolysaccharide is a potent stimulator of microglia to produce proinflammatory cytokines within the brain [12]. Cytokine receptors are distributed throughout the brain with high densities in the hippocampus [13]. Thus, the hippocampus is thought to be particularly vulnerable to immune-related alterations [14]. It is likely that the interaction of proinflammatory cytokines with neuronal elements during

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development may alter the brain in a manner that makes it more susceptible to seizure-induced hippocampus-dependent memory impairment in adulthood.

We hypothesize that microglia may be primed by an early-life immune challenge to overreact to a second seizure insult later in life, leading to exacerbated hippocampal-dependent memory impairment. The goal of the present study was to investigate a "two-hit" rat model in which there were combined effects on the developing brain of neonatal immune challenge and a second kainic acid (KA) insult. We sought (1) to assess microglial alternation following neonatal LPS exposure, (2) to assess neuroinflammatory responses and hippocampus-dependent behavioral performance after the subsequent KA insult in adulthood, and (3) to elucidate the role of microglia "primed" by early-life inflammation in later seizure-induced brain injury.

2. Materials and methods

2.1. Animals and drugs

Time-pregnant Sprague–Dawley rats were purchased from Shandong University Animal Center and monitored for the parturition date that was taken as P0 at which time all litters were culled to 10. Rat pups were housed with their dams until weaning. Grown-up rats were maintained in quiet, uncrowded facilities and given unlimited access to food and water. All rats were kept in a room maintained at constant temperature (22°C) and relative humidity (60%). The light/dark cycle was 12/12 h with photophase onset at 7:00 a.m. local time. All experimental procedures were conducted in accordance with the guidelines set by the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize the number of animals used as well as their suffering.

All compounds were purchased from Sigma-Aldrich (Sigma, St. Louis, MO) and were administered between 09:00 and 10:00 a.m. at 1 ml/kg unless otherwise stated.

2.2. Neonatal LPS exposure

On P3 and P5, all pups were separated from their respective mothers at the same time and placed in an incubator maintained at 34 °C. Pups were injected intraperitoneally (i.p.) with 50 μg/kg LPS (*Escherichia coli*, serotype 055:B5) diluted in normal saline (NS). A previous study [15] has shown that this dosing schedule of LPS administration causes no discernable change in the general health of the pups or maternal behavior. Control pups received corresponding volumes of NS. Once each pup within the litter had been injected, all animals were returned to their breeder as a group in the shortest possible time. All pups from a single litter received the same treatment. All studies were limited to males, and a maximum of two pups per litter were assigned to a single experimental group at each time point to control for possible litter effects. Pups were weaned at P28, housed three to four per cage in same sex groups, and remained undisturbed until further treatment and additional testing.

2.3. Kainic acid-induced seizures

At P45, kainic acid (KA) dissolved in saline (15 mg/kg) was administered i.p. to rats that were injected with saline or LPS neonatally. Controls received equal volumes of NS. Thereafter, animals were monitored continuously for 3 h. A seizure severity grade was assigned based on the maximal response achieved on a scale from 0 to IV as follows: 0, no response; I, wet dog shake (WDS) and/or behavioral arrest; II, WDS, staring, pawing, and clonic jerks; III, WDS, staring, pawing, clonic jerks, rearing, and falling; and IV, continuous grade III seizures for longer than 30 min (status epilepticus). After KA administration, seizure onset time (SOT), defined by the occurrence of forelimb clonus, rearing, and loss of balance [16], was recorded to the nearest second for each animal by an individual blind to the postnatal treatment of the animal. All rats received chloral hydrate (400 mg/kg, i.p.) at the end of the observation period after KA or saline injections to suppress convulsions and, importantly, to equalize seizure duration. Only rats with grade IV seizures were included in the following experiments.

2.4. Experimental protocol

A summary of the experimental design is presented in Fig. 1.

To study the time course of LPS-induced microglia alternation by immunohistochemical analysis using Iba1 as a marker of microglia, rats were sacrificed 4, 16, or 40 days after the last administration of NS or LPS on P5 (n = 5 for NS group and n = 6 for LPS group at each time point).

To establish whether the tetracycline derivative, minocycline, inhibits early-life LPS-induced microglia alternation, pups administered saline or LPS neonatally were treated with phosphate buffered saline (PBS) or minocycline. Minocycline (45 mg/kg) was diluted in PBS and buffered to a pH of 7.0 with sodium hydroxide. Minocycline or PBS alone was administered i.p. immediately after the first LPS injection on P3, then 24 h before and immediately after LPS injection on P5, and then every 24 h for 3 days. Three groups of rat pups were studied, including normal controls treated with PBS (SS), neonatally LPS-exposed pups treated with PBS (LS), and neonatally LPS-exposed pups treated with minocycline (LM). On P9 (4 days after administration of NS or LPS on P5) and P21, rats were sacrificed and evaluated for hippocampal microglial activation (n = 8 for each group at each time point).

To investigate the effects of early-life LPS exposure on seizure-induced memory impairment and the associated neuroinflammatory responses, as well as to further explore whether suppression of neonatal inflammation-induced microglial alternation would prevent changes in susceptibility to later seizure-induced brain injury, a separate study was conducted. Rat pups injected with saline or LPS

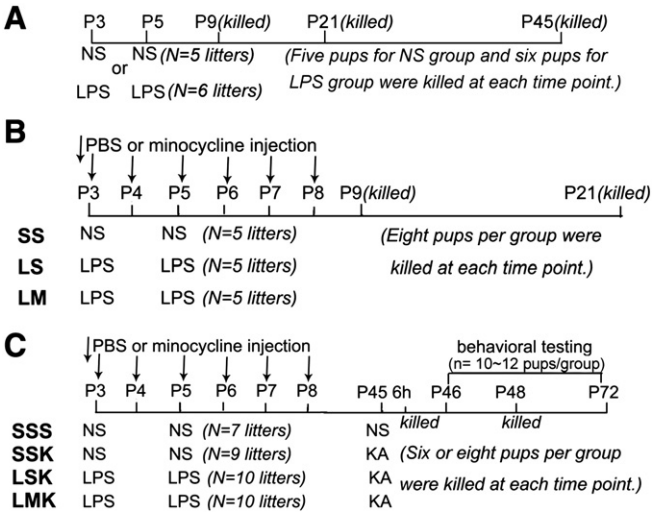

Fig. 1. Schematic of experimental groups. A, B, and C were designed to study the time course of LPS-induced microglia activation to establish whether minocycline inhibits LPS-induced microglia activation and to investigate whether neonatal LPS-induced microglia priming could influence later seizure-induced neurobehavioral outcomes, respectively. P = postnatal day; NS = normal saline; LPS = lipopolysaccharide; PBS = phosphate-buffered saline; ↓ = PBS or minocycline injection; h = hours; SS = normal controls treated with PBS; LS = neonatally LPS-exposed pups treated with PBS; LM = neonatally LPS-exposed pups treated with minocycline; SSS = normal controls; SSK = adult seizures treated with PBS neonatally; LSK = "two-hit" animals treated with PBS at the time of neonatal LPS exposure; LMK = "two-hit" animals treated with minocycline at the time of neonatal LPS exposure.

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