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# A Microglia-Cytokine Axis to Modulate Synaptic Connectivity and Function

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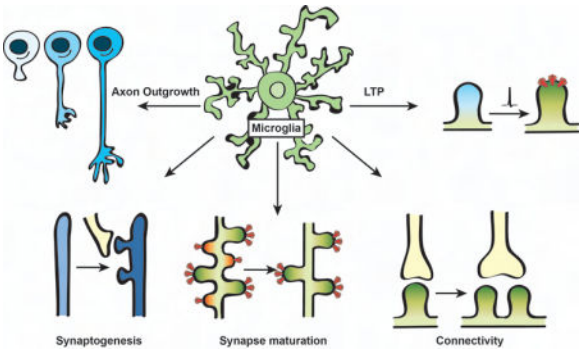
## Abstract

Microglia have recently been recognized as key regulators of synapse development, function, and plasticity. Critical to progressing the field is the identification of molecular underpinnings necessary for microglia to carry out these important functions within neural circuits. Here, we focus a review specifically on roles for microglial cytokine signaling within developing and mature neural circuits. We review exciting new studies demonstrating essential roles for microglial cytokine signaling in axon outgrowth, synaptogenesis and synapse maturation during development, as well as synaptic transmission and plasticity in adulthood. Together, these studies identify microglia and cytokines as critical modulators of neural circuits within the healthy brain, with implications for a broad range of neurological disorders with disruptions in synaptic structure and function.

## Graphical abstract




## Introduction

Nearly 100 years ago, Pío del Río-Hortega stained fixed tissue with silver carbonate to reveal mysterious brain cells that he called “microglia” [1]. From this simple tissue preparation, he made the keen observation that these resident brain macrophages were

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uniquely dynamic with robust “plasticity of their protoplasm” and a high degree of physical interaction with other nervous system cells. Fast forward to the 21st century, del Río-Hortega’s suspicions are being realized with exciting work defining key functional roles for microglia within neural circuits in the healthy brain.

Some of the first evidence that suggested microglia were playing important functions within the healthy brain were seminal 2-photon live imaging studies in mice, demonstrating that microglial processes in the intact healthy brain were highly dynamic and continuously surveying their extracellular environment [2,3]. This surveillance activity was later shown to be highly sensitive to neural activity whereby microglia modulated the motility of their processes in response to changes in neural activity and sensory experience [4–9]. Further live and static imaging revealed remarkable activity-dependent physical interactions and contact between microglial processes and synaptic elements (dendritic spines and presynaptic boutons) under steady-state conditions [4,5,10]. Indeed, given an estimated 94% of microglial processes are in contact with synaptic elements at any given point in time [4], this begs the question--what is the function(s) of microglia at synapses? It is now increasingly clear that microglia-derived molecules regulate synaptic connectivity including regulation of axon outgrowth, synaptogenesis, synapse maturation, synaptic pruning, basal synaptic transmission, and functional synapse plasticity [11]. Here, we review exciting new work on emerging roles for microglial cytokine signaling necessary for modulating synaptic connectivity and function.

## Cytokines: An introduction

Cytokines are an exceptionally large and diverse group of small signaling proteins that, upon binding to their cognate receptors, activate cellular pathways to modulate a large variety of physiological and pathological processes [12]. Based on their structural homology, cytokines are subdivided in different classes: chemokines, lymphokines, tumor necrosis factors (TNFs), colony stimulating factors (CSFs), interferons (IFNs) and interleukins (ILs). These classes are further divided into subgroups based on structural and functional properties. For example, chemokines are classified into four groups based on the presence of conserved cysteine residues in their sequence (C, CC, CXC, CX3C). IFNs are subdivided into three main groups according to their function and the molecular pathways used for signaling (type I IFNs including α- and β-IFN family members, and type II or γ-IFN family members). Moreover, subgroups of CSFs are defined based on their primary target cell types, including macrophages, granulocytes and monocytes, while TNFs and ILs comprise groups with larger diversity.

Each of these cytokine classes have canonical and well know functions in peripheral tissues. Canonically, chemokines and lymphokines enable chemotactic recruitment of various cell types in different tissues, TNFs and CSFs mainly influence growth and survival of cells, and IFNs and ILs primarily regulate aspects of cellular differentiation and inflammation [12]. However, depending on the cellular context and surrounding molecular cues, such as the presence or absence of other cytokines and cytokine receptors, the same cytokine can exert pleiotropic biological effects (e.g. cell proliferation vs. regulation of inflammatory state). Further adding to the complexity, while many of these cytokines and their receptors were

identified in the context of innate and adaptive immune cell function, it is now clear that immune and non-immune cell types throughout the body utilize these signaling pathways for homeostatic function. Exemplifying this is the nervous system in which an increasing number of studies demonstrate that cytokines regulate development and function of multiple resident cell types (neurons, astrocytes, myelinating glia, and microglia) in health and disease [13]. While roles for cytokines in the healthy and diseased nervous system now represent a large body of literature, we will focus specifically on those microglial cytokines and cytokine receptors that modulate synaptic connectivity and function in the healthy central nervous system (CNS). Among the molecules that will be reviewed most extensively are the fractalkine receptor (CX3CR1) and it's canonical either secreted or membrane-bound ligand fractalkine (CX3CL1), tumor necrosis factor alpha (TNFα), and interleukin 1β (IL-1β).

## Microglial cytokine signaling: establishing synaptic connectivity

Prior to forming synapses, newborn axons must grow towards their eventual synaptic targets. Recent work in the embryonic brain has suggested a key role for microglia and microglial cytokine signaling in regulating initial axon outgrowth. When microglia were absent in the embryonic brain by genetic targeting the transcription factor PU.1 or pharmacological blockade of the cytokine colony stimulating factor 1 receptor (CSF1R), a trophic factor necessary for microglial survival, there was exuberant outgrowth of dopaminergic axons in the E14.5 and P0 brain [14]. In contrast, reduced dopaminergic axon outgrowth was observed in a mouse model of maternal immune activation (MIA), in which pregnant dams were injected with lipopolysaccharide (LPS), a component of the envelope of Gram-negative bacteria that binds Toll-like receptor 4 (TLR4) on microglia and induces production of a broad range of cytokines. To explore the molecular mechanism(s), mice deficient in microglia-enriched molecules including the adaptor protein DAP12, the engulfment receptor complement receptor 3, or the chemokine receptor CX3CR1 were analyzed [12]. Interestingly, *Cx3cr1*$^{-/-}$ embryos were the only mutants that had defects in dopaminergic axon outgrowth, which was similar to the decreased outgrowth observed in MIA. Determining how MIA and CX3CR1 signaling may be linked mechanistically to alter axon outgrowth and identifying whether these developmental defects persist into adulthood to affect circuit function are important future directions. Also, CX3CR1 is a receptor that typically binds CX3CL1, which is expressed primarily by neurons. It is unknown if this or another ligand is involved in these developmental effects mediated by CX3CR1.

In addition to axon outgrowth, recent studies have suggested a role for microglia and microglia-derived cytokines in synaptogenesis. One study used a genetic diphtheria toxin strategy to deplete microglia by ~50% and provided evidence that synaptogenesis was impaired in the P11 somatosensory cortex [15]. Another study used a similar strategy to deplete microglia in postnatal and adult mice and showed that learning-induced synaptogenesis in the motor cortex was impaired [16]. While the effects in the motor cortex were largely attributed to microglial brain-derived neurotrophic factor (BDNF), it is unknown whether BDNF regulates synaptogenesis more globally or what molecules regulate synaptogenesis in the somatosensory cortex. Potential soluble synaptogenic factors released by microglia include cytokines. Indeed, an *in vitro* study in postnatal rat hippocampal

neurons demonstrated increased numbers of excitatory and inhibitory synapses upon exposure to microglia-derived IL-10, which was antagonized by the addition of IL-1β, another microglial-derived cytokine [17]. Determining how these cytokines orchestrate downstream signaling cascades to affect synaptogenesis and identifying whether similar mechanisms apply *in vivo* will be important next steps.

## Microglial cytokine signaling: synapse maturation

During development, synaptic connections first form in excess to form immature neural circuits. These nascent circuits then undergo synaptic pruning in which less active synapses are eliminated. The remaining synapses in the circuit are then functionally strengthened and elaborated into mature neural circuits. Some of the first evidence suggesting microglia play roles in these maturation processes was work demonstrating that microglia engulf and prune away less active synapses during brain development [4,5,10]. While complement-dependent phagocytic signaling was identified to regulate microglial synaptic engulfment and pruning [10], cytokine signaling has also been implicated in this developmental process. For example, astrocyte-derived transforming growth factor beta (TGFβ) was shown to modulate complement expression on neurons and consequently influence complement-dependent synaptic engulfment by microglia [18].

Other work has focused on microglial cytokine signaling more specifically by assessing synaptic maturation in *Cx3cr1*$^{-/-}$ mice. In the developing hippocampus, *Cx3cr1*$^{-/-}$ mice had transient increases in dendritic spine density (Figure 1a) [19]. Accompanying this delay in maturation of spine density was a defect in functional maturation of hippocampal synapses as measured by the ratio of spontaneous and miniature excitatory postsynaptic current amplitude (sEPSC/mEPSC amplitude ratio), long term depression (LTD) induction, and seizure susceptibility. Similar defects in functional synapse maturation were also observed within the developing barrel cortex in *Cx3cr1*$^{-/-}$ mice as measured by the ratio of Nmethyl-D-aspartate (NMDA) receptor subunits GluN2A and GluN2B and the relative ratio of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and NMDA receptor content at thalamocortical synapses [20•]. Interestingly, in both studies, the effects on structural and functional maturation of synapses were accompanied by transient reductions in microglial density near synapses in *Cx3cr1*$^{-/-}$ mice. Once microglial numbers reached wild type levels in older *Cx3cr1*$^{-/-}$ mice, defects in spine density and/or functional synapse maturation were largely resolved (Figure 1A). Going forward, it will be important to identify whether synaptic defects in *Cx3cr1*$^{-/-}$ mice are exclusively due to modulation of microglial recruitment to synapses or whether CX3CR1 signaling has direct downstream effects on other microglial molecules that regulate synaptic pruning and maturation.

While the original study in the hippocampus identified transient defects in synapse maturation that were largely attenuated in older animals, one parameter that was not shown to attenuate in *Cx3cr1*$^{-/-}$ mice was the sEPSC/mEPSC amplitude ratio [21]. In a follow-up study, the same group assessed more long-term effects of loss of CX3CR1 on functional neural circuits [21]. In this later study, persistent defects in sEPSC/mEPSC amplitude ratio were observed in adult *Cx3cr1*$^{-/-}$ mice, which were indicative of less multisynapse boutons (MSBs) on CA1 pyramidal neurons. This result was confirmed by ultrastructure in which

single presynaptic terminals within CA1 contacted multiple spines along the same dendrite in wild type mice and the frequency of these MSBs was reduced in *Cx3cr1*$^{-/-}$ mice (Figure 1B). This study also further revealed reduced functional connectivity between the hippocampus and prefrontal cortex as measured by fMRI, which were correlated with defects in social interactions and increased repetitive behaviors in *Cx3cr1*$^{-/-}$ mice. Precisely how CX3CR1 regulates these functional properties of synapses and neural circuits, the relative involvement of CX3CL1, and how changes in synapse function ultimately translate to behavioral alterations in knock-out mice will be critical next steps

## Microglial-derived cytokine signaling: regulating synaptic transmission and functional plasticity

Microglia are in close apposition to and physically interact with synapses, dynamically sense changes in neural activity, and express several molecules known to modulate synaptic transmission [22]. Likewise, there has been a large amount of literature demonstrating that cytokines affect a large array of synaptic properties and physiology [23]. Much of this work has suggested that cytokine signaling from other cell types such as neurons and astrocytes directly affects synapses, although it is intriguing to speculate that these effects are related to changes in microglia. Some of the first evidence suggesting that microglia-derived cytokine signaling could regulate synaptic function was work demonstrating roles for tumor necrosis factor alpha (TNFα) in regulating the amount of surface AMPA receptors, synaptic strength, and homeostatic plasticity [24–26]. While originally proposed to be astrocyte-derived, transcriptomics has revealed little to no TNFα transcripts in astrocytes and near exclusive expression in microglial cells [27,28]. More recently, using cell-specific Cre lines *in vivo*, it was demonstrated that microglia-derived TNFα drives the internalization of synaptic AMPA receptors, decreases synaptic strength, and suppresses behavioral sensitization following repeated cocaine administration (Figure 2a) [29].

In addition to TNFα, one of the most robust microglial cytokine pathways identified to regulate functional synapse plasticity is signaling between microglial CX3CR1 and its ligand CX3CL1 (Figure 2B), which is largely expressed by neurons in a secreted or membrane-bound form [30]. Some of the first evidence that this receptor-ligand signaling modulated synaptic function was work in the hippocampus in which acute application of CX3CL1 depressed glutamatergic transmission, which was blocked in *Cx3cr1*$^{-/-}$ mice [31,32]. It was later discovered that local application of CX3CL1 to acute hippocampal slices at the time of long-term potentiation (LTP) induction resulted in impaired LTP [33]. While *Cx3cr1*$^{-/-}$ hippocampus showed no defects in LTP, CX3CL1-induced LTP impairments were blocked in *Cx3cr1*$^{-/-}$ slices. Further, CX3CL1-induced LTP impairment required signaling through a purinergic receptor, adenosine receptor 3 (A(3)AR) (Figure 2b, top) [33,34]. Interestingly, another chemokine signaling molecule CXCL16, which, similar to CX3CL1, is cleaved by ADAM10 into a soluble form, was also recently implicated in modulating glutamatergic and GABAergic transmission through A(3)AR and microglia [35]. Precisely how purinergic and chemokine receptor signaling are mechanistically linked remains an important open question. One possibility is that chemokine signaling works in the same pathway as purinergic signaling to modulate rapid microglial recruitment to

synapses, which has been suggested by two recent students in acute brain slices. For example, CX3CR1-deficient microglia were no longer attracted to a pipette containing 2-MeSADP, a purinergic receptor agonist [36]. Another study demonstrated increased physical contact between microglia and neurons following excitotoxic challenge were increased upon CX3CL1 treatment [37]. Conversely, these contacts were largely inhibited in slices prepared from mice deficient in CX3CR1 or the microglial purinergic receptor P2Y12. Determining how these two pathways converge through downstream signaling to regulate microglial recruitment to synapses will be important to advance our understanding.

In contrast to the studies described above, a very recent study provided in vivo evidence that CX3CR1-deficient microglia within the visual cortex had an amplified response to laser-induced injury while basal microglial dynamics and interactions with synapses were unaltered [65•]. They also provide data that *Cx3cr1*$^{-/-}$ mice have normal activity-dependent plasticity within the visual cortex. Therefore, CX3CR1-dependent modulation of synaptic plasticity may be context or region-specific. Consistent with this idea, another group has shown that adult *Cx3cr1*$^{-/-}$ mice have reduced hippocampal LTP accompanied by impairments in learning and memory andhippocampal neurogenesis [38••]. Further, IL-1β was elevated in *Cx3cr1*$^{-/-}$ mice and LTP and behavioral deficits were attenuated upon treatment with an IL-1β receptor antagonist (Figure 2b, bottom). Similar to CX3CL1/CX3CR1-purinergic receptor signaling, it remains to be determined how CX3CR1 modulates IL-1β. It is also unknown why results differ between this study and the study which showed no LTP defects in *Cx3cr1*$^{-/-}$ hippocampal slices and acute application of CX3CL1 diminished LTP [33]. Possibilities include differences in LTP induction paradigms and age of animals used for electrophysiological recordings, which may be important for IL-1β expression.

Work demonstrating a link between CX3CL1/CX3CR1 signaling and IL-1β in the regulation of LTP is consistent with past studies demonstrating that acute application of IL-1β impairs LTP [39–41]. This is further supported by data from an early life infection model in which postnatal pups are given a dose of *Escherichia coli* followed by an immune challenge with LPS in later life. This paradigm elicits increased microglial IL-1β levels and resulted in learning and memory impairments in rats, which are blocked upon inhibition of soluble IL-1β production with a caspase I inhibitor [42,43]. Given recent transcriptomics data sets, at least under basal conditions, IL-1β is nearly exclusively expressed by microglial cells in the CNS. However, this cell type-specific expression of IL-1β may change with age or inflammation [27,28]. It is also important to note that IL-1β-induced LTP impairments are dose dependent. While at higher, more disease-relevant concentrations IL-1β impairs LTP, at lower, more physiological doses it is required for LTP induction and learning and memory [44–47]. Together, these data suggest that maintaining the appropriate balance of physiological levels of IL-1β is critical for learning and memory and subtle alterations may have large effects on functional synapse plasticity. Identifying what cell types express the receptor for IL-1β and determining how IL-1β has differing effects based on concentration are important open questions.

## Conclusions

Microglia are now emerging as key regulators of structural and functional synapses in the healthy brain. Among the molecular pathways identified, cytokines and cytokine receptors have emerged as important regulators of microglia function at synapses. During embryogenesis, microglial CX3CR1 signaling has been identified as regulator of axon outgrowth. Microglial IL-10 has been suggested to promote synaptogenesis *in vitro*. During postnatal development, microglial CX3CR1 signaling regulates microglial density within neural circuits, which, in turn, modulates synaptic pruning and maturation. Throughout the lifespan, microglial CX3CR1, TNFα and IL-1β have been implicated in regulating synaptic transmission and functional plasticity.

Together, this exciting work has opened up a new way of thinking about microglial cytokine signaling within neural circuits during non-pathogenic conditions and several new questions have emerged. First, we need to identify the precise mechanisms, including intercellular signaling, by which cytokines regulate microglia function at synapses. This also includes identifying potential interactions between other microglial-derived molecular pathways shown to modulate synapses such as complement, purinergic signaling, and BDNF. Second, there is a large body of work demonstrating cytokines derived from multiple other cell types affect synapses. For example, there has been recent exciting evidence that neuronal chemokine CCR5 modulates hippocampal synaptic plasticity and learning and memory [48]. Whether cytokine signaling from other cell types, such as neuronal CCR5 signaling, is modulated by or affects microglial function at synapses is an open question. Last, there is a large body of work indicating that abnormally reactive microglia and dysregulated cytokine levels are often concomitant with alterations in synaptic structure and function [49–53]. This includes disorders thought to have a developmental underpinning such as autism and schizophrenia as well as neurodegenerative diseases such as Alzheimer’s disease (AD). For example, altered levels of cytokines such as IL-1β, IL-6, IL-4, IFN-γ, and TGF-β and abnormally reactive microglia have been observed in individuals with autism and in mouse models of autism [54–58]. In the context of neurodegenerative disease, CX3CR1 deficiency inhibits several aspects of pathology in mouse models of AD including neuronal cell loss, betaamyloid accumulation, and cognitive deficits [13,59–61]. In the context of neurodegenerative disease, CX3CR1 deficiency inhibits several aspects of pathology in mouse models of AD including neuronal cell loss, beta-amyloid accumulation, and cognitive deficits [59–61,66].

Important in our progress to tackling these important questions is the development of new tools. One challenge has been measuring cell-type specific protein localization and levels of cytokines, particularly those that are secreted, as well as their receptors in tissue. Another challenge has been achieving cell-specific gene ablation. Mice expressing floxed alleles for cytokines and their receptors are often lacking. Similarly, while the emergence of new mice expressing CX3CR1-driven Cre and CreER expression have been invaluable for the field [16,62], these mice are a knock-in at the CX3CR1 locus and, therefore, either heterozygous are homozygous null for CX3CR1. This is further complicated by CX3CR1 expression by, not only microglia, but also a subset of peripheral immune cells. Additional tools to specifically manipulate microglia such as Cre/CreER driven by microglia-specific promoters

such as TMEM119 and P2RY12 would be tremendous resources for the field [27,63,64]. Going forward, developing these new tools, determining cell-type specific effects, and identifying downstream signaling by which microglial cytokine signaling modulates synapses will be profoundly important. Elucidating these basic biological mechanisms will be important for understanding microglia function within neural circuits and for developing novel, microglia-based therapeutics for neurological disorders.

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• Special Interest

•• Outstanding Interest

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## Highlights

- Microglial cytokine signaling regulates axon outgrowth and synaptogenesis
- CX3CR1 regulates microglial recruitment to synapses necessary for synaptic maturation
- Microglial CX3CR1, TNFα, and IL-1β modulate synaptic transmission and plasticity
- Microglial cytokine signaling at synapses has important implications for disease

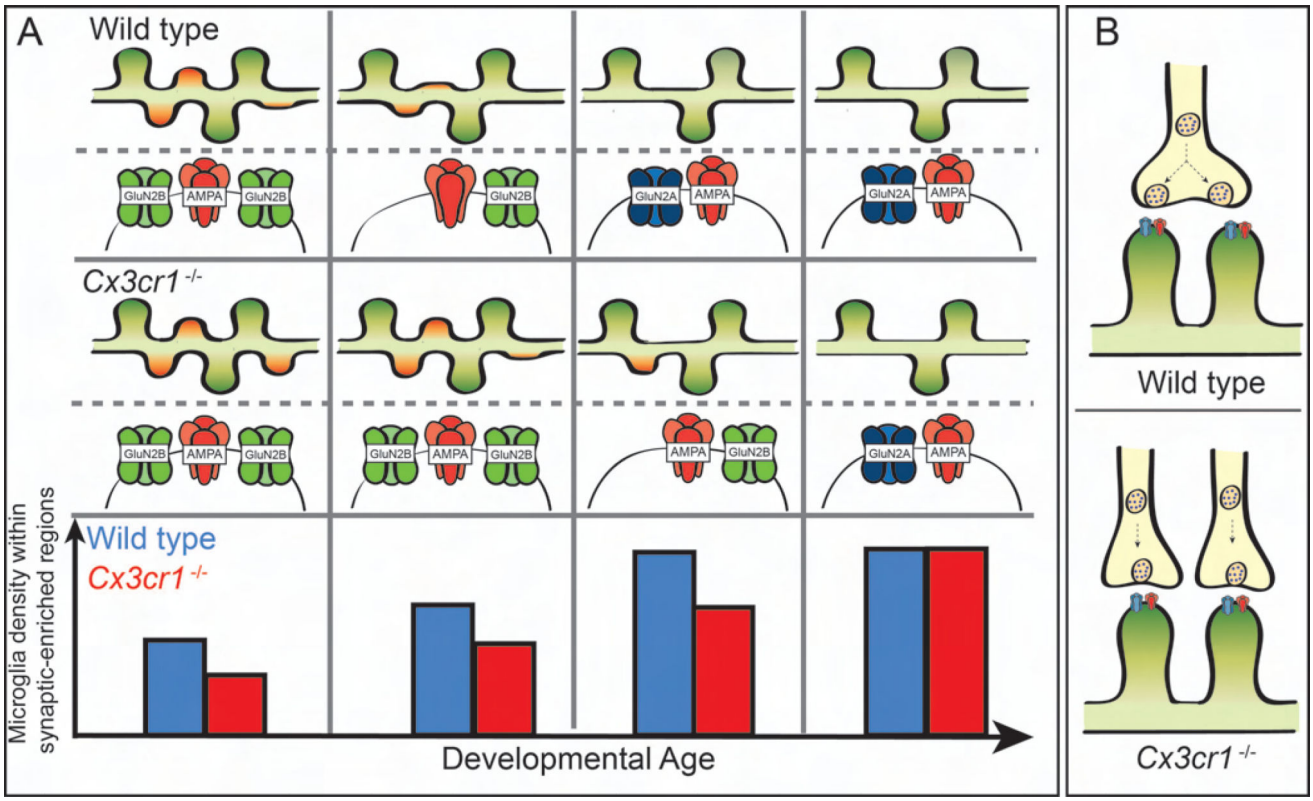


**Figure1. CX3XR1-deficient mice have delayed synapse maturation and persistent defects in synaptic connectivity**

**(a)** Top panel, during development in wild type animals, a subset of dendritic spines are pruned away (red spines) leaving the remaining subset to strengthen into mature neural circuits (green spines). In addition, there is a developmental shift from GluN2B (green) to GluN2A (blue)-containing postsynaptic NMDA receptors and an increase in AMPA/NMDA ratio. Middle panel, loss of CX3CR1 leads to a transient delay in spine pruning in the hippocampus and maturation of postsynaptic receptors in the hippocampus and barrel cortex. Bottom panel, once microglia density reaches wild type levels in the CX3CR1 deficient brain, spine density and postsynaptic receptor maturation are indistinguishable from wild type brains. **(b)** Adult animals deficient in CX3CR1 have decreased multi synapse boutons (MSBs) along the same dendrite in the hippocampus.

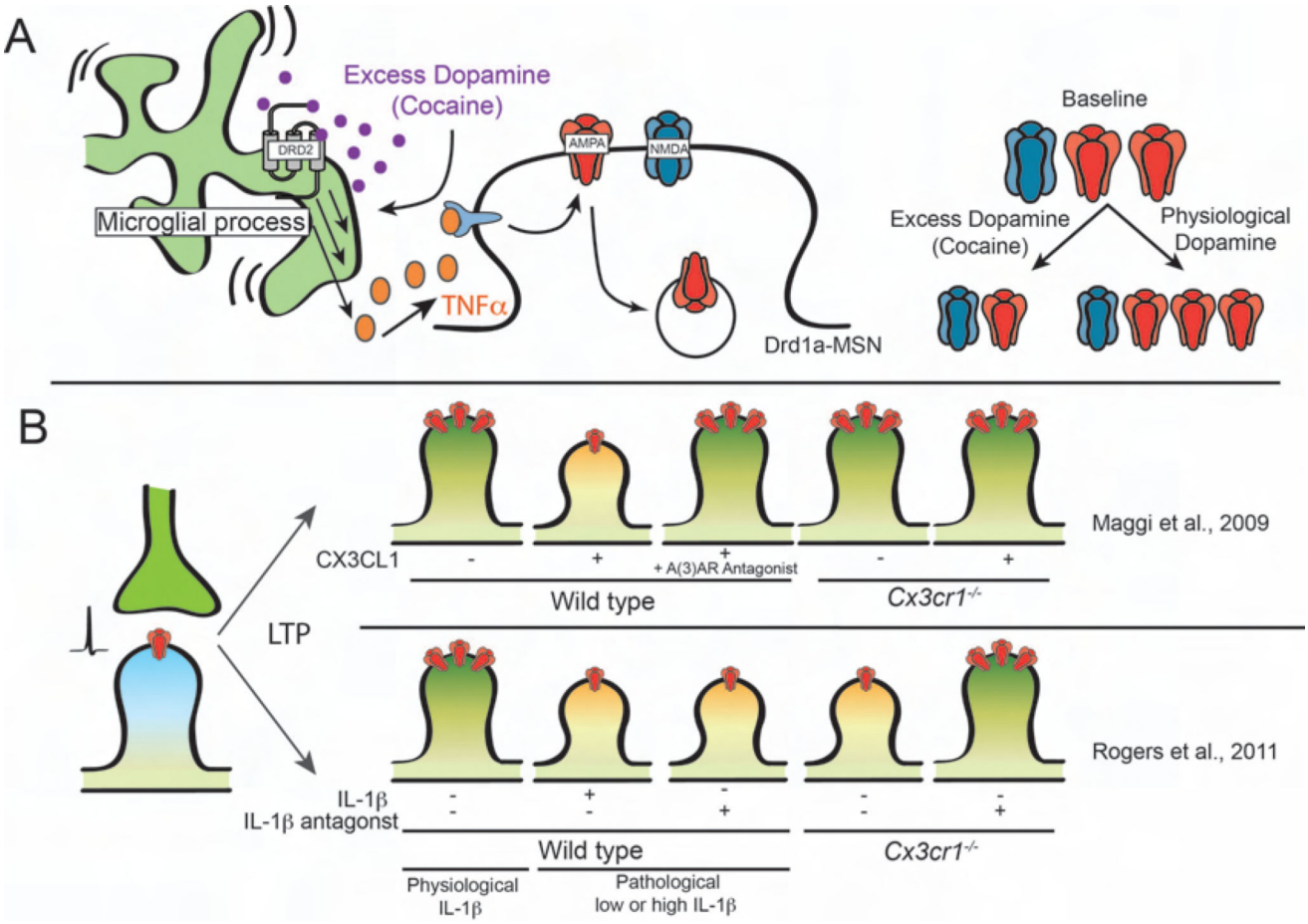


**Figure 2. Microglial cytokine signaling regulates functional synapse plasticity**

**(a)** Summary of microglial-derived TNFα effects on AMPA receptor (red) internalization within the nucleus accumbens following cocaine administration. **(b)** Summary CX3CL1 and IL-1β effects on LTP. Red receptors represent membrane-associated postsynaptic AMPA receptors necessary for LTP. Note the differing results assessing LTP in *Cx3cr1*$^{-/-}$ (Maggi *et al.*, 2009, top vs. Rogers *et al.*, 2011, bottom).