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Chemokine CCL2 modulation of neuronal excitability and synaptic transmission in rat hippocampal slices

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Abstract

In addition to its well-characterized effects in immune system, chemokine CC motif ligand 2 (CCL2, formerly known as monocyte chemoattractant protein-1) is believed to play an important role in brain physiological and pathological processes. It has been shown that CCL2 and its cognate receptor CCR2 are constitutively expressed in several brain regions including the hippocampus, and the expression is up-regulated under pathological conditions. Whereas most investigations have so far focused on its involvement in CNS pathology, few studies have examined the effects of CCL2 on neuronal and synaptic physiology. In this study, we tested the effects of CCL2 on neuronal excitability and excitatory synaptic transmission in the CA1 region of rat hippocampal slices using whole-cell patch clamp techniques. Bath application of CCL2 depolarized membrane potential and increased spike firing in CA neuronal cells. Bath application of CCL2 also produced an increase of excitatory postsynaptic currents (EPSCs) recorded in Schaffer-collateral fibers to CA1 synapses. Quantal analysis revealed that CCL2 increased the frequency of spontaneous EPSC occurrence and mean quantal content. Taken together, our data indicate that CCL2 enhances neuronal excitability and synaptic transmission via presynaptic mechanisms. These results support the emerging concept that chemokines function as neuromodulators in the CNS.

Keywords

chemokine; CCL2; EPSCs; synaptic transmission; hippocampal slices

Introduction

Chemokines constitute a large family of chemoattractant cytokines that have been classified into four subfamilies C, CC, CXC and CX3C based on the number and spacing of the conserved cysteine residues in the N-terminus of the proteins (Murphy *et al.* 2000). Chemokine CC motif ligand 2 (CCL2, known previously as monocyte chemoattractant protein-1) is a well-characterized β or CC family member of chemokines with powerful effects on the recruitment of cells of monocytic origin to sites of inflammation or injury (Fuentes *et al.* 1995, Gu *et al.* 1999, Gangur *et al.* 2002, Cartier *et al.* 2005, Deshmane *et al.* 2009). Besides its well-established role in the immune system, increasing evidence indicate

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that CCL2 may play a role in the central nervous system (CNS) (Gerard & Rollins 2001, de Haas *et al.* 2007, Melik-Parsadaniantz & Rostene 2008). Studies have shown that CCL2 is expressed in the CNS, and its expression levels are elevated in brain astrocytes and microglia under pathological conditions. The elevated CCL2 is an important mediator of the neuroinflammatory responses in brain trauma (Glabinski *et al.* 1996), ischemic brain injury (Kim *et al.* 1995, Minami & Satoh 2003) and various neurodegenerative disorders including, but not limited to, multiple sclerosis (Simpson *et al.* 1998, Mahad & Ransohoff 2003), Alzheimer's disease (Ishizuka *et al.* 1997, Sokolova *et al.* 2009) and human immunodeficiency virus type 1 (HIV-1)-associated dementia (Conant *et al.* 1998, Gonzalez *et al.* 2002, Zink *et al.* 2001, Dhillon *et al.* 2008). The biological effects of CCL2 are mediated via interaction with its cognate receptor, chemokine CC motif receptor 2 (CCR2), one of the most prominent chemokine receptors associated with neuroinflammatory processes in the CNS (Banisadr *et al.* 2002b, Sokolova *et al.* 2009).

Recent studies have shown that CCL2 is constitutively expressed in neurons of the CNS (Banisadr *et al.* 2005a, de Haas *et al.* 2007, Melik-Parsadaniantz & Rostene 2008). The expression of CCL2 was observed in the NT2 neuronal cell line (Coughlan *et al.* 2000) and in developing human neurons (Meng *et al.* 1999). Animal studies have shown that CCL2 and CCR2 are constitutively expressed in CNS neurons (Banisadr *et al.* 2005a, Banisadr *et al.* 2005b, Gosselin *et al.* 2005). CCL2 mRNA and proteins were detected in different CNS regions of normal adult rats (Banisadr *et al.* 2005b, Gosselin *et al.* 2005). Immunohistochemical studies further revealed that CCL2 is constitutively expressed in neurons of discrete brain regions such as cerebral cortex, hippocampus hypothalamus, substantia nigra, cerebellum and spinal cord (Banisadr *et al.* 2005a, Gosselin *et al.* 2005). Most importantly, the expression of CCL2 is co-localized with classical neurotransmitters (Banisadr *et al.* 2005a), and cell membrane depolarization can evoke Ca²⁺-dependent release of CCL2 (Jung *et al.* 2008, Dansereau *et al.* 2008). These results suggest that CCL2 may function as a neuromodulator in the CNS (Jung *et al.* 2008, Melik-Parsadaniantz & Rostene 2008). However, very few published studies have, to our knowledge, investigated the effects of CCL2 on neuronal and synaptic physiology in normal brain. To this end, we studied effects of CCL2 on neuronal excitability and excitatory synaptic transmission in the CA1 region of rat hippocampal brain slices. Our results showed that CCL2 depolarized neuronal membrane potential, enhanced spike firing and excitatory postsynaptic currents (EPSCs). Parts of these results were presented in abstract form at the 2009 Society for Neuroscience 39th Annual Meeting in Chicago, IL, USA.

Materials and Methods

Chemicals and reagents

Chemicals used for making artificial cerebrospinal fluid (ACSF) were purchased from Sigma-Aldrich (St. Louis, MO). Drugs used in this study were CCL2 (R&D Systems, Minneapolis, MN), picrotoxin, tetrodotoxin (TTX), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), and DL-2-amino-5-phosphonopentanoic acid (APV), RS102895, and picrotoxin. Picrotoxin and RS102895 were dissolved in dimethyl sulfoxide (DMSO), and the final DMSO concentration in ACSF was less than or equal to 0.1%. CCL2, TTX, CNQX and APV were first dissolved in distilled water at 1000× and then diluted in ACSF to the final concentration immediately before use. Drugs were applied via bath perfusion and the time necessary for the drug solution to reach the surface of the hippocampal slice was about 1 min. All drugs were purchased from Sigma-Aldrich unless otherwise indicated.

Animals

Fifteen to 30 d old male Sprague-Dawley rats used for electrophysiological and immunohistochemical experiments were purchased from Charles River Laboratories (Wilmington, MA). Animals were housed at constant temperature (22°C) and relative humidity (50%) under a regular light-dark cycle (light on at 7 AM and off at 5 PM) with free access to food and water. All animal use procedures were strictly reviewed by the Institutional Animal Care and Use Committee (IACUC) of the University of Nebraska Medical Center (IACUC No. 00-062-07).

Hippocampal brain slices

Hippocampal brain slices were prepared as previously described (Xiong *et al.* 1996). Briefly, animals were anesthetized with isoflurane and decapitated. The brains were quickly removed from cranial cavity and placed into an ice-cold (~4°C) oxygenated ACSF containing (in mM): NaCl 124.0, KCl 3.0, CaCl₂ 2.0, MgCl₂ 2.0, NaH₂PO₄ 1.25, NaHCO₃ 26.0, and glucose 10.0. ACSF was saturated with 95% O₂ and 5% CO₂ and had a pH of 7.35–7.45. The hippocampi were dissected free, and transverse hippocampal slices (400 µm in thickness) were cut using a tissue chopper. The slices were kept in continuously oxygenated ACSF at room temperature for at least 1 h before use.

Electrophysiology

During electrophysiological experiments, single hippocampal slices were transferred into the recording chamber and superfused with ACSF at a constant flow rate of 2.5 ml/min. The temperature of the ACSF was maintained at 30°C ± 1°C with an automatic temperature controller (Warner Instrument Corp., Hamden, CT). Whole-cell patch clamp recordings were made from CA1 neurons using a “blind” method. The neuronal cells recorded were voltage-clamped at –70mV and EPSCs were evoked by electrical stimulation of the Schaffer collateral/commissural pathway (0.05 Hz, 40 µs, 30–100 µA) with a bipolar tungsten electrode insulated except the tip and amplified by an Axopatch-1D amplifier (Axon Instruments, Inc., Union City, CA). A constant negative voltage step was applied to the recording electrode 100 ms preceding the stimulation pulse to monitor accessing resistance. The recording electrodes were made from borosilicate glass capillaries (World Precision Instruments, Sarasota, FL) and had a resistance of 6–9 MΩ when filled with pipette solution containing (in mM): K-gluconate 130.0, K-methylsulphate 17.5, NaCl 8.0, HEPES 10.0, Mg-ATP 2.0, GTP 0.2, EGTA 0.1, pH 7.25–7.35 (adjusted with KOH), osmolarity 292–308 mOsm. Picrotoxin (50µM) was routinely added to ACSF to block GABA_A receptor-mediated inhibitory postsynaptic currents.

Cells with resting membrane potential more negative than –50 mV were used for the study. Each recording trial consisted of an average of three consecutive sweeps. Data were low-pass filtered at 1 kHz and digitized at 5 kHz using a Digidata 1322A interface (Axon Instruments, Inc.). pCLAMP 8.1 software (Axon Instruments, Inc.) was used for data acquisition and analyses. During recording, a hyperpolarizing voltage pulse was delivered to constantly monitor series resistance, and the cells with > 20% changes in series resistance were excluded from the analysis. Also, cells with accessing resistance > 20 MΩ at any time during the recording were excluded from the analysis. The sample size (n) in each experimental group represents the number cells recorded from different hippocampal slices prepared from animals. Only one cell was tested in a slice after the whole-cell recording was successfully made. For quantal analysis, both spontaneous mini EPSCs (sEPSCs) and electrically evoked EPSCs (eEPSCs) were recorded. The eEPSCs were generated using sub-threshold stimulation intensity (0.2Hz, with ~50% failures) and at least 360 sweeps were recorded in each cell. The peak amplitudes of both eEPSCs (a_{pk}) and sEPSCs (q_{pk}) were

measured and analyzed. The mean quantal content (m_{pk}) was calculated as $m_{pk} = a_{pk} / q_{pk}$ (del Castillo & Katz 1954, Boyd & Martin 1956).

Data Analyses

Data were analyzed and displayed using Clampfit 8.1 (Axon Instruments, Inc.), Mini Analysis Program (Synaptosoft Inc, Decatur, GA,) and Origin 7.5 (OriginLab® Northampton, MA). All numerical data was expressed as mean $\pm$ SEM unless otherwise indicated. Statistical significance was determined using ANOVA, two-tailed Student's *t*-test, or the Kolmogorov–Smirnov test. A minimum *p* value of 0.05 was estimated as the significance level for all tests.

Results

Effects of CCL2 on neuronal passive membrane properties and electrical activities

Although CCL2 has been shown to be expressed in neuronal cells in the CNS, few studies have investigated its influence on neuronal passive properties and electrical activities. To these ends, we examined the effects of CCL2 on neurons recorded in the CA1 region of rat hippocampal slices using patch clamp techniques in a whole-cell recording configuration. Membrane potentials were recorded from the CA1 neurons in a current clamp mode. After rupture of patches, the recorded neuronal cells were allowed to stabilize for 3–5 min before experimental tests. The stabilized CA1 neurons exhibited resting membrane potentials between -52 mV and -68 mV with an average of -61.5 ± 4.2 mV (mean $\pm$ SD, $n = 62$). Bath application of CCL2 (2.3 nM) produced membrane depolarization in 10 out of 14 cells recorded. The average membrane potentials before and during bath application of CCL2 were -60.8 ± 3.8 mV and -56.4 ± 4.1 mV, respectively, with an average change of membrane potential of 4.4 ± 0.09 mV (mean $\pm$ SD, $n=10$, Figs. 1A, 1B). The difference is statistically significant ($p < 0.05$), indicating that CCL2 depolarizes the CA1 neuronal membrane. The CCL2-induced depolarization peaked at 7–10 min and lasted for 10–18 min. In addition to its depolarizing effect, CCL2 was found to cause slight membrane hyperpolarization in two cells, which was blocked by picrotoxin (20 μ M) and had no effects on another two cells (data not shown). While it depolarizes cell membrane, CCL2 had no apparent effects on membrane input resistance as monitored by injecting constant hyperpolarizing current pulses (Fig. 1C). The average input resistances before and during bath application of CCL2 were 202.1 ± 16.6 M Ω and 190.0 ± 16.9 M Ω ; the difference is not statistically significant (mean $\pm$ SD, $p > 0.05$, $n = 10$). Moreover, CCL2 was also found to increase neuronal spontaneous spike firing. As shown in Fig. 2, the average spike firing frequency was 0.99 ± 0.32 Hz before application of CCL2 (control, $n=7$). In contrast, bath application of CCL2 increased the spike firing frequency to an average of 2.37 ± 0.24 Hz ($n=7$). The spike firings returned to the level close to the control after washout of CCL2, with an average of 1.12 ± 0.15 Hz. The difference is statistically significant ($p < 0.05$), indicating CCL2 enhancement of neuronal firing in the hippocampus. To further examine the effects of CCL2 on neuronal electrical activity, we analyzed the effects of CCL2 on latency, amplitude, duration (measured at the halfway of AP amplitude) and after hyperpolarization (AHP) of the APs evoked by a depolarizing current pulse. As shown in Table 1, CCL2 had no significant effects on these parameters.

Enhancement of EPSCs by CCL2 in hippocampal slices

The results presented above showed that CCL2 depolarizes neuronal membrane and increases neuronal firing frequency. To further determine its effects on synaptic transmission we tested the effects of CCL2 on the EPSCs recorded in the Schaffer-collateral to the CA1 synapses. After establishment of whole-cell patch recording on the CA1 neurons, the cells were voltage-clamped to -70 mV (in some cells to -75 or -80 mV) to suppress

spontaneous spike currents; and the EPSCs were evoked by electrical stimulation of Schaffer-collateral pathway. Bath perfusion of hippocampal slices with CCL2 produced an increase of EPSCs (Fig. 3A). The CCL2-mediated increase of EPSCs was dose dependent and appeared 3–5 min after CCL2 reached the chamber. It peaked at 10–15 min and washed out in 15–25 min. At concentrations of 0.023 nM, 0.23 nM and 2.3 nM, the resultant percentages of increase on EPSCs magnitudes were $101.8\% \pm 6.1\%$ ($n = 5$), $126.5\% \pm 13.8\%$ ($n = 7$), $194.8\% \pm 13.1\%$ ($n = 17$), respectively (Fig. 3B). In contrast, bath application of heat-inactivated (boiled) CCL2 had no apparent effect on EPSCs (Fig. 3C, $n=6$). As the concentration of 2.3 nM was found to significantly increase EPSPs ($p < 0.05$, Fig. 3C), this concentration was adopted for the subsequent tests.

CCL2-induced enhancement of EPSCs was blocked by NMDA and AMPA receptor antagonists

As excitatory synaptic transmission in the hippocampus is primarily mediated by AMPA and NMDA receptors, we next examined whether the CCL2-induced enhancement of EPSCs can be blocked by co-application of AMPA and NMDA receptor antagonists. As shown in Fig. 4, co-application of AMPA receptor antagonist CNQX and NMDA receptor antagonist APV blocked EPSCs and CCL2-associated enhancement of EPSCs. The blockade of CCL2 enhancement of the EPSCs was observed in all 9 cells tested, indicating that CCL2 enhances glutamatergic synaptic transmission in the CA1 region of rat hippocampus.

Blockade of CCL2-induced enhancement of EPSCs by a CCR2 antagonist RS102895

CCR2, a cognate chemokine receptor for CCL2, is expressed in the hippocampus. To examine whether CCL2-associated enhancement of EPSCs is mediated via CCR2, we tested effects of RS102895, a specific CCR2 antagonist with an IC_{50} of 0.36 μ M for inhibition of human recombinant CCR2 (www.scbt.com/datasheet-204243-rs-102895.html), on CCL2-induced enhancement of EPSCs. While it had no apparent effects on EPSCs when applied alone by bath (Fig. 5), RS102895 (10 μ M) significantly blocked CCL2-induced increase of EPSCs ($n = 8$, $p > 0.05$, Fig. 5), indicating that CCL2 increases the EPSCs via CCR2Rs.

CCL2 increases occurrence of sEPSCs and decreases failure of eEPSCs

As CCL2 exhibited a clear action in enhancing the EPSCs recorded in the CA1 region of hippocampal slices, we next performed quantal analyses to determine whether CCL2 acts on presynaptic terminal or postsynaptic membrane, or both. sEPSCs and eEPSCs were recorded and the effects of CCL2 on sEPSCs and eEPSCs were examined. The neuronal cells recorded in the CA1 region exhibited sEPSCs while the membrane potential was held potential at -70 mV (Fig. 6). Bath application of CCL2 increased the frequency of the sEPSC occurrence, but not the amplitude (Fig. 6). The average frequency of sEPSC occurrence and sEPSC amplitude recorded before and during bath application of CCL2 (2.3 nM, 30 min) were 3.22 ± 0.23 Hz and 6.44 ± 0.56 Hz, 9.32 ± 0.46 pA and 9.86 ± 0.56 pA, respectively ($n = 10$). Statistical analysis indicates that bath application of CCL2 significantly increased the frequency of sEPSC occurrence but not the amplitude (Fig. 6, $n = 10$). The frequency of sEPSC occurrence recorded during bath application of CCL2 was as much as 2 times higher than that recorded before application of CCL2, with the most dramatic increase of those sEPSCs with amplitudes between 3 – 9 pA (Fig. 6B, 6E right). In contrast, the mean amplitude of sEPSCs did not change significantly as illustrated in the cumulative probability plot (Fig. 6D, also Fig. 6E left). These results suggest that CCL2 enhances EPSCs via presynaptic mechanisms. We also measured the eEPSCs at near threshold stimulus intensities to reveal failures of synaptic transmission and quantal fluctuations. Bath application of CCL2 decreased the number of failures from 153.7 ± 15.4 in control group ($n = 5$) to 106.4 ± 21.8 in CCL2-treated group ($n = 5$) and increased the mean quantal content from 1.45 ± 0.25 ($n = 5$) examined in control to 2.05 ± 0.34 ($n = 5$)

measured during bath application of CCL2. The differences are statistically significant (mean $\pm$ SD, $p < 0.05$), indicating that CCL2 enhances synaptic transmission by increasing neurotransmitter release.

4. Discussion

It has become evident that chemokine CCL2 and its receptor CCR2 are constitutively expressed by not only glial cells but also neurons, suggesting that they might play a role in the CNS (de Haas et al. 2007, Melik-Parsadaniantz & Rostene 2008). Although increasing evidence indicates that this chemokine is an important mediator for induction of neuroinflammatory responses in a variety of CNS pathological conditions, the functions of CCL2 in normal brain are poorly understood. In the present study, we observed that bath application of CCL2 depolarized membrane potential, increased neuronal spike firing and enhanced both spontaneous and evoked EPSCs in the CA1 region of rat hippocampal slices. In addition to its excitatory effects on hippocampal neurons, we have also observed for the first time that CCL2 enhanced the EPSCs recorded in the Schaffer-collateral to CA1 synapses via pre-synaptic mechanisms.

Despite its well-characterized expression and function in immune system, recent studies have shown that CCL2 and its receptor CCR2 are constitutively expressed in the nervous system (Banisadr et al. 2005a, Banisadr et al. 2005b, Gosselin et al. 2005, Jung et al. 2008). While the functional significance of neuronal expression of CCL2 remains to be determined, several lines of evidence indicate that CCL2 may function as a neuromodulator in the CNS (Jung et al. 2008, Melik-Parsadaniantz & Rostene 2008). It has been shown that CCL2 modulates Ca^{2+} dynamics and electrophysiological properties of cultured cerebellar purkinje neurons (van Gassen *et al.* 2005), inhibits GABA-mediated response in cultured spinal neurons (Gosselin et al. 2005), potentiates NMDA- and AMPA-induced inward currents and enhances spontaneous EPSCs in spinal slices (Gao *et al.* 2009). It has also been shown that CCL2 enhances excitability of nociceptive neurons via depolarization of membrane potential, reduction of rheobase and accommodation in response to current injection, inhibition of voltage-gated, non-inactivating K current (Sun *et al.* 2006). Our results revealed that CCL2 excites hippocampal neurons in vitro and heat-inactivated (boiled) CCL2 failed to produce such an excitation. The excitatory effects of CCL2 we observed in rat hippocampal slices are similar to those previously observed in neurons of dorsal root ganglion (White *et al.* 2005, Sun et al. 2006) and spinal cord (Gao et al. 2009). Our results strongly support the emerging concept that CCL2 may function as a neuromodulator in the CNS (Melik-Parsadaniantz & Rostene 2008, Jung et al. 2008).

To investigate CCL2 modulation of synaptic transmission in the CNS, we tested the effects of CCL2 on electrically evoked EPSCs recorded in the CA1 region of rat hippocampal brain slices. Bath perfusion of hippocampal slices with CCL2 produced an enhancement of the EPSCs in a dose-dependent manner. The CCL2-induced enhancement of the evoked EPSCs was blocked by CCR2 receptor antagonist RS102895. This indicates that CCL2 enhances the EPSCs via CCR2, a chemokine receptor specifically expressed in the hippocampus (van der Meer *et al.* 2000, Banisadr *et al.* 2002a, Banisadr et al. 2005b).

Because excitatory synaptic transmission in the hippocampus is mainly mediated via AMPA and NMDA receptors, we further examined whether the CCL2-induced enhancement of EPSC can be blocked by AMPA and NMDA receptor antagonists. Our results showed that addition of both AMPA receptor antagonist CNQX and NMDA receptor APV to the perfusate completely blocked CCL2-mediated increase of EPSCs. This suggests that CCL2 may modulate excitatory synaptic transmission in the hippocampus. Nevertheless, the

potency of CCL2 in the modulation of AMPA-mediated EPSCs and NMDA receptor-mediated EPSCs were not investigated in the present studies.

The attribution of CCL2 action site to presynaptic terminals was demonstrated by quantal analysis of synaptic transmission in the CA1 region of hippocampal slices. Our data clearly showed that CCL2 significantly increases the mean frequency of sEPSC occurrence without alteration of the mean amplitude. Our results also revealed that CCL2 increases quantal content of synaptic transmission. The frequency of sEPSCs depends on the probability of releasing excitatory neurotransmitters from presynaptic terminals (del Castillo & Katz 1954); whereas, the amplitude of sEPSCs is dependent on several factors such as the amount of transmitter released, the postsynaptic sensitivity, and the driving force for the ions conducting the EPSCs (Van der Kloot 1991). Our observations of an increase in the frequency of sEPSC and mean quantal content strongly indicate that CCL2 acts on presynaptic site. However, it is not clear whether CCL2 acts directly on presynaptic terminals, via an inhibitory interneuron, or by inhibition of glial cell uptake of glutamate. A hypothetical model of CCL2 enhancement of EPSCs is shown in Fig. 7.

The biological significance of CCL2 enhancement of neuronal excitability and synaptic transmission remains to be determined. Recent studies have shown that CCL2 and its cognate receptor CCR2 are constitutively expressed in regionalized neuronal cells including hippocampal neurons and co-localized with neurotransmitters (Banisadr et al. 2005a, Banisadr et al. 2005b), therefore, suggesting that CCL2 may function as a neuromodulator in the nervous system (Melik-Parsadaniantz & Rostene 2008). Indeed, CCL2 was found to modulate Ca^{2+} dynamics and electrophysiological properties of cultured cerebellar Purkinje cells (van Gassen et al. 2005) and to increase dopaminergic neuronal excitability and resultant dopamine release (Guyon *et al.* 2009). In dorsal root ganglia neurons, CCL2 was found to be packaged into large dense-core vesicles whose release could be induced from the soma by depolarization in a Ca^{2+} -dependent manner (Jung et al. 2008). These findings, together with the results reported in this study, strongly support the emerging concept that chemokines function as neuromodulators in the nervous system (Melik-Parsadaniantz & Rostene 2008, Jung et al. 2008). If this concept is well supported by more experimental results, it will not only open our understanding of the neuromodulatory role that CCL2, and perhaps other chemokines, play in health and disease, but also provide target(s) for the development of therapeutic potentials.

In summary, we have, for the first time, demonstrated that CCL2 enhances neuronal excitability and EPSCs in the CA1 region of rat hippocampal slices. The CCL2 enhancement of EPSCs was blocked by a CCL2 antagonist RS102895, indicating that CCL2 mediates its excitatory effects on synaptic transmission via its cognate receptor CCR2. Quantal analysis revealed that CCL2 enhanced EPSCs via presynaptic mechanisms as it increased the mean frequency of sEPSCs occurrence and mean quantal content without change of the mean amplitude of sEPSCs. Although the biological significance of CCL2 enhancement of EPSCs is unclear at present, it may represent a neuromodulatory action for CCL2 in the nervous system (Melik-Parsadaniantz & Rostene 2008, Jung et al. 2008).

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Abbreviations used

CCL	CC chemokine ligand
CCR	CC chemokine receptor
ACSF	artificial cerebrospinal fluid
EPSC	excitatory postsynaptic current
sEPSCs	spontaneous mini EPSCs
ePSCs	electrically evoked EPSCs
AMPA	α -amino-3-hydroxyl-5-methyl-4-isoxazole-propionate receptor
NMDAR	<i>N</i> -methyl- <i>D</i> -aspartate receptor
CNQX	6-cyano-7-nitroquinoxaline-2,3-dione
APV	2-amino-5-phosphonovalerate
AHP	after hyperpolarization
TTX	tetrodotoxin
DMSO	dimethyl sulfoxide

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Figure 1.

Bath application of CCL2 produced neuronal membrane potential depolarization. **A.** A representative whole-cell recording of membrane potential from a CA1 neuron in rat hippocampal slice showing membrane potential depolarization induced by bath application of CCL2 (2.3 nM). Down deflections were membrane voltage changes in response to constant negative current injection. The horizontal bar above the membrane potential trace indicates the time of bath application of CCL2. **B.** Summarized bar graph illustrating averaged membrane potentials measured before CCL2 application (Ctrl), at peak of depolarization (CCL2) and after washout (washout) of CCL2. Note that CCL2 produced a significant change in membrane potential. **C.** CCL2 had no significant effect on membrane input resistance. * $p < 0.05$ compared with control, $n=10$.

Figure 2.

CCL2 increases spontaneous spike firings. **A.** An example of CCL2-induced increase of spontaneous spike firings recorded from a CA1 neuronal cell in a rat hippocampal slice before (Ctrl), during (CCL2) and after (washout) bath perfusion of CCL2. Bath application of CCL2 produced an increase of spike firings and the spike firing returned to control level after washout of CCL2. **B.** A summary bar graph showing that the CA1 neuronal spike firing frequency was significantly increased following bath application of CCL2. * $p < 0.05$ compared with control, $n=7$.

Figure 3.

Enhancement of EPSCs by CCL2 in the CA1 region of hippocampal slices. **A.** Time course and amplitude (% of basal) of the EPSCs recorded from a neuronal cell in the CA1 region in response to constant current pulse stimulation of Schaffer-collateral fibers (250 μ A, 20 μ s, 0.05Hz). Each data point in the graph plots the average of the peak amplitudes of three consecutive EPSCs. Note that bath application of CCL2 (2.3 nM), as indicated by a horizontal bar, increased EPSCs. Above this graph are representative individual EPSCs taken from different time points as marked by letters *a*, *b* and *c*, respectively. **B.** A dose-responsive curve showing that CCL2-induced enhancement of EPSCs was in a dose-dependent manner. The bar graph in **C** shows the average of EPSC amplitudes taken before, during and after bath application of CCL2 (2.3 nM). Note CCL2 significantly increased EPSCs recorded from the CA1 neurons in the hippocampal slices ($n=17$). * $p < 0.05$.

Figure 4.

An example showing CCL2 enhancement of EPSCs was blocked by co-application of NMDA and AMPA receptor antagonists. All current traces were recorded from the same cell with a hold potential of -70mV . Bath co-application of APV ($50\text{ }\mu\text{M}$) and CNQX $10\text{ }\mu\text{M}$ completely blocked the effects of CCL2 enhancement of EPSCs.

Figure 5.

Blockade of CCL2-mediated enhancement of EPSCs by a CCR2 receptor antagonist RS102895. **A.** Time course showing that CCL2 (2.3 nM) failed to increase EPSCs in the presence of CCR2 receptor blocker RS102895 (10 μM) in the perfusate. The representative individual EPSCs taken from different time points are shown above the time course. The bar graph (**B**) displays average EPSC amplitudes (% of basal) measured at different testing periods as indicated by numbers 1–4. While it alone had no apparent effect on EPSCs, bath perfusion of hippocampal slices with RS102895 (RS) significantly blocked CCL2-induced enhancement of EPSCs ($p > 0.05$, $n=8$). Holding potential: -70mV .

Figure 6.

CCL2 enhances EPSCs via presynaptic mechanisms. **A.** Example traces of sEPSCs recorded from a rat CA1 pyramidal neuron. Note that CCL2 increased the frequency of sEPSC occurrence. **B.** Histograms of the frequency of sEPSC events recorded from the same CA1 pyramidal neuron as shown in **A**. CCL2 significantly increased the frequency of sEPSCs ($p < 0.05$). $V_h = -70\text{mV}$. **C.** The eEPSC amplitude distributions before and during bath application of CCL2 on the same neuron as shown in **A**. CCL2 decreased the number of failures of eEPSCs. The mean amplitudes were $9.32 \pm 0.46\text{ pA}$ and $9.86 \pm 0.56\text{ pA}$, respectively. **D.** Cumulative amplitude distribution of sEPSCs before (□), during (●) and after (Δ) perfusion of CCL2. (Kolmogorov–Smirnov test, $p > 0.05$). **E.** Averaged data from 5 experiments showing that CCL2 had no significant effects on mean amplitude of sEPSCs (left two bars), but it significantly increased mean frequency of sEPSC occurrence. These results indicate a presynaptic site of CCL2 action.

Figure 7.
A schematic diagram illustrating potential mechanisms underlying CCL2-induced enhancement of EPSCs in the hippocampus.

Table 1

Effects of CCL2 on evoked electrical activities in CA1 neurons

	Latency (ms)	AP amplitude (mV)	AP duration (ms)	AHP (mV)
Control	18.9 ± 11.1	70.3 ± 6.4	1.6 ± 0.6	6.6 ± 5.9
CCL2	23.4 ± 15.1	72.6 ± 9.6	1.8 ± 0.4	4.2 ± 3.3
Washout	28.3 ± 17.5	71.7 ± 10.3	1.5 ± 0.4	3.4 ± 0.3

Values are mean ± SEM (n=7). There was no statistically significant difference between control and CCL2 on latency, amplitude, duration (measured at the halfway of AP amplitude) and after hyperpolarization (AHP) of the APs evoked by a depolarizing current pulse. AP: action potential.