Maternal infection and fever during late gestation are associated with altered synaptic transmission in the hippocampus of juvenile offspring rats

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Lowe GC, Luheshi GN, Williams S. Maternal infection and fever during late gestation are associated with altered synaptic transmission in the hippocampus of juvenile offspring rats. Am J Physiol Regul Integr Comp Physiol 295: R1563–R1571, 2008. First published August 27, 2008; doi:10.1152/ajpregu.90350.2008.—Prenatal exposure to infection is known to affect brain development and has been linked to increased risk for schizophrenia. The goal of this study was to investigate whether maternal infection and associated fever near term disrupts synaptic transmission in the hippocampus of the offspring. We used LPS to mimic bacterial infection and trigger the maternal inflammatory response in near-term rats. LPS was administered to rats on embryonic days 15 and 16 and hippocampal synaptic transmission was evaluated in the offspring on postnatal days 20–25. Only offspring from rats that showed a fever in response to LPS were tested. Schaffer collateral-evoked field excitatory postsynaptic potentials (fEPSPs) and fiber volleys in CA1 of hippocampal slices appeared smaller in offspring from the LPS group compared with controls, but, when the fEPSPs were normalized to the amplitude of fiber volleys, they were larger in the LPS group. In addition, intrinsic excitability of CA1 pyramidal neurons was heightened, as antidromic field responses in the LPS group were greater than those from control. Short-, but not long-term plasticity was impaired since paired-pulse facilitation of the fEPSP was attenuated in the LPS group, whereas no differences in long-term potentiation were noted. These results suggest that LPS-induced inflammation during pregnancy produces in the offspring a reduction in presynaptic input to CA1 with compensatory enhancements in postsynaptic glutamatergic response and pyramidal cell excitability. Neurodevelopmental disruption triggered by prenatal infection can have profound effects on hippocampal synaptic transmission, likely contributing to the memory and cognitive deficits observed in schizophrenia.

lipoopolysaccharide; pregnancy; CA1 pyramidal cells; glutamatergic response; neuronal excitability

ALTHOUGH THE ETIOLOGY OF NEURODEVELOPMENTAL disorders, such as schizophrenia, remains quite controversial, epidemiological studies suggest an increased incidence of maternal infections, both viral and bacterial, associated with the later development of schizophrenia in the offspring (39, 47). How maternal infection can lead to offspring neurodevelopmental disorders remains to be determined, but the inflammatory response to infection has been implicated (11). Accordingly, the release of inflammatory molecules, such as cytokines, is known to produce physiological changes in the host, such as fever, which, in turn, could produce an unfavorable environment for fetal brain development. Moreover, cytokines could cross the placenta (53) and disrupt fetal brain development since they have been shown to inhibit neuronal survival (33) and dendritic growth (17). One brain region that has been repeatedly shown to be affected in schizophrenia is the hippocampus (4, 8, 14, 22, 27, 37). This area is known to play critical roles in several types of memory (44), as well as in fear and anxiety (6, 24), which are all known to be disrupted in this disease (12, 19–21, 38). How prenatal maternal infection affects the neurodevelopment of the hippocampus and how these changes relate to schizophrenia is not understood.

Several rodent models that mimic prenatal maternal infection of bacterial or viral origin have been developed to investigate possible alterations of hippocampal function in the offspring. It is believed that the hippocampus of the offspring undergoes significant neuroanatomical changes following late gestational inflammation. It has been reported that the injection of the bacterial endotoxin LPS to pregnant mice on embryonic day 17 (E17) is associated with increased hippocampal pyramidal cell layer density and smaller pyramidal cells in the offspring (18). This study also reported an enhancement in novel-object recognition, a deficit in associative learning and memory but no alterations in spatial learning in the Morris water maze, which are all hippocampus-dependent tasks. More recently, it has been reported that administration of the viral mimic polyinosinic: polycytidylic acid to near-term mouse dams led to an impairment in reversal learning in the water T-maze in the adult offspring (34), a behavior that also involves the hippocampus. Another study using injections of IL-6, a key fever-promoting cytokine, at a late gestational period resulted in a significant deficit in spatial memory along with increases in certain subunits of N-methyl-d-aspartate (NMDA) and γ-aminobutyric acid A (GABA_A) receptors in the hippocampus of the adult offspring (42). While anatomical and behavioral changes have already been found in the hippocampus, it remains unknown as to how synaptic transmission in the hippocampus is affected by prenatal maternal infection.

Understanding what is occurring at the synaptic level will help to understand the consequences of maternal infection during pregnancy on hippocampal function in the offspring. We have used a model of maternal infection in rats to determine whether neurodevelopmental disruption during pregnancy can lead to specific changes in hippocampal synaptic transmission in the offspring. We administered LPS to near-term pregnant rats (E15–16) to stimulate their immune response and then characterized synaptic transmission using field and whole cell patch-clamp recordings in hippocampal slices from the juvenile offspring. We found that gestational inflammation with LPS and associated short-lasting fever in the dams...
lead to important changes in synaptic transmission in the hippocampus of offspring. The changes in synaptic transmission observed in this model may shed some light into the underlying synaptic dysfunction present in schizophrenia.

MATERIALS AND METHODS

Animals

Timed-pregnant Sprague Dawley rats (Charles River, St-Constant, QC, Canada) were housed individually in a controlled environment (22°C, 12:12-h light-dark cycle, lights on from 8:00 to 20:00). Food and water were provided ad libitum. All procedures were performed in accordance with the guidelines established by the Canadian Council on Animal Care and were approved by the McGill University Animal Care Committee.

Maternal Infection with LPS

Pregnant rats were handled several days consecutively before the injections to minimize stress to the animals. A rectal probe for rats (Physitemp Instruments, NJ) was used to monitor their core body temperature. Body temperatures were measured immediately before the injection and every 2 h thereafter up to 8 h postinjection. On the day of birth, a small quantity of indelible ink was injected into one of the paws of each pup to identify animals from different birth groups. Pups were cross-fostered with surrogate saline-treated or untreated dams into mixed litters of 12. Since sex differences in the hippocampus of prepubertal offspring rats have been reported in previous studies investigating the effects of gestational immune activation (26, 42), we decided to limit possible variability due to sex differences and only male pups were retained for the study. For the LPS group, only pups from mothers who showed a significant fever response (i.e., >0.5°C) were used.

Electrophysiology

Brain slice preparation. Brains were removed from 20- to 25-day-old offspring from LPS- or saline-treated mothers and submerged in ice-cold oxygenated (95% O2/5% CO2) artificial cerebrospinal fluid (aCSF) containing (in mM): 126 NaCl, 24 NaHCO3, 10 glucose, 3 KCl, 2 MgSO4, 1.25 Na2HPO4, 2 CaCl2, 0.4 ascorbic acid. The frontal and cerebellar sections/regions were removed and 45° cuts from the midline were made on the ventral surface. The brain was mounted onto a vibratome stage with the ventral side down and a sagittal cut was made along the midline. Horizontal slices of the dorsal hippocampus were made at 350-μm thickness with a Vibroslice (Campden Instruments) and kept in bubbled aCSF at room temperature for at least 1 h before recording.

Field potential recordings. Slices were placed in a humidified interface chamber perfused (1–1.5 ml/min) with aCSF at 28°C for at least 30 min before recording. The slice surface was also exposed to warm, humidified 95% O2/5% CO2. Recording pipettes (1.5–2.5 MΩ) were pulled from borosilicate glass capillary tubing (Warner Instrument, Hamden, CT) and filled with internal solution containing (in mM): 144 K-glucurate, 0.2 EGTA, 10 HEPES, 2 ATP, and 0.3 GTP; pH 7.2 (285–295 mosmol/l). The blind whole cell configuration was used to obtain recordings in the submersed slice. Whole cell recordings in voltage-clamp and current-clamp modes were performed using a patch-clamp amplifier (model 2400; A-M Systems). Resting membrane potentials were measured in current-clamp mode once a stable recording was obtained. Cells from control slices were only used for experiment when the resting membrane potential was more negative than −55 mV and spikes overshot 0 mV. The membrane potential output signal was filtered online at 1 kHz and sampling was set at 10 kHz in the pClamp 9 software (Axon Instruments, Union City, CA).

The first action potential (AP) that resulted from a 500-ms-long depolarizing pulse from holding potential (VH) (−60 mV) was used to assess the AP characteristics: spike threshold, amplitude, and width. Spike amplitude was taken as the peak of the AP measured from the AHP that followed the first AP that resulted from a single spike. Spike amplitude was determined from the slope of the curve of voltage following small hyperpolarizing current steps of 5 pA from VH. The depolarization sag was measured as the difference between the peak and plateau regions of the response resulting from 4-s-long hyperpolarizing steps from Vh increasing by 10 pA. The afterhyperpolarization (AHP) resulting from a single spike was determined from the AHP that followed the first AP that resulted from a 500-ms-long depolarizing pulses from VH. Spike-train AHPs were recorded after eliciting 10 APs by applying 10 suprathreshold depolarizing current steps from VH at 100 Hz. The amplitude of the AHP was taken as the difference between the peak AHP and VH, and latency of the AHP was taken as the time of the peak from the beginning of the recording.

Drugs

DL-2-Amino-5-phosphonopentanoate was purchased from Sigma Aldrich (Oakville, ON, Canada) and 6,7-dinitroquinoxaline-2,3-dione was purchased from Tocris Bioscience (Bristol, UK). All drugs were diluted in aCSF from previously prepared stock solutions that were prepared in deionized H2O and stored at −80°C.

Data Analysis

All electrophysiological data were analyzed using pCLAMP 9.2 software (Axon Instruments). All results are expressed as means ± SE unless otherwise specified. For statistical analyses we performed Student’s t-test (two-tailed) using Prism 4 (GraphPad Software, San Diego, CA) and Origin 5.0 (Microcal Software, Northampton, MA). P < 0.05 was considered to be statistically significant. For field recordings, N represents the number of slices, and a maximum of two slices from each animal was used. For whole cell recordings, N represents number of cells and a maximum of two cells per slice per animal was used.

Cell Counting

Brains were removed from 26-day-old rats and submerged in ice-cold oxygenated aCSF. The frontal and posterior sections (including the cerebellum) of the brain were removed, leaving a 3- to 5-mm-thick section containing the hippocampus, which was fixed in...
4% paraformaldehyde at 4°C overnight and then cryo-protected and later stored at −80°C. We obtained two 16-μm-thick horizontal slices from each of three sections (each separated by 100 μm) of the dorsal hippocampus along the longitudinal axis. Slices were mounted with Vectashield containing DAPI (Vector Laboratories, Burlingame, CA) to label nuclei. Pictures of three subregions of CA1 or CA3 pyramidal cell layer from each slice were taken on a fluorescence microscope (Nikon Instruments) at a magnification of ×40 and the analysis was done blind to the treatment groups. The counting frame was a 200 μm × 75 μm rectangle enclosing a section of the cell layer. The upper blade of the dentate gyrus was used as a landmark for the most lateral subregion of CA1 or the most medial subregion of CA3 to be counted. The number of nuclei from the three subregions within the CA1 or CA3 pyramidal cell layer was summed from each slice, and thus the total number of nuclei per hippocampus side consisted of the total counts from six slices.

RESULTS

Body temperature responses to LPS. We administered LPS intraperitoneally to pregnant rats on E15 and E16 and monitored their core body temperatures before and after the injections. We found that LPS produces a variety of temperature responses. Out of 103 dams that received LPS, 59 displayed an increase in body temperature, 33 displayed a decrease in body temperature, and 11 showed no change in body temperature (Fig. 1A). LPS is known to induce a cytokine-mediated elevation in body temperature in rats, and this response, although attenuated, also occurs in pregnant rats (5, 16, 32). Because of these varying responses to LPS, we decided to limit further variability in our subjects by only selecting pups from mothers that displayed febrile responses to LPS on E15. The animals that responded to LPS exhibited a marked increase in body temperature at 4 h compared with control rats (P < 0.05), and this increase remained significant up to 8 h before slowly returning to baseline body temperature (Fig. 1B). On E16, the body temperature response to LPS was attenuated and attributed to tolerance to LPS after the first exposure (43). As expected, all of the 59 control dams that were given saline did not show any significant changes in body temperatures following the injections on either day.

One day after the dams gave birth (P1), we counted the number of animals per litter, sexed them, and weighed each of the male pups. We found that LPS did not have any effect on litter size and the weights of pups at birth (LPS, n = 31 litters of 12.1 ± 0.4 pups and n = 200 pups weighing 6.79 ± 0.03 g; control, n = 38 litters of 12.4 ± 0.4 pups and n = 258 pups weighing 6.91 ± 0.04 g). Thus, the dose of LPS we administered to late gestational rats did not cause significant changes in litter size or weight in the pups.

Synaptic transmission in CA1. We first investigated whether synaptic potentials in the hippocampus of offspring from the LPS-treated group were altered. We electrically stimulated the Schaffer collaterals and recorded fEPSPs in the CA1 area of hippocampal slices from 20- to 25-day-old offspring. The initial slopes of the fEPSPs at the same magnitude of stimulation currents were smaller in the LPS group compared with control (LPS, n = 15; control, n = 14; P < 0.05) (Fig. 2, A, i), suggesting a general decrease in excitatory synaptic transmission subsequent to maternal LPS treatment. This decrease may, in part, be due to a presynaptic reduction in synaptic excitability, since the amplitudes of the fiber volleys [an indication of the amount of presynaptic fiber stimulation (3)] were dramatically reduced in the LPS group compared with control (LPS, n = 15; control, n = 14; P < 0.05) over most of the stimulation intensities (Fig. 2, A, ii). Hence, higher current stimulation intensity was needed to elicit fiber volleys of the same amplitude (Fig. 2, B, i). Interestingly, when the fEPSPs were normalized to fiber volleys (i.e., comparing fEPSPs in response to fiber volleys of the same amplitude), it was found that the amplitude and initial slope of the fEPSPs of the LPS group were greater compared with those of control at all the fiber volley amplitudes examined (at 0.2 mV: LPS, n = 15; control, n = 12; P < 0.05; and at 0.4 mV: LPS, n = 14; control, n = 13; P < 0.05) (Fig. 2, ii).
B, ii and iii). This implies that in response to an equivalent presynaptic stimulation, fEPSPs elicited in the LPS group were larger in size, suggesting an increase in postsynaptic excitability. Together, maternal inflammation by LPS during late gestation produces in offspring a decrease in the excitability of the Schaffer collaterals but a concomitant postsynaptic increase in response to excitatory synaptic transmission. We also measured fEPSPs in CA3 and dentate gyrus by electrically stimulating the mossy fibers and perforant path, respectively, and found no difference in these regions (data not shown), suggesting that maternal LPS treatment causes changes in basal synaptic transmission that are specific to CA1.

**Short- and long-term plasticity in CA1.** We assessed short-term plasticity by measuring paired-pulse facilitation of the fEPSP. We applied paired stimuli to the Schaffer collaterals at varying interpulse intervals and determined the paired-pulse ratio as the slope of the second fEPSP divided by that of the first fEPSP. A paired-pulse ratio value >1 indicates facilitation, which is believed to result from an increase in transmitter release due to residual calcium in the presynaptic cleft after the first stimulation (10, 23, 52). Facilitation in hippocampal CA1 neurons can be observed at interpulse intervals between 30 and 100 ms (29). The amount of facilitation of the second fEPSP was found to be significantly diminished in the LPS group compared with control (LPS, n = 16; control, n = 9; at 10 ms, P < 0.05; and at 100 ms, P < 0.05) (Fig. 3A), suggesting that presynaptic short-term plasticity is decreased in the offspring after maternal LPS treatment during late gestation. We next determined whether long-term plasticity would be affected as well. We found that the amount of long-term potentiation (LTP) in CA1 elicited with a 1-s 100-Hz tetanus was not different between LPS and control groups (Fig. 3B).

**Recurrence inhibition in CA1.** We next investigated whether GABAergic inhibition in offspring was disrupted following maternal immune challenge. To do so, we assessed fast GABAergic inhibition of CA1 pyramidal cells by examining paired-pulse depression of the population spike. We applied paired stimuli to the Schaffer collaterals at varying interpulse intervals and recorded the responses in the somatic layer of CA1. Orthodromic stimulation of fibers within the stratum radiatum can produce two types of inhibition in CA1 pyramidal cells: one that is through the direct activation of GABAergic interneurons and is called feedforward inhibition, while the other is through an indirect activation of GABAergic interneurons following pyramidal cell excitation of these interneurons and is called feedback (or recurrent) inhibition (2). Depression of the second population spike can be observed at interpulse intervals between 5 and 50 ms, when recurrent inhibition by GABAA receptors is active (1, 40, 45). No difference in paired-pulse depression of the population spike was found between LPS and control groups (Fig. 4), implying that the feedback and feedforward GABAergic inhibition by GABAA receptors are not likely affected in the offspring after late-gestational maternal LPS treatment.

**Antidromic responses in CA1.** We investigated further the increase in postsynaptic excitability noted above following normalization to fiber volleys. To this end, we examined antidromic responses by stimulating the axons of CA1 pyramidal cells and recording in the CA1 somatic layer. To prevent contamination by excitatory and recurrent inhibitory synaptic transmission, synaptic activity was abolished with 5 μM dL-2-amino-5-phosphonopentanoate and 10 μM 6,7-dinitroquinolinol-2,3-dione. Antidromic responses in CA1 were dramatically increased in the LPS group compared with control over all stimulation intensities (LPS, n = 10; control, n = 8; P < 0.05) (Fig. 5), suggesting that late-gestational maternal LPS treatment leads to an enhancement in the excitability of CA1 pyramidal cells in the offspring.
intrinsic properties of CA1 pyramidal neurons could contribute to excitability of the CA1 area, we investigated whether certain field recordings uncovered some important changes in the dromic and orthodromic synaptic responses observed during /\textsuperscript{H}N1005.

Intrinsic properties of CA1 pyramidal cells. Since the antidromic and orthodromic synaptic responses observed during field recordings uncovered some important changes in the excitability of the CA1 area, we investigated whether certain intrinsic properties of CA1 pyramidal neurons could contribute to these changes. Using whole cell recordings, we first determined that the resting membrane potential of CA1 pyramidal cells in the LPS group was significantly more depolarized compared with control, while input resistance was not different (Table 1). Next, the firing characteristics were investigated. In current-clamp mode, the threshold voltage to spike generation in response to a 500-ms-long depolarization (from -60 mV) was not different between LPS and control groups (Table 1). However, the amplitude of APs in the LPS group was significantly smaller than those from control (Table 1). Spike width, which depends on a variety of potassium currents but primarily the fast-activating calcium-dependent potassium current (25, 46) is an indicator for spike repolarization and an important determinant of cell excitability. Spikes tended to be wider in the LPS group than control, while input resistance was not different (Table 1).

![Fig. 3. Maternal infection with LPS disrupts short-term facilitation without altering long-term potentiation (LTP) in the offspring. A: paired stimuli at varying interpulse intervals (10, 25, 50, 100, 200, 400, 800, 1,600, 3,200, and 6,400 ms) were applied to the Schaffer collaterals to record facilitation of the second fEPSP. Ratio of the slope of the 2nd to the 1st fEPSP (fEPSP2/fEPSP1) was determined as the paired-pulse ratio, and this was found to be attenuated in the LPS group (\(n = 16\)) compared with control (\(n = 9\)). Right inset shows example responses to paired stimuli at 10, 25, 50, 100, and 200 ms. Vertical bar: 2.5 mV; horizontal bar: 50 ms. B: amount of LTP was not different in the LPS group (\(n = 6\)) compared with control (\(n = 7\)). Each data point represents an average of 3 consecutive data. Right inset shows example responses at time 1 (630s) and time 2 (2700s). Vertical bar: 2 mV; horizontal bar: 5 ms. Values are means \pm SE. *P < 0.05 vs. control.](http://ajpregu.physiology.org/)

![Fig. 4. Maternal infection with LPS does not affect offspring GABAergic inhibition in CA1. Paired stimuli at varying interpulse intervals (5, 10, 25, 50, and 100 ms) were applied to the Schaffer collaterals to record depression of the 2nd population spike. Ratio of the amplitude of the 2nd to the 1st population spike (PS2/PS1) was determined as the paired-pulse ratio, and this was not altered in the LPS group (\(n = 10\)) compared with control (\(n = 11\)). Right inset shows example responses to paired stimuli at 10, 25, 50, and 100 ms. Vertical bars: 2 mV (control), 5 mV (LPS); horizontal bar: 25 ms. Values are means \pm SE.](http://ajpregu.physiology.org/)

![Fig. 5. Maternal infection with LPS produces enhanced excitability of CA1 pyramidal cells in the offspring. Axons of CA1 pyramidal cells in stratum oriens were stimulated and antidromic responses recorded in the somatic layer of CA1 in the presence of AP-5 and 6,7-dinitroquinoxaline-2,3-dione. Antidromic responses in the LPS group (\(n = 10\)) were greater than those from control (\(n = 8\)). Inset: typical antidromic responses in control and LPS (thicker line) groups at 200 \(\mu\)A stimulation. Vertical bar: 2.5 mV; horizontal bar: 5 ms. Values are means \pm SE. *P < 0.05 vs. control.](http://ajpregu.physiology.org/)

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<th>Table 1. Resting and firing characteristics of CA1 pyramidal cells</th>
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<td><strong>Control</strong></td>
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<td>Resting membrane potential, mV</td>
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Values are means \pm SE; \(n\) of cells is shown in parentheses. *P < 0.05 vs. control. NS, not significant; AP, action potential; AHP, afterhyperpolarization. Depolarization sag was measured from 100 pA hyperpolarizing pulse from holding potential.
they were not different between LPS and control groups (Table 1). A train of APs enables greater intracellular accumulation of calcium to open calcium-dependent potassium channels responsible for the slow AHP (41). In contrast, slow AHPs were found to be slightly larger and had faster latencies to peak in the LPS group compared with control (Fig. 6, A, i and ii). When slow AHP-dependent accommodation in response to graded depolarization was measured, no differences in spike frequency were noted, suggesting that the increase in AHP amplitude measured was not sufficient to have an effect on spike frequency (Fig. 6B).

Pyramidal cell density. Since the fiber volley is an indication of the amount of presynaptic axon activation (3), the reduced fiber volleys recorded in CA1 in the LPS group would suggest that fewer or less excitable Schaffer collateral axons in the offspring result from maternal infection. We therefore investigated whether fewer CA3 axons result in the offspring after late-gestational maternal LPS treatment by determining the cell density in CA3 (see METHODS). We did not find any differences in CA3 pyramidal cell number between LPS and control groups. In the left hippocampus, 1,101 ± 19 (SD) and 1,100 ± 58 (SD) cells were counted in LPS [n (number of hippocampi) = 3] and control (n = 4) conditions, respectively. Likewise, 1,075 ± 14 (SD) and 1,129 ± 80 (SD) cells were counted in the right hippocampus of LPS (n = 3) and controls (n = 4). The reduction in presynaptic input to CA1 observed in LPS group is probably not due to fewer Schaffer collateral axons but more likely due to decreased excitability of these axons.

Both the increase in the fEPSP (after normalizing to fiber volley) and in the antidromic population spike may be due in part to a larger number of pyramidal neurons in CA1, which was reported in a previous study to occur in mice after maternal LPS administration on E17 (18). We therefore investigated whether such an increase in cell population was also present in the left hippocampus, 1,473 ± 39 (SD) and 1,443 ± 3 (SD) cells were counted in LPS [n (number of hippocampi) = 4] and control (n = 3) conditions, respectively. Likewise, 1,431 ± 29 (SD) and 1,464 ± 5 (SD) cells were counted in the right hippocampus of LPS (n = 3) and controls (n = 3). Thus, in our model, maternal inflammation following LPS administration during late gestation does not have an effect on the number of CA1 pyramidal cells in the hippocampus of the offspring and is probably not contributing to the enhanced fEPSPs and antidromic responses in CA1.

DISCUSSION

Several studies in animals have shown that maternal inflammation during gestation leads to anatomical changes in the hippocampus of offspring and alterations in specific behaviors that depend on that structure (18, 34, 35, 42). However, little is known about how synaptic transmission is altered in the hippocampus to bring about these behavioral abnormalities. In the present study we assessed the effects of maternal infection and fever elicited by LPS on E15 and E16 in rats on 1) hippocampal synaptic transmission and 2) CA1 pyramidal cell excitability in the juvenile offspring. This is the first study to investigate the effects of fever that arise during maternal inflammation, and its effects on hippocampal synaptic transmission in the offspring. Our electrophysiological investigation indicates a decrease in presynaptic transmission with a compensatory increase in postsynaptic excitability specific to the CA1 region.

Importance of distinguishing between LPS-induced fever from hypothermia. In contrast to most other studies, one important variable we have accounted for during maternal infection is fever. We have systematically used pregnant rats that responded with a fever (~57% of dams) instead of hypothermia (~32% of dams), since fever and hypothermia are thought to be very different immunogenetic responses regulated by different cytokines. In humans, fever is regarded as a physiological adaptive response that promotes survival following infection, while hypothermia is a maladaptive response that more often leads to death than does fever (28). Fever is known to be a result of induction of prostaglandins mediated by a number of cytokines including IL-6 (13), while hypothermia has been hypothesized to be mediated through the cytokine TNF-α (28). Given that fever and hypothermia are generated...
through different cytokine pathways with potentially different consequences in the offspring, it was thought to be important to select offspring only from mothers that showed fever after LPS to reduce potential variability.

**Reduced input to CA1 pyramidal cells and postsynaptic compensation.** Field recordings uncovered an enhancement in excitatory synaptic transmission in hippocampal CA1 pyramidal cells from the offspring of LPS-treated dams, as evidenced by larger fEPSPs. Fiber volleys were smaller and required higher stimulation intensity to evoke the same amplitude in the LPS group compared with control. Since the fiber volley is an indication of the amount of presynaptic axon activation (3), this finding would suggest that fewer or less excitable Schaffer collateral axons in the offspring result from maternal infection. However, a reduction in CA3 axon excitability is the most likely explanation, since no difference in CA3 cell density was found in the offspring from LPS-treated mothers. In addition, presynaptic transmitter release is affected because paired-pulse facilitation of the fEPSP was found to be less in the LPS group. The evidence seems to point to a decrease in presynaptic excitability in CA1, which would lead to a reduction in glutamatergic input to CA1 pyramidal cells. Surprisingly, in the LPS group, postsynaptic responses in CA1 were increased when fiber volley amplitudes were normalized. The heightened postsynaptic response was probably not due to a greater number of CA1 pyramidal cells, because cell density in this area was not found to be different between the LPS and control groups. Impairment in postsynaptic GABA<sub>A</sub> receptor function probably did not contribute to this enhancement, since no changes were noted in the paired-pulse depression of the population spike. The increase in the excitatory postsynaptic response could be a result of compensation to the weaker excitatory synaptic input to CA1 and may be caused by increased α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) glutamate receptor number or responsiveness. Similar postsynaptic increases in Schaffer collateral-evoked fEPSPs in CA1 have been previously reported in the hippocampus following a reduction in overall hippocampal activity (15). In that study, the amplitude of miniature excitatory postsynaptic currents was also found to be larger, suggesting that this compensation was mediated at the level of AMPA glutamate receptors. It remains to be determined whether AMPA receptors show any change in our study. Another recent study has reported an increase in the AMPA/NMDA receptor ratio in hippocampal CA1 after maternal LPS administration on E19 (26) without changes in the AMPA receptor-mediated current. It is possible that this later time of LPS injection (see below) and/or differences in thermoregulatory response (e.g., they did not distinguish between fever and hypothermia) could explain the contrasting results.

**Enhanced excitability of CA1 pyramidal cells.** One of the most dramatic changes we observed in the offspring after maternal infection was the substantial increase in CA1 pyramidal cell responses following antidromic stimulation. The enhanced antidromic spike may be explained by a number of factors. For example, it may be the result of a direct increase in the number and/or gating of sodium (Na<sup>+</sup>) channels at the level of the axon because axonal Na<sup>+</sup> channels directly contribute to the antidromic population spike (48). Hence, one factor may be a heightened sensitivity of pyramidal neurons to fire antidromically because of an increase in the number and/or the activation curve of Na<sup>+</sup> channels in the axon resulting in the firing of a greater number of pyramidal neurons. The LPS-mediated effects may be specific to axonic and not somatic Na<sup>+</sup> channels, since no difference was found at the soma level in the spike threshold or spike height (a decrease in soma spike height was actually found) between both groups measured with whole cell recordings. Interestingly, since it is well known that distinct types of Na<sup>+</sup> channel localize the soma and axons of hippocampal pyramidal cells (51), it is tempting to speculate that Na<sup>+</sup> channels at the axon are specifically affected in the LPS group and that this may be due to changes in myelination. Indeed, it has been reported that LPS injected at late gestational stages in rats (9) or mice (49) produces a robust decrease in myelin in the hippocampus of the offspring and demyelination is known to trigger changes in the type and levels of Na<sup>+</sup> channel (50).

We also determined that the AHP size and the number of pyramidal cells probably did not contribute to the enhancement in antidromic excitability, since AHPs were found to be larger and pyramidal cell density was unchanged in the offspring after maternal infection. The only other variable that we measured that may have contributed to the enhanced antidromic population spike was the significantly more depolarized membrane potential observed in the LPS group (~2 mV difference). Hence, this depolarization may participate in the greater excitability of the neurons in the LPS group in response to antidromic activation.

**Reduction in short- but not long-term plasticity.** In the LPS group, we uncovered a deficiency in short-term plasticity as evidenced by the impairment in paired-pulse facilitation of the fEPSP. Since presynaptic short-term plasticity was altered and disturbances in basal synaptic transmission in CA1 pyramidal cells were found in the LPS group, we wondered how LTP in the offspring may be affected by maternal infection. LTP is a cellular model of learning and memory, and its behavioral correlates, such as spatial memory in the Morris water maze and novel-object recognition, have been found to be altered in the offspring after maternal infection (18, 34, 42). We did not find any difference in the amount of LTP achieved in the LPS group vs. control. This finding is in contrast to that reported recently by Lanté et al. (26), who showed LPS given to rat dams on E19 led to an impairment of LTP in the 28-day-old offspring. However, in addition to the later infection time (E19), this study did not control for fever and hypothermia. At E19, hippocampal neurons are migrating or have already migrated to their final locations in the hippocampus, whereas at E15–16 they have just differentiated (7). The fact that different outcomes result from different times of gestational LPS injection suggests that the time window for the inflammation in the mother is critical in determining the appearance and/or extent of the changes seen in the offspring. One study showed that inflammation with polyethylene glycol acid induced early in gestation produced more significant anatomical changes in the hippocampus of the mice offspring than inflammation induced later in gestation (34). It seems that the specific changes in behavior or anatomy depend on the coordination between the time of inflammation and the stage of development of the particular brain structure affected, and earlier gestational inflammation appears to have a more detrimental effect than later (36).
Perspectives and Significance

We have demonstrated that maternal immune stimulation and fever by LPS on E15 and E16 lead to significant consequences in hippocampal synaptic function. The changes observed in the juvenile rats, namely a decrease in presynaptic excitability in CA1, together with an increase in CA1 synaptic transmission and CA1 pyramidal cell excitability, underscores the profound impact that infection during pregnancy could have on the brains of human offspring. It appears that a period of prenatal exposure to inflammation will interfere with normal hippocampal development and contribute to abnormal hippocampal function, thus promoting the cognitive deficits observed in neurodevelopmental disorders such as schizophrenia.

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