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Impairment of long-term depression induced by chronic brain inflammation in rats

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ABSTRACT

Although deficits in synaptic plasticity have been identified in aged or neuroinflamed animals with memory impairments, few studies have examined the cellular basis of plasticity in such animals. Here, we examined whether chronic neuroinflammation altered long-term depression (LTD) and studied the underlying mechanism of LTD impairment by neuroinflammation. Chronic neuroinflammation was induced by administration of lipopolysaccharide (LPS) to the fourth ventricle. Excitatory postsynaptic potentials were recorded extracellularly in the rat hippocampal CA1 area to examine alterations in synaptic plasticity. Chronic administration of LPS induced remarkable memory impairment in the Morris water maze test. *N*-methyl-D-aspartate receptor (NMDAR)-dependent LTD was almost absent in LPS-infused animals. The AMPA receptor (AMPAR)-mediated synaptic response was reduced in the LPS-infused group. These results suggest that reduction in NMDAR-dependent LTD might arise because of alterations in postsynaptic AMPARs *as well as NMDARs* and that such changes may be present in mild and early forms of Alzheimer-type dementia.

Introduction

Chronic neuroinflammation plays an essential role in the pathogenesis of Alzheimer's disease (AD) [1–3]. Recent reports suggest that AD and other forms of dementia arise because of pathological processes in which synaptic loss and dysfunction begin several years prior to severe neuronal loss [4]. A number of experiments have now consistently reported that chronic neuroinflammation can be reproduced in rats by infusion of lipopolysaccharide (LPS) into the fourth ventricle [5–8]. LPS activates microglia to initiate a series of inflammation-induced changes within the hippocampus and entorhinal cortex [5,6].

The *N*-methyl-D-aspartate receptors (NMDARs) play an important role in modulation of excitatory synaptic transmission because of high permeability to calcium ions and an ability to activate downstream calcium-dependent signal transduction processes. Because of the high Ca^{2+} permeability, activation of NMDARs is often the first event in glutamate-induced neuronal injury [9]. The AMPA receptors (AMPARs) are the other major subtype of ligand-gated ionotropic glutamate receptors. AMPARs are essential for synaptic plasticity such as long-term potentiation (LTP) and long-term depression (LTD), both of which are mechanisms involved in learning, memory, and experience-dependent plasticity [10,11].

Although deficits in synaptic plasticity have been identified in neuroinflamed animals with memory impairments [6], few studies have examined the cellular basis for plasticity in such animals. Here, we examine whether chronic neuroinflammation within the hippocampus alters NMDAR-dependent and NMDAR-independent LTD in Schaffer collateral/commissural-CA1 synapses, and investigate alterations in NMDAR- and AMPAR-mediated responses induced by chronic LPS infusion.

Materials and methods

Fourteen male Fisher-344 rats (SLC Inc., Shizuoka, Japan) were housed singly in colony rooms with a 12 h light–dark cycle (lights on at 7 a.m.). Animal experiments were conducted in accordance with approved institutional animal care procedures. Rats were

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anesthetized with isoflurane and placed in a stereotaxic frame (Kopf Instruments, Tujunga, CA) fitted with an isoflurane gas anesthesia system, and an incisor bar was set 3.3 mm below the ear. The scalp was incised and retracted and holes were created at appropriate locations in the skull using a dental drill. The coordinates for fourth ventricle infusions were as follows: 2.5 mm posterior to Lambda on the midline and 7.0 mm ventral to the dura. Either LPS (Sigma, St. Louis, MO; prepared from *Escherichia coli*, serotype 055:B5) or artificial cerebrospinal fluid (aCSF) were chronically infused (0.25 μl/h for 28 days) through a cannula implanted in the fourth ventricle of the brain, which was attached to an osmotic minipump (Alzet, Palo Alto, CA; model 2004), as described elsewhere [12].

Rats were trained to learn the position of the camouflaged escape platform using a standardized procedure that required the use of distal cues in a maze environment [13] in a Morris water maze, as described elsewhere [12]. The animals were subjected to two or three trials per day, with intertrial delays of 60 s, for a total of nine consecutive days. The location of the platform remained constant and in each training trial the rats were allowed to swim for 90 s or until they found the platform. A probe trial (every sixth trial) was conducted 24 h after every fifth training trial to assess the development of spatial bias in the maze; thus, the entire training procedure included four probe trials for each rat. During these probe trials, the rats swam with the platform retracted to the bottom of the pool for 30 s, at which time the platform was raised to its normal position for completion of the trial. At completion of the protocol using the hidden platform, all rats were assessed for cue learning using a visible platform. The location of this platform varied from trial to trial in a single session of six training trials.

After the behavioral test, rats were sacrificed and hippocampal slices were prepared. Under deep halothane anesthesia, brains were removed and transferred to ice-cold dissection buffer. A hippocampal block was removed and sectioned at a thickness of 400 μm using a vibratome. Slices were incubated for 1 h at 33 °C in aCSF composed of 124 mM NaCl, 5 mM KCl, 1.25 mM NaH_2PO_4, 26 mM $NaHCO_3$, 10 mM dextrose, 1.5 mM $MgCl_2$, and 2.5 mM $CaCl_2$. The aCSF was bubbled with a mixture of 5% CO_2 and 95% O_2 (both v/v). All recordings were performed in a submersion recording chamber perfused with aCSF (30 ± 0.5 °C, 2 mL/min).

Synaptic responses were evoked by stimulating the Schaffer collateral/commissural pathway with 0.2 ms pulses delivered through a concentric bipolar stimulating electrode (FHC, Bowdoinham, ME) and were recorded extracellularly in the CA1 stratum radiatum. Baseline responses were measured using a half-maximal stimulation intensity of 0.033 Hz. NMDAR-dependent LTD was induced by a standard 1 Hz (15 min) protocol, and NMDAR-independent LTD was evoked using a paired-pulses with 50 ms interstimulus interval delivered at 1 Hz for 15 min in the presence of an NMDAR antagonist (50 μM D,L-2-amino-5-phosphonopentanoic acid; APV) in the aCSF.

For measurement of paired-pulse facilitation (PPF), we used interstimulus intervals of 25, 50, 100, 200, 400, 1000, and 2000 ms. Input–output curves were generated by gradually increasing stimulus intensity. Pharmacologically isolated NMDAR-mediated synaptic responses were measured using 0 mM $MgCl_2$ aCSF with 10 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX). Fifty micromolar APV was added at the end of each experiment to confirm NMDAR-mediated responses. For the comparison of NMDAR- and AMPAR-mediated synaptic transmission between the two groups, areas under the curves were calculated. All drugs were obtained from Sigma.

One-way repeated ANOVA was conducted to assess the degree of impairment of spatial working memory induced by chronic LPS infusion. Synaptic responses were quantified as the initial slopes of the extracellularly recorded excitatory postsynaptic potentials (EPSPs) in the CA1 and were used as indices of synaptic strength. Only data from slices with stable recordings (<5% change over the baseline period) were included in analysis. All data are presented as averages ± SEMs normalized to preconditioning baselines. For statistical comparisons, LTD magnitude was taken as the average during the last 3 min of recording. Statistical significance was assessed using Student's *t* test or ANOVA, followed by Newman–Keuls *post hoc* analysis. Any *p* values less than 0.05 were considered significant, unless otherwise specified.

Results

Search error was used to assess performance accuracy in the water maze; this technique is described in detail elsewhere [13]. As shown in Fig. 1A, aCSF-infused rats quickly became proficient

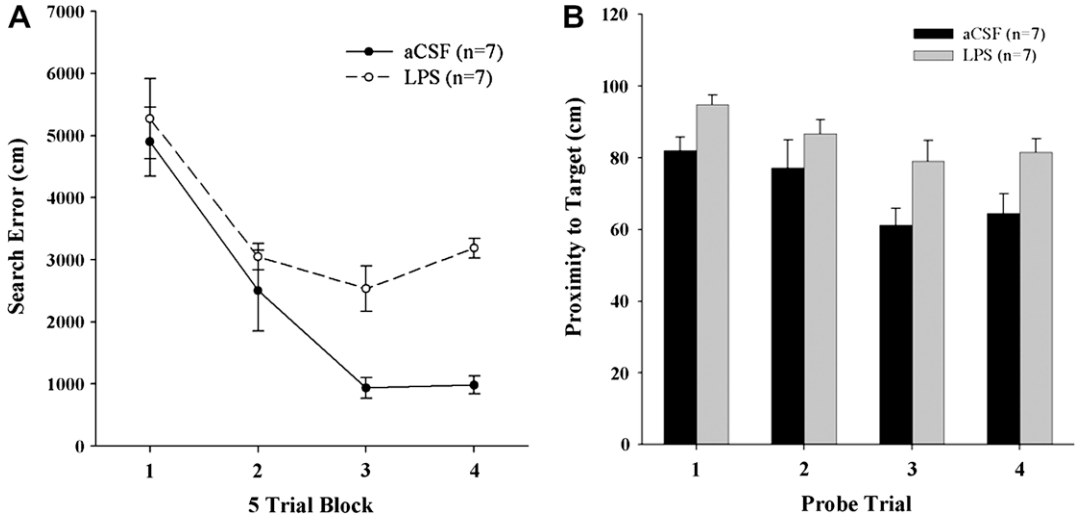

Fig. 1. Performance of aCSF- and LPS-infused rats in the spatial version of a Morris water maze. (A) Search error in finding a hidden platform in a spatial learning task during five training trial blocks. The aCSF-infused rats became proficient at locating the submerged platform during training. The LPS-infused rats did not show any improvement over the course of training when compared with aCSF-infused animals ($p < 0.001$). (B) Proximity-to-target during the 30 s probe trial. The mean distance from target location in the probe trial decreased over the course of training in control rats ($p < 0.05$). In contrast, LPS-infused animals showed no improvement over the course of training. aCSF: aCSF-infused rats; LPS: LPS-infused animals.

at locating the submerged platform during training trials, but LPS-infused rats did not show any improvement with training when compared with control aCSF-infused rats. ANOVA analyses showed that between-group effects (aCSF, LPS) were significant ($F[1,12] = 15.2$, $p < 0.002$) and that training effects (block) were also significant ($F[3,36] = 11.8$, $p < 0.001$). There were significant interaction effects between group and training ($F[3,36] = 3.2$, $p < 0.04$).

There were apparent differences in probe performance trials, as assessed by proximity-to-target and mean distance from target location data. ANOVA analyses showed that between-group effects (aCSF, LPS) were significant ($F[1,12] = 8.9$, $p < 0.012$); and that proximity-to-target (probe) over the course of training decreased significantly in the LPS-infused group but not in controls ($F[3,36] = 35.5$, $p < 0.001$). Interaction effects between group and probe were significant ($F[3,36] = 3.0$, $p < 0.05$). These results suggest that LPS-infused rats failed to locate the hidden platform over the course of training (Fig. 1B).

After completion of the behavioral test, we first measured NMDAR-dependent LTD in the CA1 area of hippocampal slices prepared from behaviorally characterized rats treated with chronic infusions of LPS or aCSF. The magnitude of NMDAR-dependent LTD in the aCSF-infused group was 80.8 ± 2.5% of baseline ($n = 6$). Chronic infusion of LPS into the fourth ventricle for 28 d inhibited NMDAR-dependent LTD The LPS-infused group had lower NMDAR-dependent LTD (95.5 ± 3.6% of baseline; $n = 7$) when compared with the aCSF-infused group 58–60 min after application of a standard 1 Hz, 15 min stimulation ($p < 0.008$; Fig. 2A and B). Short-term depression immediately after stimulation with 1 Hz for 15 min was reduced in the LPS-infused group (LPS-infused group 79.6 ± 3.1% of baseline; aCSF-infused group 54.0 ± 4.5% of baseline, $p < 0.0003$).

Next, we examined the NMDAR-independent form of LTD, induced by delivering paired pulses (50 ms interstimulus interval) in the presence of an NMDAR antagonist (50 μM APV). However, there was no significant between-group difference (aCSF-infused group 87.4 ± 2.7% of baseline; $n = 6$; LPS-infused group 95.7 ± 4.4% of baseline; $n = 5$, $p < 0.55$).

We next determined whether the difference in LTD mechanisms between aCSF- and LPS-infused animals could result from changes in basal synaptic transmission. PPF ratios, reflecting presynaptic changes, were similar in the two groups, suggesting that the reduced NMDAR-dependent LTD observed in the Schaffer collateral/commissural-CA1 synapse may result from an alteration in a postsynaptic rather than a presynaptic event (Fig. 3A).

Input–output responses, which reflect mainly AMPAR-mediated synaptic activity, were reduced in LPS-infused animals (aCSF-infused group 6.2 ± 6.7 arbitrary units, LPS-infused group 4.2 ± 4.2 arbitrary units, $p < 0.019$) (Fig. 3B and D). To examine whether NMDAR-mediated synaptic responses were changed by chronic LPS infusion, we measured pharmacologically isolated NMDAR-mediated synaptic responses using 0 mM $MgCl_2$ aCSF with 10 μM CNQX. Unexpectedly, the NMDAR-mediated synaptic response in the LPS-infused group did not differ from that of the aCSF-infused group.

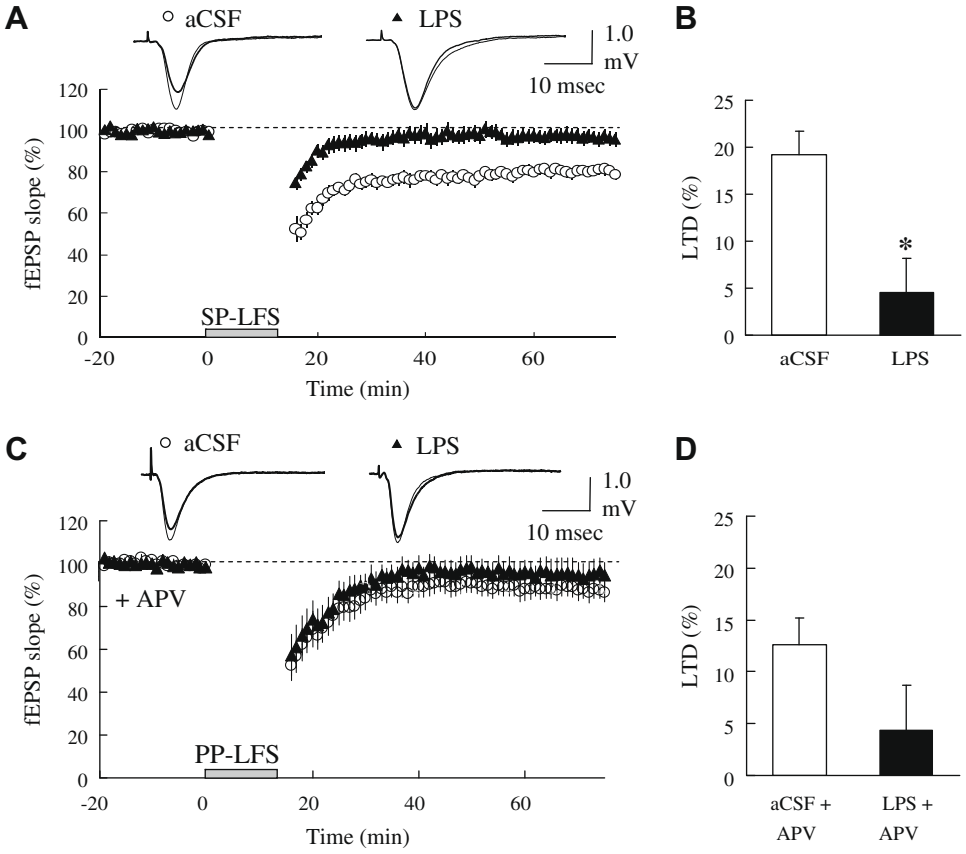

Fig. 2. Impaired NMDAR-dependent LTD after chronic LPS infusion. (A) Field EPSP slope (% of baseline) was plotted against time. NMDAR-dependent LTD was induced by a standard 1 Hz (15 min) protocol (SP-LFS). The magnitude of NMDAR-dependent LTD was reduced in the LPS-infused group. (B and D) Comparison of mean LTD magnitude during the last 3 min after 1 Hz single-pulse or 1 Hz paired-pulse stimulation (PP-LFS). (C) Field EPSP slope (% of baseline) was plotted against time. NMDAR-independent LTD was induced using a paired-pulses with a 50 ms interstimulus interval delivered at 1 Hz for 15 min in the presence of 50 μM APV in the aCSF. Traces are examples of responses recorded 1 min before (thin traces) or 60 min after (thick traces) SP-LFS or PP-LFS. aCSF: aCSF-infused group; LPS: LPS-infused group. Values in parentheses: numbers of slices tested. Error bars indicate SEMs. $^{*}p < 0.008$, compared to the aCSF-infused group.

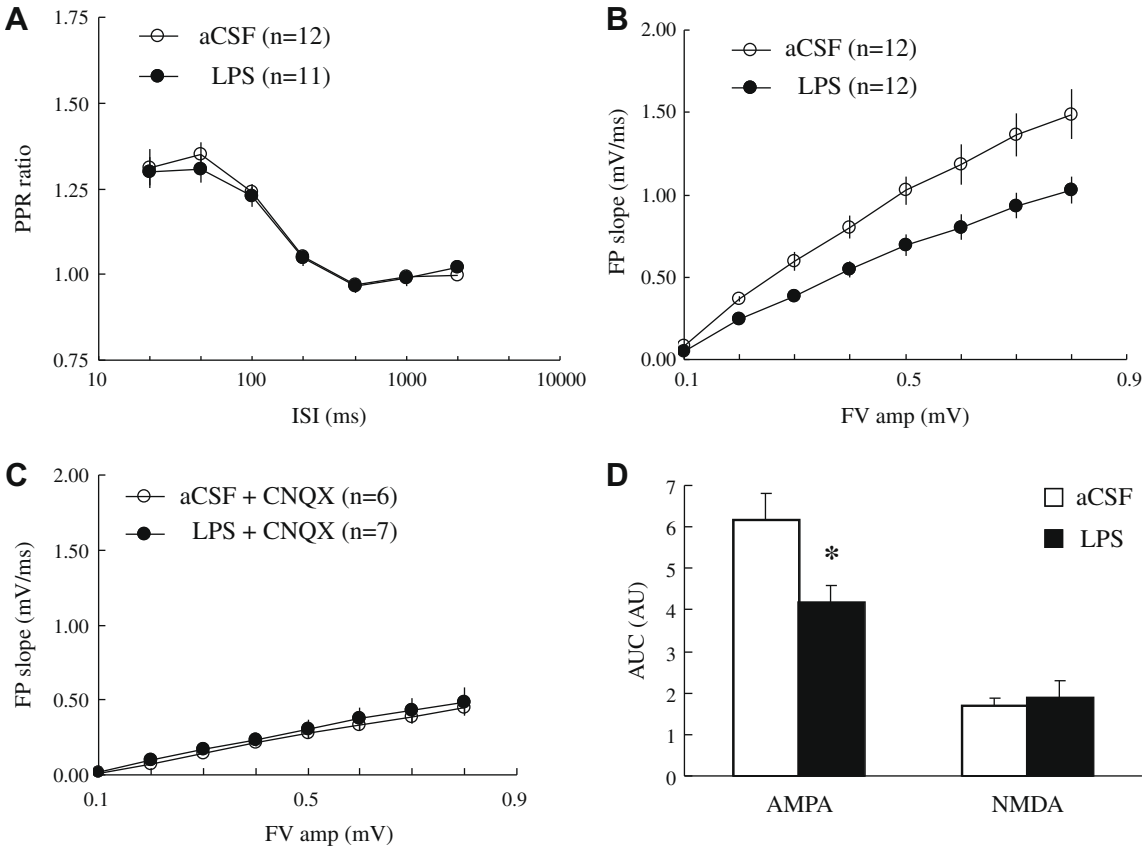

Fig. 3. Impaired AMDAR-mediated synaptic transmission after chronic LPS infusion. (A) Similar paired-pulse facilitation (PPF) ratios in the two groups. (B) AMPAR-mediated basal synaptic transmission in the LPS-infused group was lower than in the aCSF-infused group. (C) No between-group difference in NMPAR-mediated synaptic transmission. (D) Areas under the curves. To compare AMPAR- (B) and NMDAR-mediated synaptic transmission (C), areas under the curves were calculated. aCSF: aCSF-infused group; LPS: LPS-infused group. Values in parentheses: numbers of slices tested. Error bars indicate SEMs. $^{*}p < 0.019$ compared to the aCSF-infused group. AUC: area under the curve, AU: arbitrary unit.

Discussion

The present study shows that chronic infusion of LPS into the fourth ventricle impaired spatial memory of rats as measured in the Morris water maze. This impairment of memory by chronic neuroinflammation is consistent with our previous results, and data from other groups, showing that LPS infusion impairs animal performance on the T-maze alternation task or in the Morris water maze [5,12,14,15].

The impairment in rat memory caused by chronic brain LPS infusion may arise because of synaptic loss and dysfunction prior to severe neuronal loss [4]. The novelty of our finding resides in the fact that NMDAR-dependent LTD was impaired in hippocampal slices from animals treated with LPS, as a consequence of chronic neuroinflammation. The decreased NMDAR-dependent LTD observed in LPS-infused rats may result from either a loss of NMDAR, NMDAR dysfunction or cell death. Our results show that short-term depression which is depend on Ca^{2+} influx through NMDAR was also reduced in animals treated with LPS, suggesting that NMDARs were disrupted by chronic LPS infusion. Biochemical evidence that LPS infusion over 4 weeks reduced the number of NMDAR1-immunoreactive cells within the hilar region of the dentate gyrus and in the pyramidal layer of the CA3 area without evidence of neuronal loss [8] are also in line with the present results.

As a consequence of NMDAR dysfunction, Ca^{2+} influx and extrusion across the plasma membrane, cytosolic buffering, and uptake into organelles, may be altered in neurons. Our (unpublished) data showing that voltage dependent calcium channel-dependent LTP in LPS-infused rats was impaired support this idea. However, it remains to be determined whether diminished NMDAR-dependent LTD in LPS-infused animals results from a change in activity required for NMDAR recruitment or from downregulation of the machinery coupling NMDARs to LTD and further studies such as determining the protein levels of NMDAR and AMPAR in the neuron and/or glial cell are required.

However, pharmacologically isolated NMDAR-mediated synaptic transmission in LPS-infused animals did not differ from that in aCSF-infused rats in the current study. This result appears difficult to reconcile with our finding of impaired NMDAR-dependent LTD, but may be understood when it is considered that AMPARs predominantly mediate basal synaptic transmission whereas NMDARs contribute minimally to the basal synaptic response because of their strong voltage dependence. The reduced AMPAR-mediated synaptic response seen in the present study may result from increased removal or decreased function of AMPARs induced by chronic brain inflammation. The expression of LTP is believed to be enhanced by increased insertion of AMPARs into postsynaptic membranes, and functional enhancement, whereas LTD expression requires removal and/or functional inhibition of these receptors [11,16,17]. Recent reports using transgenic AD animal models strongly suggest that decline in AMPAR-mediated synaptic transmission mediated by Aβ results in synaptic failure [18].

The present results show that chronic neuroinflammation led to impairment of spatial memory and attenuation of NMDAR-dependent LTD The results suggest that reduced NMDAR-dependent LTD might arise because of alterations in postsynaptic AMPARs *as well*

as NMDARs and that such alterations may underlie mild and early forms of Alzheimer-type dementia.

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