

Feature Review

Toll-like receptor signaling in neural plasticity and disease

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Toll-like receptors (TLRs) are a family of innate immune system receptors that respond to pathogen-derived and tissue damage-related ligands. TLR signaling in immune cells, glia and neurons can play roles in the pathogenesis of stroke, Alzheimer's disease (AD) and multiple sclerosis (MS). Recent findings suggest that TLR signaling also influences multiple dynamic processes in the developing and adult central nervous system including neurogenesis, axonal growth and structural plasticity. In addition, TLRs are implicated in the regulation of behaviors including learning, memory and anxiety. This review describes recently discovered and unexpected roles for TLRs in neuroplasticity, and the implications of these findings for future basic and translational research studies.

Toll-like receptors

Toll-like receptors (TLRs) are transmembrane pattern-recognition receptors that initiate signals in response to diverse pathogen-associated molecular patterns (PAMPs) [1]. The first Toll protein was discovered in *Drosophila melanogaster*, where it controls dorso-ventral patterning [2]. A mammalian homologue for Toll, TLR4, was later found to recognize bacterial lipopolysaccharide (LPS), a major cell wall component of gram-negative bacteria [3]. Subsequently, many additional homologues have been identified across diverse species (for a comprehensive evolutionary overview, see [4]). Until recently, it was believed that while *Drosophila* Toll plays both immune and developmental roles, mammalian TLRs mediate immune responses of two kinds: i) Orchestration of the immediate specific and global tissue response of the innate immune system to pathogens until the acquired immune response is fully functional. This orchestration is driven primarily by cytokine and chemokine production. ii) Facilitation of adaptive immunity by activating antigen-presenting cells such as macrophages and dendritic cells. However, recent findings suggest that mammalian TLRs also possess developmental roles during embryogenesis, as well as physiological and metabolic roles in adults. For example, TLR5-deficient mice exhibit hyperphagia and develop hallmark features of metabolic syndrome, including hyperlipidemia, hypertension, insulin resistance and increased adiposity [5].

TLRs are expressed in a variety of mammalian immune system-related cell types including B cells [6], mast cells

[7], natural killer cells [8], regulatory T cells [9], macrophages, monocytes, dendritic cells [10], neutrophils [11] and basophils [12], in addition to non-immune cells such as epithelial [12] and endothelial cells [13]. TLRs are also present in the brain where, until recently, their expression was believed to be limited to microglia [14], astrocytes [15] and oligodendrocytes [16]. However, we now know that neurons as well as neuronal progenitor cells also express TLRs [17].

TLRs rely on receptor dimerization to achieve specificity in agonist recognition. Although most TLRs form homodimers, certain TLRs such as TLR2 can also form heterodimers with TLR1 or TLR6 [1]. In the context of PAMPs, the different TLRs respond to specific classes of pathogens. TLR4 predominantly recognizes LPS from gram-negative bacteria, whereas TLR2 dimerizes with TLR1 or TLR6 to recognize lipopeptides from gram-positive bacteria [1]. TLR5 is expressed in the intestine where it senses bacterial flagellin protein [18,19]. TLR11 generates an innate immune response upon sensing a parasite-specific surface motif consisting of an acidic loop on profilin from *T. gondii* [20,21]. TLRs 3, 7, 8 and 9 are almost exclusively localized to intracellular membranes where they are ideally positioned for activation by nucleic acids of bacterial and viral origin [1]. TLR3 is activated in response to viral double-stranded RNA (dsRNA) [1]. Human TLR8 and its murine orthologue TLR7 recognize viral ssRNA as well as various synthetic imidazoquinolines, that is compounds with a double cyclic organic backbone, which have different affinities toward TLR7 and TLR8 [22]. TLR9 recognizes unmethylated CpG DNA found in bacteria as well as viral genomes [1].

In addition to the pathogen-derived ligands that activate the different TLRs, endogenous TLR ligands referred to as damage- (or danger-) associated molecular patterns (DAMPs) have been identified. Numerous endogenous ligands have been described and include low molecular weight hyaluronic acid (LMW-HA), fibrinogen, fibronectin, β -defensins, heparin sulfate proteoglycans and heat-shock proteins [23,24]. Importantly, the signaling outcomes seem to differ between PAMP and DAMP-induced TLR activation. This is probably due to the need to differentiate between pathogen-induced TLR activation that requires immune intervention and tissue damage-induced TLR activation that requires a balance between immune intervention and tissue damage repair [21,25,26]. Endogenous

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TLR activation is one of the most exciting fields of TLR-related research today because it is realized that TLRs are not solely dedicated to eliciting pathogen-related immune responses but also bear physiological as well as pathological roles unrelated to infection.

Following ligand binding, TLRs activate signaling components to initiate immune responses for host defense. The cytoplasmic region of TLRs shares a Toll/IL-1 receptor (TIR) domain, which mediates interactions between TLRs and TIR domain-containing adaptor proteins by either heterophilic or homophilic interaction of their TIR domains. The signaling pathways activated by TLRs are broadly classified into myeloid differentiation factor 88 (MyD88)-dependent and MyD88-independent pathways, with MyD88 the universal adaptor protein recruited by all TLRs except for TLR3, which utilizes TIR domain-containing adapter-inducing interferon- β (TRIF) to mediate signaling, and TLR4, which utilizes both MyD88-dependent and TRIF-dependent signaling pathways [1].

TLRs are classically studied in relation to immunity, however recent evidence implicates TLRs as mediators of central nervous system (CNS) plasticity. Whereas studies with pathogen-derived TLR ligands showed that TLR activation in the brain adversely affects cognition, recent findings suggest that TLRs regulate cognitive function in the absence of a pathogen-derived ligand. This review summarizes our current knowledge of TLRs in developmental and adult neuroplasticity during physiological as well as neuropathological conditions.

TLR signaling in CNS cells

Activation of a given TLR engages different signaling pathways in different neural cell types. For example, TLR4 activation results in distinct signaling outcomes in astrocytes, microglia, neurons and neural progenitor cells (NPCs), and these pathways differ from TLR-induced signaling in dendritic cells (Figure 1a). TLR4 activation in dendritic cells signals through a myeloid differentiation

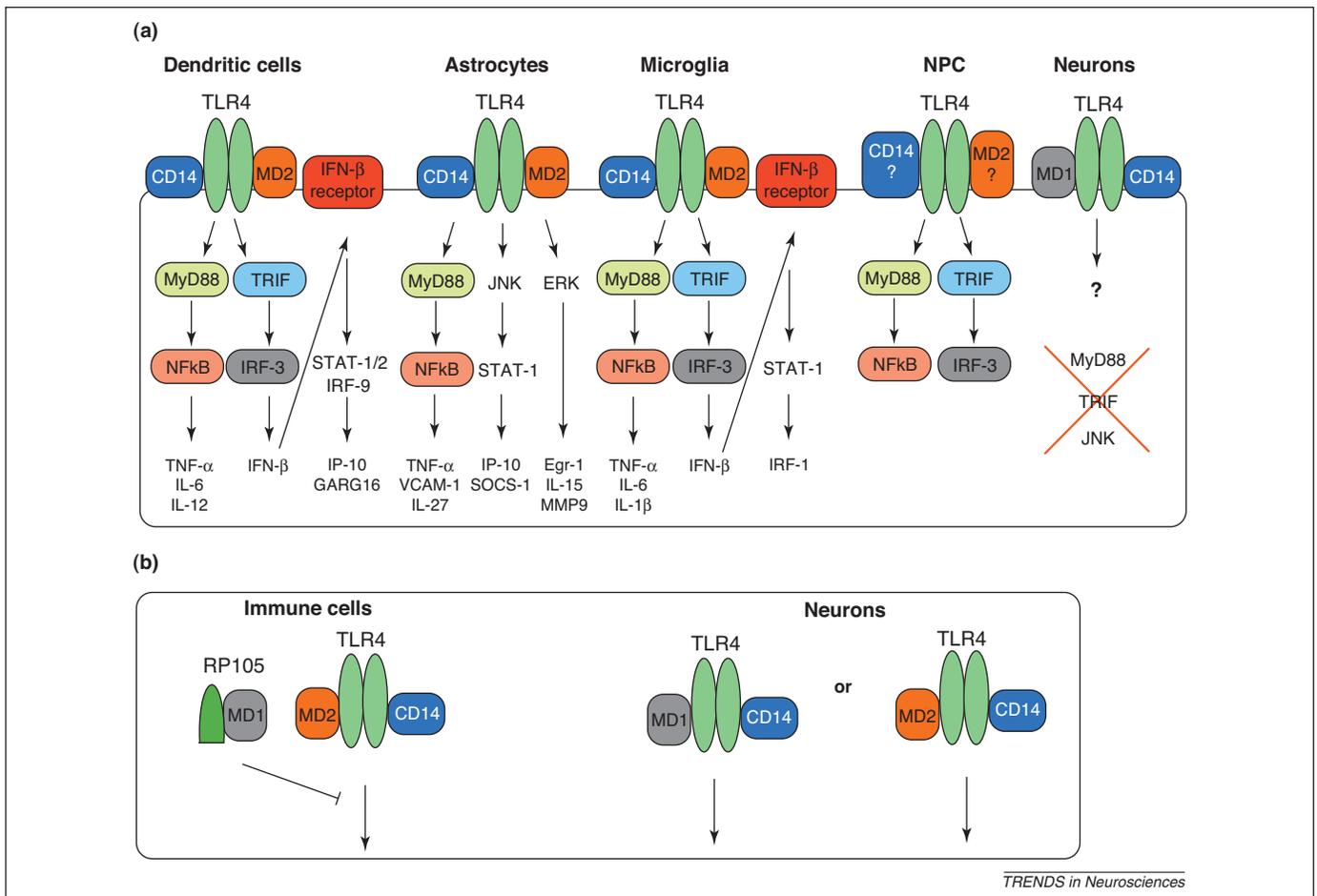


Figure 1. TLR4 signaling in dendritic cells compared to neural cells. **(a)** In dendritic cells, LPS induced TLR4 signaling occurs through both MyD88-dependent and -independent pathways. A MyD88-dependent pathway leads to rapid activation of NF κ B and mitogen-activated protein (MAP) kinases and production of inflammatory cytokines. A MyD88-independent pathway induces slow activation of NF κ B, MAP kinases and IRF-3. The activation of IRF-3 induces production of IFN- β . Secreted IFN- β stimulates IFN- β receptor, which leads to activation of STAT-1 and STAT-2, resulting in induction of IFN-inducible genes such as IP-10 and GARG-16 in dendritic cells [27]. In astrocytes, LPS induces early recruitment of MyD88 and subsequent activation of MyD88-dependent pathway leading to NF κ B activation. NF κ B is activated after degradation of I κ B- α , p65 phosphorylation and nuclear translocation of NF κ B subunits (p65 and p50), followed by p38-dependent transcriptional activity of the NF κ B complex and induction of target genes including TNF- α , IL-27, IL-15, matrix metalloproteinase (MMP)-9 and VCAM-1. Induction of TRIF-dependent IFN- β is not detected in astrocytes, but a MyD88-independent pathway induces delayed, MAPK, JNK and p38-mediated tyrosine phosphorylation of STAT1, and activation of downstream genes IP-10 and SOCS-1. Phosphorylation of ERK1/2 is involved in Egr-1, IL-15 and MMP-9 induction. STAT1 can exert a negative control on the expression of certain genes, such as MMP-9. Microglial signaling initiated by TLR4 consists of at least two pathways, MyD88-mediated NF κ B activation and TRIF-mediated IRF-3 activation. Activated IRF-3 induces IFN- β expression, which via IFN- β receptors initiates STAT1 signaling that activates IRF-1. TLR4 on NPCs exposed to LPS mediates signaling through both the MyD88-dependent pathway that results in NF κ B activation, as well as through TRIF, which activates IRF-3. Neurons treated with LPS show no activation of NF κ B, TRIF or JNK pathways [37,38]. **(b)** The TLR4 signaling complex in immune cells and neurons. In human and rodent immune cells, TLR4 dimers bind to the adaptor proteins CD14 and MD-2. An additional complex comprised of RP105 and MD-1 serves as an inhibitor of TLR4. In rodent neurons, TLR4 dimers bind CD14 and either MD-1 or MD-2 [30,46].

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primary response gene 88 (MyD88)-dependent pathway to activate nuclear factor kappa-light-chain-enhancer of activated B cells (NF κ B) and produce cytokines such as tumor necrosis factor- α (TNF- α), interleukin 6 (IL-6) and IL-12. The MyD88-independent TRIF pathway is also activated resulting in nuclear translocation of Interferon regulatory factor (IRF)-3 and synthesis of interferon β (IFN- β), which then activates its receptor coupled to Signal Transducer and Activator of Transcription-1/2 (STAT-1/2) and IRF-9 and a secondary wave of transcription of cytokines and chemokines such as interferon gamma-induced protein 10 kDa (IP-10) and Glucocorticoid Attenuated Response Gene 16 (GARG16) [27]. In astrocytes, however, TLR4 activates the MyD88- but not the TRIF-dependent pathway. In these cells, MyD88-mediated signaling leads to NF κ B-induced transcription of TNF- α , vascular cell adhesion molecule 1 (VCAM-1) and IL-27, whereas other signaling mediators such as c-Jun N-terminal kinases (JNK) activate STAT-1 to transcribe IP-10 and suppressor of cytokine signaling proteins-1 (SOCS-1). Extracellular-signal-regulated kinases (ERK) is also activated by LPS in these cells independently of MyD88 [28]. TLR4 activation in microglia resembles its activation of dendritic cells, with both MyD88- and TRIF-dependent signaling pathways active. These in turn induce NF κ B activation that promotes transcription of cytokines such as TNF- α , IL-6 and IL-1 β , and IRF-3 activation, resulting in IFN- β -mediated activation of STAT-1 and subsequently IRF-1 [29].

In contrast to other neural cells, the signaling outcomes of TLR4 activation in neurons are largely unknown. Recent findings indicate that in addition to TLR4, dorsal root ganglia (DRG) neurons express cluster of differentiation-14 (CD14), and myeloid differentiation protein (MD)-1, an MD-2 homologue, but lack the expression of Radioprotective 105 kDa (RP105) and have very low expression of MD-2 [30]. RP105 is a TLR4 homologue that lacks the TIR-domain, and is therefore unable to mediate signaling and inhibits TLR4. In immune cells, TLR4 binds MD-2, whereas RP105 binds MD-1. Neurons express an unusual assortment of TLR4 receptor complex components with MD-1 or MD-2 in addition to CD-14 (Figure 1b). It is possible that this combination of proteins in the TLR4 receptor is responsible for the non-canonical signaling mediated by TLR4 in neurons. Neurons do not translocate NF κ B to the nucleus, transcribe IFN- β or activate JNK [17], suggesting that neither of the known TLR4-induced signaling such as MyD88 and TRIF are activated in these cells as a result of TLR4 activation by LPS. However, it is known that activation of NF κ B in neurons influences their plasticity and survival. For example, activation of NF κ B in hippocampal neurons induces the expression of manganese superoxide dismutase and protects neurons from being damaged and killed by oxidative and excitotoxic insults [31,32]. NF κ B has important roles in synaptic plasticity and learning and memory [33]. Whether or not there are roles for NF κ B in mediating effects of signaling from TLRs on neuronal plasticity is unknown, and further research is required to answer this question.

TLRs in the generation and growth of neurons

During embryonic development, extensive neurogenesis occurs in the subventricular zone [SVZ, also referred to

as the subependymal zone (SEZ) in the adult] of the lateral ventricles [34]. Neurogenesis in this region slows significantly at early postnatal stages and continues modestly in the adult. NPCs derived from the SVZ tangentially migrate along the rostral migratory stream to the olfactory bulb where they radially migrate and differentiate into neurons of the granule and glomerular layers [35]. In the adult mammalian brain, neurogenesis also occurs in the subgranular zone (SGZ) of the dentate gyrus (DG) in the hippocampus. Neurons arising from the SGZ differentiate and integrate into the DG as granule cells. The following sections review evidence that TLRs regulate NPC fate and the differentiation and growth of neurons in various stages of development and in the adult brain.

TLRs and NPC proliferation

TLRs 2, 3 and 4 are expressed in NPCs, and recent evidence indicates that these receptors influence NPC proliferation [36–38]. TLR4 inhibits NPC proliferation, as TLR4 deficiency increases NPC proliferation in the SGZ of the DG of adult mice, albeit without a corresponding increase in neuronal survival [38]. However, TLR2 deficiency does not alter proliferation of NPC in the adult hippocampus [38] or in the SVZ of the embryonic brain [37]. Indeed, dual inhibition of TLR2 and TLR4 using neutralizing antibodies increases NPC self-renewal, an effect conferred by TLR4 [38]. TLR3 deficiency also increases proliferation of embryonic NPCs in the SVZ during early but not late embryonic developmental stages [36]. Interestingly, MyD88 deficiency also enhances NPC proliferation in the SGZ of the DG [38], which suggests that TLR signaling components are necessary to affect NPC proliferation, even in the absence of exogenous TLR ligands. Although IL-1 signaling also relies on MyD88, it has not yet been determined whether MyD88-mediated effects on NPC proliferation involve IL-1 signaling, TLR signaling or a combination of the two pathways. Similar to their effects on SVZ/SEZ and hippocampal NPCs, TLRs also alter proliferation of retinal progenitor cells (RPCs). During murine retinal development, multipotent RPCs give rise to neurons and Müller glia. TLR4 deficiency increases early postnatal RPC proliferation [39]. Further, deficiency of downstream TIR adaptor proteins such as MyD88 and TRIF, two signaling mediators for TLR4, also enhances RPC proliferation.

TLR2 activation has differential effects on embryonic and adult NPCs. TLR2 activation depresses embryonic NPC proliferation [37], whereas in adult NPCs, TLR2 activation does not alter self-renewal ability [38,40]. Although TLR3 and TLR4 deficiencies enhance NPC proliferation, activation of these TLRs diminishes NPC self-renewal. TLR4 activation in adult NPCs activates both MyD88-dependent and -independent pathways [39] and ultimately results in inhibition of NPC proliferation [38]. In addition, TLR4 activation by LPS inhibits the proliferative capacity of RPCs in neonates [39]. TLR2 and TLR4 activation also stimulates TNF- α synthesis and release from adult NPCs, which might contribute to the inhibition of NPC proliferation [40]. TLR3 activation reduces embryonic NPC proliferation to a greater extent than adult NPCs, correlating with the diminished control of TLR3 on NPC proliferation during the transition from early

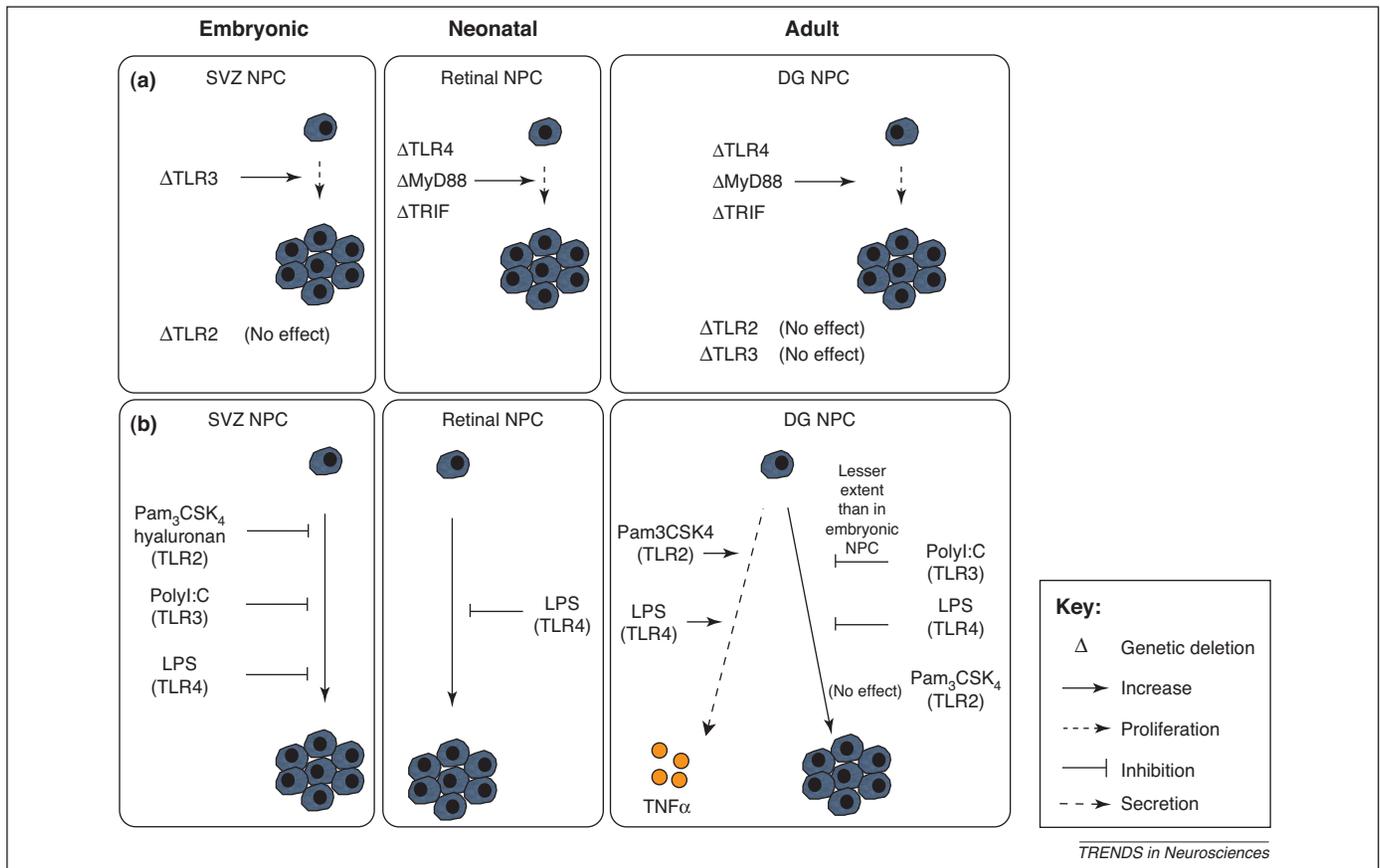


Figure 2. TLRs and NPC proliferation. (a) (Left panel) Deficiency of TLR3 but not TLR2 increases proliferation of SVZ-derived embryonic NPCs [36,37]. (Middle panel) Deficiency of TLR4, MyD88 or TRIF increases proliferation of RPCs in neonates [39]. (Right panel) Deficiency of TLR4, MyD88 or TRIF but not TLR2 or TLR3 increases proliferation of DG- derived adult NPCs [36,38]. (b) (Left panel) Activation of TLR2 by Pam₃CSK₄, TLR3 by PolyI:C or TLR4 by LPS inhibits proliferation of SVZ-derived embryonic NPCs [36–38]. (Middle panel) Activation of TLR4 using LPS inhibits proliferation of RPCs in neonates [39]. (Right panel) Activation of TLR4 by LPS, and to a lesser extent activation of TLR3 with PolyI:C (but not TLR2 using Pam₃CSK₄), inhibits DG-derived adult NPC proliferation [36–38,40,42]. Activation of TLR2 and TLR4 with the above ligands also induces the release of TNF- α in these cells. This figure describes work performed in mice.

embryogenesis toward early postnatal ages [36]. **Figure 2** summarizes the effects of TLR deficiency (**Figure 2a**) or activation (**Figure 2b**) on NPC and RPC proliferation in embryos, neonates and the adult.

TLR4 activation in NPCs induces MyD88-dependent and -independent pathways, but little is known of the transcriptional targets that are downstream of these signaling mediators [38]. NPC proliferation is altered by TLR or TIR adaptor protein deficiency [36,38] as well as TLR activation [37,38]; however, the balance between TLR activation and inhibition and the resulting molecular mechanisms controlling NPC proliferation remain unclear. Another area that requires more investigation is the influence of TLR activation on the rostral migratory stream and neuronal distribution in the olfactory bulb. Differential rates of proliferation in the embryonic SVZ or adult SEZ can alter neuronal number in the adult olfactory bulb, which leads to changes in olfaction. Therefore, it will be of interest to determine whether olfaction is affected by TLR signaling.

TLRs and NPC fate

TLRs are also implicated in the modulation of NPC differentiation. TLR2 deficiency alters the differentiation profile of NPCs, which results in diminished numbers of neurons expressing the early neuronal markers doublecortin and β -III tubulin, and increased differentiation into cells

expressing the astrocytic markers glial fibrillary acidic protein (GFAP) or S100 calcium binding protein B (S100 β) [38]. TLR2 deficiency does not affect the fate of oligodendrocytes [41]. Therefore, although TLR2 deficiency does not alter the proliferative capacity of NPCs, it suppresses neuronal differentiation, shifting NPCs toward an astrocytic fate. These effects are intrinsic to NPCs; wild type NPCs grown on TLR2-deficient mixed astrocyte-neuronal cultures retain a normal differentiation ratio of neurons to glia [38]. In contrast, TLR4 deficiency enhances neuronal differentiation from NPCs but only marginally reduces differentiation into astrocytes [38]. Dual inhibition of TLR2 and TLR4 using neutralizing antibodies increases neuronal differentiation, which suggests that the predominant effects on differentiation are mediated by TLR4 [38]. Deficiency in MyD88, the common signaling mediator for TLR2 and TLR4, also enhances neuronal differentiation [38].

In contrast to TLR2 deficiency, TLR2 activation increases neuronal differentiation and simultaneously decreases astrocyte formation [38]. However, others report no effect of TLR2 activation on adult NPC differentiation to neurons or glia [40]. In contrast to TLR2, activation of TLR4 on adult NPCs using LPS reduces neuronal differentiation but has no effect on astrocyte differentiation [38]. Similarly, TLR4 activation on RPCs inhibits neuronal differentiation without altering astrocyte differentiation

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Table 1. TLRs and neuronal differentiation

Neural cells	Influence on NPC differentiation					
	Genetic manipulations			Pathological modulation		
	Δ TLR4	Δ TLR2	Δ MyD88	LPS TL4	Pam ₃ CSK ₄ TLR2	Flagellin TLR5
NPC-derived cells						
• Neurons (β III ⁺ , DCX ⁺)	↑ [38]	↓ [38]	↑ [38]	↓ [38]	↑ [38]/ – [40]	N/A
• Astrocytes (S100 β ⁺ , GFAP ⁺)	↓ [38]	↑ [38]	↓ [38]	↑ [38]	↓ [38]/ – [40]	N/A
• Oligodendrocytes (MBP ⁺ , NG2 ⁺)	– [38]	– [38]	N/A [38]	– [38,41]	– [38]/↓ [40,41]	– [41]
RPC-derived cells						
• Neurons (β III ⁺ , DCX ⁺)				↓ [39]		
• Astrocytes (S100 β ⁺ , GFAP ⁺)				– [39]		

[39]. The impact of TLRs on NPC differentiation is summarized in Table 1. The involvement of TLRs and their activation by PAMPs in NPC differentiation raises interesting questions on the relevance of this phenomenon to physiological (sterile) conditions. The opposing effects on NPC differentiation conferred by TLR2 and TLR4 deficiency might not only be the result of endogenous activation, but also from developmental effects elicited by a deficiency of the receptor. This question could be answered by experiments in which the expression of TLR2 and TLR4 is suppressed or increased in a cell type-specific manner in the adult hippocampus.

TLRs and adult neurogenesis

Adult neurogenesis is a complex process that requires NPC proliferation, and the differentiation, migration and ultimately integration of neurons into existing networks. In mammals, neurogenesis from adult stem cells is responsible for the continuous maintenance and plasticity of only two major populations of neurons, interneurons of the olfactory bulb and granule neurons in the hippocampal DG. Several TLRs are implicated in the modulation of neurogenesis in the adult mammalian brain. TLR2-deficient mice exhibit significantly reduced neurogenesis, as indicated by expression of the early neuronal markers doublecortin and β III tubulin in bromodeoxyuridine (BrdU)-labeled cells (NPCs and their progeny) in the hippocampal SGZ. These mice have a higher proportion of cells in the hippocampal hilus expressing the astrocytic markers, but not the oligodendrocyte markers, indicating that TLR2 signaling promotes differentiation of NPCs into neurons at the expense of astrocytes [38].

The impact of TLR4 on neurogenesis *in vivo* is markedly different from TLR2. TLR4 deficiency increases cell proliferation, as measured by BrdU incorporation in the DG [38]. However, these cells fail to survive and mature as neurons, indicating reduced neuronal survival in TLR4-deficient mice. By contrast, the DG of TLR4-deficient mice contains a higher basal level of neurons and fewer glia compared to wild type mice. Similar effects are also observed in the SEZ [38]. This could indicate that during embryonic development, TLR4-deficient NPCs proliferate and differentiate into neurons at a high rate, whereas in the adult, NPCs are highly proliferative but do not become fully mature neurons. Interestingly, in contrast to the hippocampus, TLR4 deficiency in RPCs does not impair neuronal survival and results in increased neuronal differentiation into rod photoreceptors and bipolar cells [39].

The role of TLR3 in adult neurogenesis has also been studied [42]. Whereas there is no alteration in total cell genesis in the DG of TLR3-deficient mice, these animals have a higher proportion of cells expressing the mature neuronal marker NeuN. Further, the hippocampal DG and CA1 of TLR3-deficient mice are enlarged, which suggests that TLR3 might be involved in regulating neurogenesis in the adult hippocampus [42].

An indication of the cumulative effects of TLRs on adult neurogenesis can be gained from MyD88-deficient mice. NPCs of MyD88-deficient mice exhibit higher proliferative capacity and increased neuronal differentiation in the SGZ of the hippocampus [38]. The latter result suggests a possible role for cytokines of the IL-1 family acting on IL-1 receptors (IL-1Rs) on neurogenesis because such IL-1-related signaling would be expected to be impaired as the result of MyD88 deficiency.

TLRs and neurite outgrowth

The effects of TLRs in the CNS also extend to the development of neuronal circuits. TLR8 expression changes during the period of neuronal differentiation and axonogenesis. In mice, TLR8 is first detected at embryonic day 12 (E12), increases in later embryonic and neonatal stages, and decreases markedly after postnatal day 21 (P21) [43]. In addition, TLR8 exhibits a dynamic spatiotemporal expression. TLR8 is present in axonal tracts during embryogenesis and shifts to a diffuse expression in the neuronal soma postnatally, suggesting a role for TLR8 in nervous system development. Activation of TLR8 by the synthetic ligand R-848 significantly reduces the length of primary neurites. Further, R-848 results in neuronal death in a dose-dependent manner, independently of its effects on neurite outgrowth, whereas inhibition of TLR8 results in neurite elongation and reduced neuronal death [43,44]. A negative effect on neuronal viability has only been demonstrated for TLR8; neuronal viability is not affected by activation of TLR3 [45], TLR4 [46] or TLR9 [47–49]. However, when microglial cells are co-cultured with neurons, activation of TLRs 2 and 4 results in neuronal cell death [50]. This provides functional importance for the marked contrast between the signaling outcomes of TLR activation in neurons compared to microglia and astrocytes. Complementary studies are warranted to assess the effects of other TLRs and their activation on neuronal viability.

The developmental expression pattern of TLR3 contrasts with TLR8. TLR3 is strongly expressed during early embryogenesis, and decreases during the early postnatal

period where it maintains a low expression [36]. Similar to TLR8, TLR3 activation by either the synthetic ligand PolyI:C, or the DAMP mRNA inhibits neurite growth from E9 chick DRG neurons as well as E14 mouse embryonic brains. However, this effect is not accompanied by increased neuronal cell death [45]. TLR3-induced neurite growth cone collapse is rapid and independent of transcriptional regulation or NF κ B activation. Further, TLR3 is strongly expressed by sensory neurons. Intrathecal PolyI:C administration to mouse pups on P4 diminishes sensorimotor function and decreases dorsal root ganglion neurofilament expression, which is important for nerve growth and regeneration [45]. Notably, neurite outgrowth is only affected by TLR3 activation; TLR3-deficient neurons do not exhibit augmented neurite outgrowth. The diverse roles of TLR8 and TLR3 in neurite development and neuronal cell death are summarized in Figure 3a, and the temporal expression of the different TLRs discussed above is illustrated in Figure 3b. Similar to TLR4 activation, the signaling pathways induced by TLR3 and TLR8 activation by PAMPs in neurons are devoid of NF κ B, AP1, JNK or ERK activation, which again stresses the striking difference between TLR activation in neurons compared to other cells

in the CNS. The signaling pathway(s) underlying the effects of TLR activation on neurite growth in neurons therefore remains an open question.

TLRs and cognition

The growing body of evidence of the involvement of TLRs in neurogenesis, neurite outgrowth and neuron survival suggests that TLRs might also impact cognitive processes in health, injury and/or disease. It was recently shown that TLR3 has broad effects on the cognitive performance of mice in hippocampal-dependent and -independent behavioral tasks [42]. Although TLR3-deficient mice have intact spatial reference memory, memory extinction is slower than control mice. TLR3 deficiency also confers superior performance in spatial working memory. Conversely, activation of TLR3 by intracerebroventricular (ICV) infusion of PolyI:C diminishes working memory performance. However, these results should be interpreted with caution because PolyI:C can also activate the intracellular helicases retinoid-inducible gene I and Melanoma Differentiation-Associated Gene 5.

Similar to memory retention in a water maze task, contextual fear memory is also enhanced in TLR3-deficient mice [41]. Interestingly, anxiety appears to be increased in

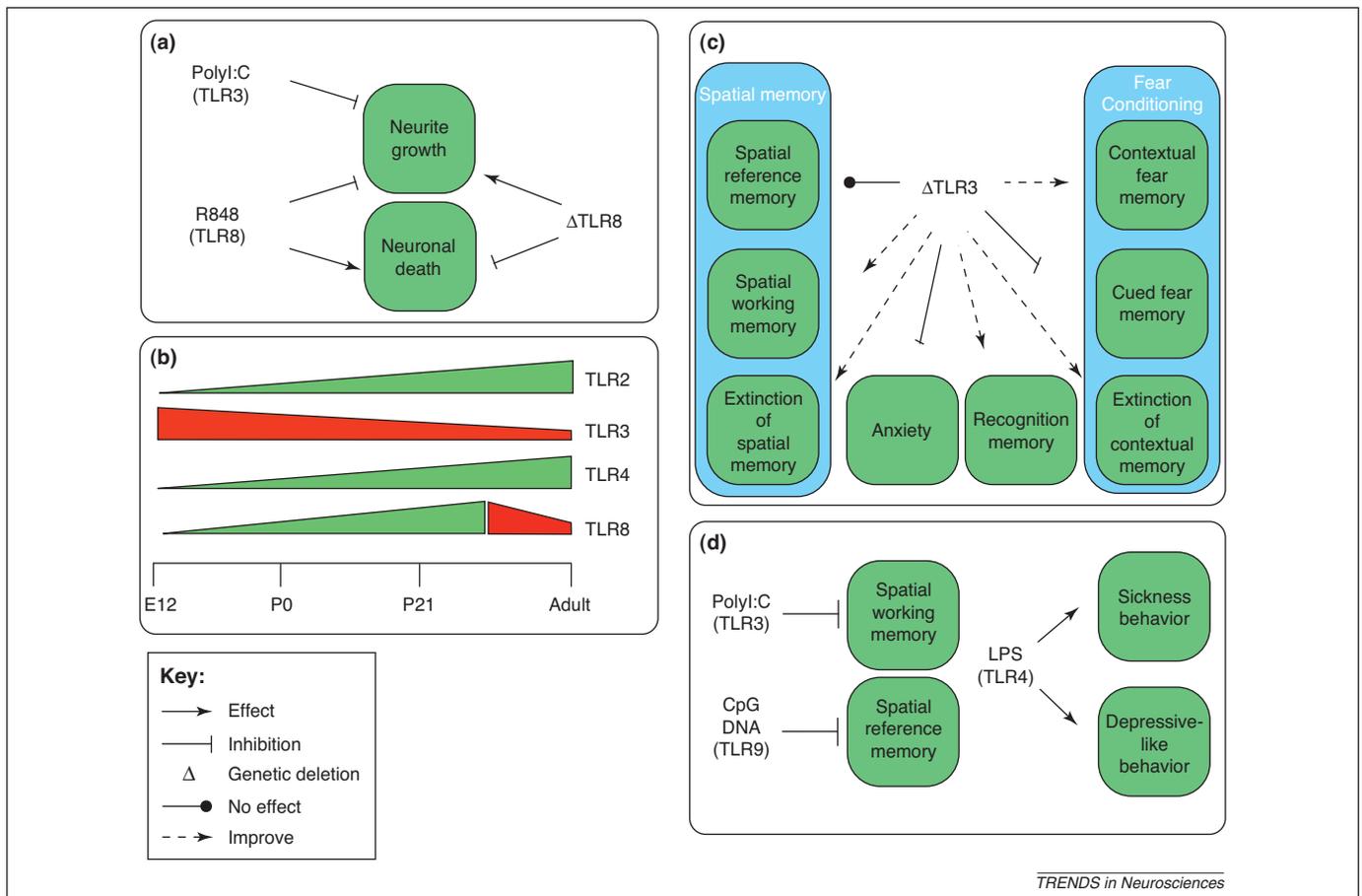


Figure 3. TLRs in CNS development, plasticity and cognition. **(a)** The impact of TLRs on neurite outgrowth and neuronal survival. TLR8 deficiency increases neurite outgrowth and inhibits cell death. TLR8 activation using R-848 inhibits neurite outgrowth and reduces neuronal survival. TLR3 activation using PolyI:C inhibits neurite outgrowth [43–45]. **(b)** Developmental CNS expression patterns for select TLRs in mice. TLR2 expression increases from early embryonic stages into adulthood. TLR3 is expressed during early embryonic stages and gradually declines into adulthood where it maintains low expression, mainly in neurons. TLR4 expression increases from early embryonic stages into adulthood. TLR8 expression increases from early embryonic stages until P21. Subsequently, TLR8 expression diminishes during adulthood and is maintained at a low level [36,37,43,44]. **(c)** TLR3 deficiency has pleiotropic effects on behavior in mice. Spatial working memory and memory extinction, but not reference memory, is improved. Contextual memory of fear and fear memory extinction are also enhanced. In addition, amygdala-dependent cued fear memory and anxiety responses are impaired. Short-term recognition memory is enhanced [42]. **(d)** ICV infusion of the TLR4 ligand LPS results in sickness coupled with depressive-like behavior [54–56,105]. ICV infusion of the TLR3 ligand PolyI:C results in spatial working memory impairment [42]. ICV infusion of the TLR9 ligand CpG DNA results in spatial reference memory impairments [57]. This figure represents work performed in mice.

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TLR3-deficient mice, and amygdala-dependent performance is blunted [42]. The effects of TLR3 on cognition are summarized in Figure 3c. These effects emphasize the role of TLR3 under normal conditions, devoid of infection or tissue damage, which excludes receptor activation through PAMPs or DAMPs. Endogenous ligands for TLR3 include mRNA [51] and stathmin [52]. However, the availability *in vivo* of such ligands to activate TLR3 under normal conditions has not yet been established. A mechanism by which mRNA can activate TLR3 has not been demonstrated, and stathmin expression is strongly enhanced under neuroinflammatory conditions suggesting a role for stathmin in TLR3 activation in pathological states. Further work is required to elucidate roles for endogenous TLR3 ligands in modifying cognitive processes. It is also important to determine whether TLR3 signaling influences cognition via developmental effects or direct effects on synaptic plasticity.

The possible roles of TLRs 2, 4 and 8 on cognition remain to be determined, but seem probable given the evidence that these receptors profoundly alter neural NPC proliferation and differentiation (TLR2 and TLR4) and neurite outgrowth (TLR8). Cognition has been assessed in transgenic mice overexpressing or deficient in other TIR-domain containing receptors, such as IL-1R [53]. However, it remains to be elucidated whether there is a link between signaling from IL-1R and other TIR-domain containing receptors such as the TLR family in the context of cognition.

In addition to the studies of TLR-deficient mice described above, the effects of specific TLR agonists on cognitive performance have also been investigated. However, these studies must be interpreted with caution due to variations in concentration and timing of treatments as well as the effects of state-dependent changes. State-dependent changes are particularly relevant for cognitive tests, as they can abrogate learning and memory by impairing the motivation to perform a task, a process that is not hippocampal dependent. For example, a high dose of LPS (0.25 $\mu\text{g/hr}$ for 28 days) impairs spatial learning associated with neuronal death [54], whereas a very high acute dose of LPS (20 μg) causes similar impairments in spatial learning associated with synaptic loss or damage [55]. At these doses, the effects of LPS on cognition are due to neuropathology rather than reversible inhibition of plasticity. In another study, acute ICV injection of low doses of LPS (1–100 ng) induced depressive-like behavior as well as sickness, which include decreased appetite, weight loss and reduced interest in the physical and social environment, which are mediated by TNF- α [56]. Therefore, the fact that low doses of LPS induce sickness behavior precludes any conclusions on the specific effects of TLR4 activation in the brain on hippocampus-dependent cognitive behavior.

TLR9 activation also exerts effects on spatial learning and memory; ICV infusion of CpG DNA (1 $\mu\text{g/day}$) over 4 weeks results in increased latency to reach the platform in a water-maze task [57]. Although this dose did not adversely affect motor function, significant brain pathology was observed including microglial activation, acute axonal damage surrounding the ventricles, ependymal disruption

and reactive astrogliosis within the hippocampus. This implies that the memory impairments are due to neurotoxicity rather than due to a pharmacological effect of activation of TLR9 on pathways known to alter synaptic transmission or plasticity-related molecular pathways. In this regard, whereas it is widely accepted that neuroinflammation causes cognitive impairment, we would like to emphasize the possible contribution of TLR-mediated signaling to non-neuroinflammation mediated pathways that affect cognitive behavior. Our current knowledge on the effects of brain-specific activation of TLRs 3, 9 and 4 is summarized in Figure 3d.

TLRs in CNS infection

The well-established ligands for TLRs in the context of infection are molecules on or liberated from bacteria, viruses and other invading pathogens. The responses of cells to such pathogen-derived ligands have been most extensively studied in immune cells, particularly dendritic cells and macrophages [58,59]. In the CNS, microglia express several different TLRs that, when activated by PAMPs, induce the production of pro-inflammatory cytokines including IL-6 and TNF- α [60,61]. Microglia can be activated during systemic infections without the blood–brain barrier (BBB) being compromised suggesting that PAMPs can cross the BBB and/or activate macrophages and microglia in circumventricular organs. Alternatively, macrophages and/or circulating cytokines can pass the BBB and intercept invading pathogens in the brain and/or activated microglial cells. There is evidence that localized activation of TLR4 in BBB-associated macrophages/microglia can trigger a ‘wave’ of microglial activation that spreads within the brain parenchyma; this transcellular wave of innate immune cell activation is believed to be propagated by TNF- α [62]. TLR activation can and does result in different outcomes in different types of CNS cells. When TLR3 is activated in human astrocytes, a comprehensive neuroprotective response was suggested to occur, in contrast to the pro-inflammatory reaction of microglial cells [63]. However, the majority of studies support the view that TLR3 activation in human astrocytes contributes to a pro-inflammatory phenotype of astrocytes [64].

This emphasizes the importance of studying cell-specific responses to TLR activation in the context of the cellular network in the CNS.

A range of infectious conditions have been associated with activation of one or more TLRs in macrophages/microglia in the CNS [61], and several of these TLRs are involved in limiting and clearing bacterial and viral infections of the CNS (Table 2). The use of animal models of meningitis and encephalitis has contributed to our understanding of the mechanisms by which the innate immune system in the brain responds to pathogens. TLR2 and TLR4 play key roles in the response of cells in the CNS to pneumococcal meningitis [65]. The immune response of mice lacking both TLR2 and TLR4 to intracisternal pneumococcal infection is more severely impaired than that of mice lacking either TLR2 or TLR4 alone. Mice lacking TLR2, 4 and 9 have similar susceptibility as mice lacking TLRs 2 and 4 to pneumococcal meningitis. Importantly, When TLR2/4 double-deficient mice received bone marrow

Table 2. Involvement of TLRs in CNS infection, injury and disease

Condition	TLR/TIR	Role and influence on disease outcome	Refs
Infections			
Meningitis (bacterial)	TLR2, TLR4	Clearance of infectious agents	[65]
	TLR9	No effect	[65]
Meningitis (viral) WNV	TLR2, TLR9	Stimulates innate anti-viral activities	[66]
	TLR3	Mediates WNV entry into the brain causing lethal encephalitis	[68]
	TLR3	Protective role against WNV infection	[69]
	MyD88	Restricts viral infection	[70]
	SARM	Restrict viral replication	[71]
Herpes simplex	TLR2, TLR9	Reduction in viral load in the brain	[66]
HIV AIDS	TLR3	Vital for natural immunity to HSV-1 in the CNS	[67]
	TLR4	Induces glial reactivity and inflammation	[103]
Injury			
Ischemic stroke	TLR2, TLR4	Enhancement of neuronal death and functional deficits	[17,79]
	TLR3, TLR9	No effect	[79]
	HMGB1 (TLR4)	Exacerbates ischemic damage	[81]
Ischemic preconditioning	TLR4	Mediates ischemic preconditioning-induced neuroprotection	[80]
Traumatic injury	TLR2, TLR4	Exacerbates neuronal injury	[78,82]
Disease			
AD	TLR2	TLR2 acts as an endogenous receptor for the clearance of toxic A β by bone-marrow-derived immune cells	[86]
	PGN (TLR2), LPS (TLR4), CpG ODN(TLR9)	Increased uptake of A β by microglial cell line <i>in vitro</i>	[88]
	TLR9	Protects against A β toxicity, reduces A β toxicity, reduces A β aggregation, ameliorates A β induced memory impairments	[90,91]
	TLR2, TLR4	Clearance (phagocytosis) of A β by microglia <i>in vivo</i>	[89]
	TLR4	Reduces microglial activation by A β	[93]
	TLR4	Increases vulnerability of neurons to A β ASP299GLY polymorphism reduces the risk of AD in humans Increases TNF- α , IL-1 β , IL-10 and IL-17 in the brains of an AD mouse model	[94] [95] [92]
MS	<i>S. pneumoniae</i> (TLR2)	Exacerbates EAE by a TLR2-mediated mechanism	[100]
	Hyaluronic acid (TLR2)	Adverse effect on OPCs; blocks remyelination	[41]
	TLR2	TLR2 deficiency does not affect EAE progression	[98]
	15-HC (TLR2)	Exacerbates MOG(35–55) induced EAE	[101]
	TLR4	Exacerbates EAE induced disease outcome	[97]
	LPS (TLR4)	Early life exposure to lipopolysaccharide suppresses EAE	[99]
	TLR9	Ameliorates EAE induced by MOG(35–55)	[104]
	TLR9	Exacerbates EAE severity induced by MOG	[98]
	MyD88	Delays onset of the disease Protects from EAE	[98] [97]

transplants from wild type mice, a cerebral immune response occurred and the pneumococcal infection was attenuated [65]. This result emphasizes that in the case of cerebral meningitis, TLR2 and 4 expression on circulating immune cells is critical for a successful immune response to infection in the brain.

Encephalitis caused by herpes simplex virus (HSV) infection results in an inflammatory response in the CNS, and TLR2 and TLR9 act synergistically to stimulate innate antiviral activities, thereby protecting against HSV infection in the brain [66]. This implies that synergism rather than redundancy exists in the TLR family of receptors in pathogen responses in general, and in the brain in particular. HSV-1 is a double-stranded DNA virus with dsRNA intermediates. Molecular genetic studies suggest that mutations in TLR3 might render some humans vulnerable to HSV encephalitis [67]. Human TLR3 also appears to be largely redundant for antiviral immunity because TLR3-deficient patients have infections with numerous viruses without developing severe disease. Nevertheless, human TLR3 is essential for primary immunity to

HSV-1 in the CNS, at least in some circumstances. This is an example of an individual TLR playing a non-redundant role in host defense due to its ability to recognize dsRNA.

Two additional infectious agents to which TLRs respond are West Nile virus (WNV) and HIV. Studies of TLR3-deficient mice have resulted in seemingly conflicting conclusions regarding the role of TLR3 in WNV pathogenesis. An initial report provided evidence that TLR3 plays a pivotal role in the entrance of WNV into the brain [68], whereas a more recent report indicated that TLR3 protects the brain against WNV [69]. This discrepancy could result from distinct routes of inoculation, passage history of the virus and/or the viral dose [69]. MyD88 restricts WNV by inhibiting replication in subsets of cells and preventing immune cell migration into the CNS. Mice deficient for MyD88 show increased lethality after WNV infection and elevated viral burden in the brain [70]. Sterile alpha- and armadillo-motif-containing protein (SARM) is the only known inhibitory mammalian TIR-domain containing adaptor protein. WNV replication is also increased specifically in the brainstem of SARM-deficient mice and is asso-

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ciated with enhanced mortality [71]. SARM deficiency is also linked to reduced levels of TNF- α , decreased microglia activation and increased neuronal death in the brainstem after WNV infection. Therefore, SARM functions to restrict viral infection and neuronal injury in a brain region-specific manner, possibly by modulating the activation of resident CNS inflammatory cells [71]. It is logical that MyD88 deficiency results in enhanced mortality following WNV infection because MyD88 is the main adaptor protein involved in TLR-related response to WNV. However, that deficiency in SARM results in a similar effect is of interest because i) SARM is highly expressed in the brain [71], and ii) SARM acts to inhibit TLR3- and TLR4-mediated responses [72]. The immunological phenotypes of MyD88 and SARM provide additional evidence that immunological responses in the brain require tight regulation.

The adverse effects of aberrant TLR activation caused by systemic infections might also alter brain development in utero. Indeed, TLR2 activation using the synthetic bacterial lipopeptides Palmitoyl-cysteinyl-seryl-(lysyl₄) (Pam₃CSK₄) and Follistatin-like 1, or LMW-HA (a TLR2 and TLR4 DAMP [73]) inhibits the proliferation of NPCs in the brains of developing mouse embryos resulting in cortical dysgenesis [37]. Related to this, a vast literature shows causative links between maternal exposure to TLR ligands and brain structure as well as behavioral deficits in offspring, deficits suggested by some to mimic maternal infections in humans that have been proposed (though not proven) to contribute to some cases of schizophrenia in the offspring (for a comprehensive review, see [74]).

The toll of brain injury

Two general types of injury to the CNS that are very common and associated with morbidity and mortality are ischemic injury (stroke) and traumatic injury to the brain or spinal cord. By inducing the production of pro-inflammatory cytokines and excitotoxins, activation of TLR4 and TLR2 in microglia probably contributes to the neuronal damage that occurs after a stroke [75–78]. TLR2 and TLR4 signaling in neurons might also contribute to their demise after a stroke because levels of TLRs 2 and 4 are increased in cerebral cortical neurons in response to ischemia/reperfusion injury, and the amount of brain damage and neurological deficits caused by a stroke are reduced in mice deficient in TLR2 or TLR4 [17]. However, in contrast to the typical MyD88–NF κ B signaling pathway that induces cytokine production in microglia, the cell death-promoting actions of TLR2 and TLR4 in ischemic neurons are mediated by JNK and the transcription factor AP-1 [17]. By contrast, TLR3 and TLR9 do not alter ischemic stroke outcome [79]. A short ischemic event (ischemic preconditioning) results in a subsequent resistance to severe ischemia. TLR4-deficient mice exhibit reduced ischemic preconditioning-induced neuroprotection compared to wild type mice [75]. TLR4 deficiency maintains levels of TNF- α , inducible nitric oxide synthase (iNOS), cyclooxygenase-2 and NF κ B below wild type levels. These data demonstrate that TLR4 signaling is involved in brain tolerance to ischemic damage [80]. Whereas the endogenous TLR ligands that might activate TLRs during a stroke are unknown, recent findings suggest a role for the DAMP high mobility group box 1 (HMGB1) in TLR4 activation [81].

Very little information is available concerning the roles of TLRs in traumatic injury to the CNS. Axotomy-induced degeneration of retinal ganglion cells was reduced in TLR4-deficient mice, and this neuroprotection was associated with decreased activation of JNK and p38 kinases and reduced iNOS levels [78]. In a stab-wound model of cortical brain injury the activation of microglia and astrocytes was reduced in mice lacking TLR2 [82]. In addition, the expression of the heme oxygenase-1 (*HO-1*) gene, a glia-expressed wound-responsive gene, was reduced after stab-wound injury in TLR2 knockout mice. This implies that TLR2 contributes to glial cell activation and HO-1 production associated with traumatic brain injury. Information is lacking regarding the roles of other TLRs in traumatic CNS injury.

Inflammation of the lumbar spinal cord following traumatic injury, or peripheral nerve injury, is associated with pain [83]. Acute activation of TLR4 by intrathecal LPS administration in rodents is associated with hyperalgesia and pain. LPS is a potent inducer of nociception/orphanin FQ (N/OFQ), an opioid-related peptide that plays a key role in pain physiology, and blockade of either TLR4 or MD-1, a member of the TLR4 complex, prevents N/OFQ expression [46]. It was recently shown that CNS TLR4 activation increases p38 phosphorylation and increases extracellular ATP production. ATP then activates the purinergic receptor P2X7 that further increases p38 phosphorylation and causes secretion of mature IL-1 β [83]. IL-1 β can modulate neuronal mechanisms of chronic pain in the dorsal horn via the MyD88 pathway. Therefore, by activating TLR4 using LPS, two signaling cascades that utilize the adaptor protein MyD88 facilitate nociception, which stresses the importance of the TLR/TIR adaptor protein family in this aspect of neuronal plasticity. In this respect, it is important to bear in mind that LPS is an exogenous, rather than endogenous, TLR4 ligand. The inflammatory process following LPS activation can be supplemented with endogenous activation once inflammation occurs in the tissue; however, it is possible that initial activation of TLR4 using an endogenous ligand will induce different outcomes. Not all TLRs are implicated in pain, however, because TLR7 is not necessary to elicit mechanical, thermal, inflammatory and neuropathic pain in mice [84]. However, TLR7 expressed on DRG neurons does mediate itch sensation (pruritus) primarily by non-histamine pruritogens [84]. TLR7 ligands probably elicit itch via a direct action on sensory neurons, however non-neuronal cells could also be involved in this process.

Emerging roles for TLRs in neurodegenerative and demyelinating diseases

Alzheimer's disease (AD) is the most common age-related neurodegenerative disorder with a devastating effect on the patients and their caregivers. AD involves local inflammatory cellular and molecular alterations associated with the characteristic pathological changes, amyloid deposits and tau tangles. Amyloid β -peptide (A β) is generated as a proteolytic product of the amyloid precursor protein (APP). A β self-aggregates and accumulates on the surface of neurons and in large 'plaques', whereas another protein, named tau, accumulates within neurons in brain regions that are critical for learning and memory including the

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Box 1. The relevance of endogenous ligands to physiological conditions

The subject of endogenous ligands has leapt in the past several years from being regarded as 'endotoxin contamination' to the forefront of research in the TLR fields. There is no doubt that under various pathological conditions from cancer, stroke to physical wounds, endogenous epitopes are exposed and detected by TLRs. However, these observations cannot explain how TLRs confer effects in the absence of a PAMP or DAMP as occurs in physiological conditions. Two signaling events are plausible under physiological conditions: i) there are ligands that are not considered 'damage associated molecular patterns', but rather 'physiologically occurring molecular patterns' that contribute to constitutive TLR activation; ii) constitutive TLR activation is not mediated by ligands, but rather by unknown co-receptors that together mediate a non-cannonical signaling pathway. The two possible explanations must be tested in order to determine which pathway is predominant under physiological conditions by TLRs.

deficient mice are not protected against EAE suggests that the endogenous levels of 15-HC in EAE-induced mice are not sufficient to efficiently exacerbate the disease in a TLR2-dependent manner. However, it is clear that signaling through MyD88 is critical in disease development and therefore identifies TLRs as targets for the development of therapeutic interventions in MS (Box 1).

Conclusion and future directions

The concept that the same protein acquires multiple roles during evolution is exemplified in TLRs, which initially emerged as a family of innate immune receptors, and are now being recognized as modulators of CNS plasticity. TLRs influence NPC proliferation, differentiation, neurite outgrowth and behavioral plasticity. However, although evidence from mice deficient in TLRs strongly implicates these receptors in neuroplasticity, the distinction between developmental and functional effects of life-long deficiency of a TLR, as well as specific roles for TLRs in neuroplasticity following infection and injury remains unclear (Box 2). Future studies focused on the role of TLRs in discrete regions of the CNS in health and disease will provide valuable insight into the expanding role of TLRs in the function and plasticity of the nervous system.

Little is known regarding if and how TLR signaling interacts with other signaling pathways involved in developmental and adult neuroplasticity. Glutamate is the major excitatory neurotransmitter in the CNS and activation of both AMPA and NMDA subtypes of glutamate receptors is essential for synaptic plasticity/learning and memory. Neurons lacking either TLR2 or TLR4 exhibit increased resistance to the adverse effects of energy deprivation, an experimental model in which activation of glutamate receptors plays a role in the cell death process [17]. Interestingly, TLR3 deficiency affects levels of activated cAMP response element-binding (CREB), a transcription factor that plays important roles in learning and memory and that is known to be activated in response to glutamate receptor stimulation [42]. Another signaling pathway that one or more TLRs may interact with is the phosphoinositide 3-kinase (PI3 kinase)/Akt pathway that is activated by neurotrophic factors such as brain-derived neurotrophic factor (BDNF) and insulin-like growth factors (IGFs). Both BDNF and IGF-1 promote neurogenesis, enhance synaptic

Box 2. Outstanding questions

- In the absence of tissue damage, what are the endogenous ligands that activate TLRs under physiological conditions? Are self-mRNA and stathmin relevant for noninflammatory conditions?
- Does TLR3 signaling affect cognition by altering the development of neuronal circuits and/or by more acute effects on synaptic plasticity? Do TLRs other than TLR3 have an impact on cognition?
- What signaling pathways are initiated by TLRs to mediate neurological effects, and do they differ among various CNS cell lineages?
- Do IL-1R and TLR signaling cooperate in shaping CNS plasticity?
- Does TLR signaling affect cognition during aging?
- Do TLRs contribute to cognitive impairment that occurs as the result of CNS infections?
- Do one or more TLR signaling pathways modify signaling cascades activated by neurotransmitters and neurotrophic factors?
- What are the transcription factors and gene targets that mediate effects of TLR activation on neurogenesis, neurite outgrowth, neuronal plasticity and behavior?
- Can specific CNS-penetrant TLR antagonists and agonists be identified and developed as therapeutic interventions for CNS injury and disease?

plasticity and protect neurons against a range of metabolic, oxidative and excitotoxic stressors [102]. Because several TLRs (2 and 4, in particular) increase the vulnerability of neurons to oxidative and metabolic stresses, it might be expected that BDNF and IGF-1 signaling would counteract the adverse effects of TLR2 and 4 signaling, although this remains to be determined.

Another largely unexplored issue concerns how, at the levels of signal transduction and gene expression, TLRs affect the proliferation, differentiation, outgrowth, plasticity and survival of neurons. The emerging findings described above suggest the existence of a complex TLR signaling network that influences neural plasticity by both direct effects on NPC and neurons, and indirect glia-mediated processes. Further studies of the signaling outcomes of TLR activation by endogenous and extrinsic ligands in neurons and glial cells will provide novel insights into the roles of these ancient receptors in the generation, plasticity and pathology of the nervous system.

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