Neuroinflammatory changes negatively impact on LTP: a focus on IL-1β

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Abstract

In recent years it has become clear that neuroinflammatory changes develop in the brain with age and that similar, though more profound changes, occur in neurodegenerative conditions and in animal models of neurodegeneration. These changes are linked with deterioration in plasticity and the evidence suggests that a key causative factor is microglial activation and the associated increase in production and release of inflammatory cytokines. Several groups have reported that interleukin (IL)-1β negatively impacts on hippocampal-dependent learning and has an inhibitory effect on LTP although this is concentration-dependent. Similarly other inflammatory cytokines, which are also produced by microglia similarly decrease LTP. The evidence supporting these findings will be reviewed here and will be discussed in the context of considering mechanisms by which the negative impact of neuroinflammation can be ameliorated.

IL-1β negatively impacts on synaptic plasticity - the historical perspective

It was recognized in the late 1980s and early 1990s that the inflammatory cytokine, interleukin (IL)-1, was an endogenous pyrogen and that it interacted with centrally-located IL-1 receptors to induce the behavioural changes that accompanied fever (Blatteis, 1988; Kluger, 1991). At around the same time, the first reports indicating that IL-1β exerted a negative effect on LTP in vitro appeared. Thus application of IL-1β to hippocampal slices inhibited LTP in CA1 (Bellinger et al., 1993) and CA3 (Katsuki et al., 1990). A similar negative effect of IL-1β on LTP in dentate gyrus was reported subsequently (Cunningham et al., 1996) and, in this case, it was proposed that the IL-1β-induced change was mediated by the inhibitory effect of the cytokine on calcium channel activity. The negative effect of IL-1β on LTP was also described in vivo; intracerebroventricular injection of IL-1β inhibited LTP in perforant path-granule cell synapses in urethane-anaesthetized rats (Kelly et al., 2001; Lynch, 1998; Murray and Lynch, 1998a; Vereker et al., 2000b), while the endogenous receptor antagonist, IL-1ra, attenuated the IL-1β-induced changes (Loscher et al., 2003).

In addition to its inhibitory effects on LTP, IL-1β has also been shown to inhibit hippocampal-dependent learning, for example spatial learning (Oitzl et al., 1993 Gahtan and Overmier, 2001; Gibertini, 1998), and contextual fear conditioning (Maier and Watkins, 1995; Pugh et al., 1998; 1999; 2000). IL-1ra has been shown to attenuate the impairment in contextual fear conditioning induced by immunodeficiency virus-1 coat protein gp120, which may be mediated by IL-1β (Pugh et al., 2000). In contrast, hippocampal-independent forms of learning like auditory-cue fear conditioning, are insensitive to IL-1β (Pugh et al., 2000). Consistent with these data that describe a negative impact of IL-1β on cognition, a mouse model in which IL-1β is overexpressed displayed impairment in contextual fear conditioning but hippocampal-independent tasks were not affected (Hein et al., 2010; Hein et al., 2012)

The signaling events induced by IL-1β have been studied in detail (Dunne and O'Neill, 2003) with clear evidence that it activates members of the MAP kinase family. It has been shown that intracerebroventricular injection of IL-1β increases activation of p38 MAP kinase and JNK in hippocampus and inhibition of p38 MAP kinase by SB203580, or JNK by SP600125, attenuated the IL-1β-induced inhibition of LTP in vivo and in vitro (Curran et al., 2003; Kelly et al., 2003). IL-1β also activates NFκB and the finding that the IL-1β-induced inhibition of LTP was suppressed by the non-specific NFκB inhibitor, SN50, suggests that a number of IL-1β-induced signaling events combine to impact on LTP (Kelly et al., 2003). Interestingly, IL-1β also impacts on BDNF-induced signaling in a
p38-dependent manner (Tong et al., 2012). The authors of this work suggested that the key effect of IL-1β was to prevent the BDNF-dependent phosphorylation of insulin receptor substrate 1 which mediates the signal transduction effects of TrkB that rely on activation of CREB, Arc and coflin.

**The effect of IL-1β on LTP is dose-dependent**

Some time after the initial findings that indicated a negative effect of IL-1β on LTP, a number of papers provided data demonstrating that the IL-1β-induced change is probably dose-dependent since blocking IL-1R by IL-1ra also decreased LTP (Goshen et al., 2007; Loscher et al., 2003; Schneider et al., 1998; Spulber et al., 2009) and a temperature-dependent effect of IL-1ra on LTP was also described (Ross et al., 2003). It was therefore suggested that physiological levels of IL-1 are necessary for induction and maintenance of LTP and this was confirmed by Avital and colleagues who showed that LTP was absent in perforant path granule cell synapses in anaesthetized IL-1R knockout mice and also in area CA1 in slices prepared from these mice (Avital et al., 2003). However transplantation of neural precursor cells that differentiated into astrocytes, obtained from wild type neonatal mice, were also effective in correcting the deficit in LTP observed in IL-1RI knockout mice (Ben Menachem-Zidon et al., 2011). Whether these studies imply that the loss of IL-1RI is associated with loss of cells or decreased cell viability remains to be determined. The studies of the Yirmiya group identified a close correlation between the ability of mice to sustain LTP and their performance in spatial learning tasks. Interestingly environmental enrichment, which has a beneficial effect on LTP, principally because of its ability to increase BDNF and stimulate neurogenesis (Bekinschtein et al., 2011; Eckert and Abraham, 2010), was capable of supporting LTP and spatial memory in IL-1R1 knockout mice (Goshen et al., 2009). Neither BDNF expression nor neurogenesis were affected in IL-1R1 knockout mice but the authors suggested that environmental enrichment induced its effect because it increased dendritic spine size (Goshen et al., 2009). Therefore the implication is that certain morphological changes can compensate for the loss of IL-1 receptors although the mechanism by which this occurs requires examination.

**When IL-1β is increased LTP is decreased**

A correlation between IL-1β concentration in hippocampus and impaired LTP has been identified in a number of models. For example, it has been widely reported that lipopolysaccharide (LPS) increases production of inflammatory cytokines in brain areas including hippocampus and many groups have reported LTP (Kelly et al., 2003; Lante et al., 2008; Maggio et al., 2013; Vereker et al., 2000a) and spatial learning (Lante et al., 2008; Shaw et al., 2001) deficits in LPS-treated animals. Strategies that attenuated the LPS-induced change in IL-1β and deficits in LTP and/or spatial learning include glucagon-like peptide-1 (Iwai et al., 2014), activation of mineralocorticoid receptors (Maggio et al., 2013), overexpression of mitochondrial transcription factor A (Hayashi et al., 2008), and treatment with N-acetylcysteine (Lante et al., 2008), CD200Fc (Cox et al., 2012), atorvastatin (Clarke et al., 2008), inhibitors of JNK (Barry et al., 2005), and p38 (Kelly et al., 2003) and the anti-inflammatory cytokine, IL-10 (Kelly et al., 2001).

The deficit in LTP in transgenic mouse models of Alzheimer's disease (AD) has also been associated with increased expression of IL-1β (Gallagher et al., 2012; Gallagher et al., 2013; Kelly et al., 2013) while administration of Aβ, which inhibits LTP, increase hippocampal concentration of IL-1β (Clarke et al., 2007; Lyons et al., 2007). Recent evidence has indicated that administration of an IL-1R blocking antibody improved synaptic plasticity in an animal model of AD (Kitazawa et al., 2011).
There are other examples in the literature which link poor ability of animals to sustain LTP with increased hippocampal concentration of IL-1β, for example aged animals (Griffin et al., 2006; Liu et al., 2012; Murray and Lynch, 1998a) and animals exposed to stress or to γ-irradiation (Lonergan et al., 2002; Vereker et al., 2001). In each of these situations, the deficit in LTP was ameliorated when hippocampal concentration of IL-1β was decreased, for example by treating animals with the polyunsaturated fatty acid, eicosapentaenoic acid, or by decreasing reactive oxygen species production (Lonergan et al., 2002; Lynch et al., 2007; Lynch, 2010; Vereker et al., 2001).

It has been reported that aged animals are more susceptible to immune challenge and this is closely correlated with increased IL-1β concentration in the brain. For example abdominal surgery negatively impacted on hippocampal-dependent tasks in aged, but not young, rats and this effect was blocked by central administration IL-1ra (Barrientos et al., 2012), while E. coli infection similarly affected hippocampal-dependent memory in 24 month-old but not 3 month-old rats (Barrientos et al., 2006). LPS, which increases IL-1β concentration in hippocampus, stimulated cytokine production to a greater extent in hippocampus of aged mice compared with young mice and this was coupled with more profound sickness behaviour (Godbout et al., 2005) and impaired performance in a spatial learning task (Chen et al., 2008).

**IL-1β, IL-18, the inflammasome and LTP**

Apart from IL-1β, IL-18 has been shown to inhibit LTP. Indeed the IL-18-induced attenuation of LTP in dentate gyrus in vitro and its ability to decrease NMDA receptor-mediated potentials were both blocked by IL-1ra, although the inhibitory effect of TNFα on LTP was unaffected (Curran and O’Connor, 2001). It was subsequently reported that the inhibitory effect of IL-18 on LTP could be attenuated by the mGluR5 specific antagonist MPEP and the group II mGluR antagonist, MTPG (Cumiskey et al., 2007c) and also by the COX-2 inhibitor, SC-236, the iNOS inhibitor 1400W and peroxisome proliferation activated receptor (PPAR)γ agonist, ciglitazone (Cumiskey et al., 2007b). Interestingly IL-18 also inhibits LTP of C-fiber-evoked field potentials induced by tetanic stimulation of the sciatic nerve in the spinal cord and the authors identified a key role for microglial activation since IL-18 was localized largely to microglia and this effect was inhibited by minocycline (Chu et al., 2012).

Both IL-1β and IL-18 are processed by caspase 1, which is activated as a consequence of inflammasome activation. Assembly of the inflammasome, which is a collection of cytosolic proteins including pro-caspase 1, is triggered by activation of members of the mammalian nucleotide binding domain, leucine-rich repeat (LRR)-containing receptor (NLR) family and results in activation of caspase 1 with the subsequent processing and release of IL-1β and IL-18 (Tschopp et al., 2003; Walsh et al., 2014). Perhaps the most studied member of the family is NLRP3, which is present in microglia and plays a key role in regulating inflammation.

There are other NLR family members including NLRP1 and NLRP2, as well as 2 others, NLRC4 which contains an N-terminal caspase activation and recruitment domain (CARD), and absent in myeloma-2 (AIM2) (Walsh et al., 2014). NLRP1 and NLRP2 have been identified in neurons and astrocytes respectively (de Rivero Vaccari et al., 2014; Minkiewicz et al., 2013) although NLRP1 is also present in microglia, as is NLRC4 (Walsh et al., 2014). AIM2 has been identified in neurons (Walsh et al., 2014). Recent evidence has indicated that there is an age-related increase in NLRP1 activation (Mawhinney et al., 2011); the authors pointed out that this increase parallels the age-related increases in IL-1β and IL-18 although neurons are not the main producers of these
cytokines in the brain. The authors reported a correlation between NLRP1 activation and age-related impairment in spatial learning in Fischer 344 rats and demonstrated that probenecid, which inhibits pannexin-1 and therefore prevents inflammasome activity, improved spatial learning in older rats. A correlation between NLRP3 activation and synaptic plasticity has also been reported. We have reported that the ATP-induced P2X₇ inflammasome assembly in LPS-primed microglia was inhibited by a specific P2X₇ receptor antagonist, GSK1370319A, and that this antagonist improved the ability of aged rats to sustain LTP (Murphy et al., 2012). Since that report, it was shown that knockout of NLRP3 in transgenic mice that overexpress amyloid precursor protein and presenilin 1 (APP/PS1 mice) prevented the loss of LTP and poor spatial learning commonly observed in these animals (Heneka et al., 2013).

Other inflammatory cytokines also block LTP
In addition to IL-1β and IL-18, several other inflammatory cytokines also inhibit LTP, including type I and type II interferons (IFN). IFNα inhibited LTP in CA1 and this was attenuated by genistein indicating a role for tyrosine kinase in mediating the effect (Mendoza-Fernandez et al., 2000), while the inhibitory effect of IFNγ on LTP was attenuated by the phosphodiesterase inhibitor, ibudilast (Mizuno et al., 2004); it was suggested that this might be a consequence of the ibudilast-induced release of IL-10 and NGF from microglia. Intracerebroventricular injection of IFNγ also blocked LTP in perforant path-granule cell synapses of urethane-anaesthetized rats perhaps as a consequence of microglial activation and the associated increase in IL-1β (Maher et al., 2006), while the inhibitory effect of IFNγ on LTP in CA1 in vitro was also associated with increased IL-1β and microglial activation (Kelly et al., 2013).

Other inflammatory cytokines that have been reported to exert an inhibitory effect on LTP in hippocampus include IL-6 (Bellinger et al., 1995) and TNFα (Cowley et al., 2012; Cunningham et al., 1996; Wang et al., 2005), although TNFα (as well as IL-1β) also inhibits LTP in spinal cord lamina and the evidence suggested that the synaptic effects were mediated by TNFα- and IL-1β-induced stimulation of glia (Gruber-Schoffnegger et al., 2013). The inhibitory effect of TNFα, at least in vitro, appeared to rely on activation of the group I metabotropic glutamate receptor since it was blocked by the inhibitor, MCPG (Cumiskey et al., 2007a) although its effect was also associated with p38 MAP kinase activation (Butler et al., 2004). IL-2 (Tancredi et al., 1990) and IL-8 (Xiong et al., 2003) also exert an inhibitory effect on LTP.

Glial activation contributes to neuroinflammatory changes
The primary source of inflammatory cytokines in the brain is glia and both microglia and astrocytes can be stimulated to produce IL-1β, TNFα and IL-6 although astrocytes probably produce more IL-6 and less TNFα than microglia (Cowley et al., 2012; Minogue et al., 2012). There is unequivocal evidence that impairment in LTP is associated with evidence of microglial activation as indicated by increased expression of cell surface markers, which is indicative of their antigen presentation function, and upregulation of inflammatory cytokine expression (Lynch, 2009; Lynch, 2010). However in the past few years it has become clear that microglia are plastic cells and mimic macrophages in the sense that they respond to different stimuli by adopting different activation states.

Macrophages become classically-activated in response to IFNγ in vitro, adopting the so-called M1 phenotype; this phenotype is associated with increased production of inflammatory mediators and, in situ, is responsible for destruction of pathogens. In contrast, macrophages become alternatively-activated when incubated with anti-inflammatory
cytokines like IL-4 and adopt the so-called M2 activation state; these cells play a role in tissue repair (Gordon, 2003). Alternatively-activated cells were initially identified by Stein and colleagues when this group established that cells treated with anti-inflammatory cytokines failed to produce NO (an archetypal indicator of classically-activated cells), were not cytotoxic and did not exhibit antigen-presentation capability (Mosser, 2003; Stein et al., 1992). Like macrophages, microglia adopt the M1 phenotype when exposed to IFNγ or LPS and adopt the M2 phenotype when incubated in the presence of IL-4. The M1 microglia is identified by an increase in mRNA expression of TNFα and iNOS, whereas the M2 phenotype is identified by increased mRNA expression of arginase I, mannose receptor, chitinase 3-like 3 and found in inflammatory zone-1 (FIZZ1), although several other markers of the different phenotypes have been suggested (Chhor et al., 2013). In particular, the M1 phenotype is associated with increased expression of several inflammatory cytokines including IL-1β and IL-6 (Chhor et al., 2013; Colton and Wilcock, 2010) and also with increased expression of markers of oxidative stress (Colton and Wilcock, 2010) which negatively impacts on synaptic plasticity (Lynch, 2004). Indeed recent direct evidence has indicated that microglia in the brain of APP/PS1 mice, which exhibit a deficit in LTP (Kelly et al., 2013) adopt the M1 activation state (Minogue et al., 2014) and the same was true in CD200-deficient mice (Denieffe et al., 2013b) in which LTP is also decreased (Costello et al., 2011).

Switching microglial phenotypes and the impact on LTP

While factors which are associated with microglia adopting the M1 activation state, like IFNγ and LPS, are linked with a deficit in LTP, factors that switch microglia from the M1 to the M2 phenotype are associated with restoration of LTP or enhanced LTP. For instance, inhibiting IL-1β with a neutralising antibody resulted in macrophages adopting the M2 state (Mirza et al., 2013) and this is consistent with the ability of IL-1ra to attenuate the IL-1β-induced inhibition of LTP (Loscher et al., 2003). In contrast IL-4 and IL-10, which induce microglia to adopt the M2 phenotype (Chhor et al., 2013; Minogue et al., 2014), are capable of attenuating the impaired LTP which is characteristic of age and LPS treatment (Loane et al., 2009; Lynch et al., 2004; Nolan et al., 2005).

Perhaps predictably, Akt, which can be activated downstream of IL-4 receptor engagement, appears to play a role in controlling the switch between macrophage phenotypes (Arranz et al., 2012). Whereas increased activation of the PI3K/Akt pathway has been observed in LTP, inhibitors of this pathway block LTP (Bruel-Jungerman et al., 2009; Jo et al., 2011; Kelly and Lynch, 2000). A role for PPARγ in switching cells from an M1 to an M2 phenotype has been reported (Chawla, 2010; Mandrekar-Colucci et al., 2012), and it has been shown that rosiglitazone, the PPARγ activator, partially reversed the age-related deficit in LTP (Cowley et al., 2012; Loane et al., 2009) and it also attenuates the Aβ-induced inhibition of LTP (Costello et al., 2005). This may be as a consequences of its ability to increase hippocampal IL-4 concentration (Loane et al., 2009).

NADPH oxidase is perhaps the key enzyme responsible for production of reactive oxygen species and its activity is increased with age (Bruce-Keller et al., 2010; Kumar et al., 2013). Recent evidence suggests that inhibition of NADPH oxidase induces microglia to switch from the M1 to the M2 phenotype (Kumar et al., 2013) This is interesting since a loss of LTP has been associated with increased reactive oxygen species production induced by H2O2 (Vereker et al., 2001) or age (Murray and Lynch, 1998a) and that the age-related changes are attenuated by antioxidative strategies, namely lipid acid and vitamins E and C (Lynch, 2001; McGahon et al., 1999; Murray and Lynch, 1998b).
One of the significant issues arising from the data that support the concept of microglia adopting the M1 phenotypes in vivo is identification of the cell source of IFNγ. The evidence indicates that resident brain cells do not produce significant amounts of the cytokine except in very specific circumstances (Suzuki et al., 2005) and that damage-associated molecular patterns (DAMPs), which also induce microglia to adopt the M1 phenotype, are produced only by dying cells. We have proposed that the source of IFNγ is infiltrating cells and report that Th1 cells, natural killer cells and macrophages infiltrate the brain of aged animals and APP/PS1 mice, release IFNγ and induce the microglia to adopt the M1 phenotype (Denieffe et al., 2013a; Kelly et al., 2013; Minogue et al., 2014). The evidence suggests that this cell infiltration is associated with increased blood brain barrier permeability. This raises the possibility that targeting the blood brain barrier might be a potentially useful therapeutic strategy in reducing neuroinflammatory changes that occur in the brain with age and neurodegenerative conditions.

Conclusions
There is little doubt that when microglia adopt the M1 phenotype and increase their production and release of inflammatory cytokines, synaptic plasticity is impaired. However careful analysis of the effect of IL-1β has revealed that the concentration of the cytokine determines the impact on LTP; specifically low concentrations and relatively acute exposure to IL-1β appears to be necessary for LTP whereas higher concentrations are detrimental. Whereas numerous factors have been shown to modulate the negative impact of inflammatory cytokines on LTP, significant clarity has developed as a result of a greater understanding of microglial phenotypes. Indeed the evidence to date indicates that factors which induce the M2 phenotype or switch M1 microglia into M2 microglia support LTP. This concept, which needs to be further assessed, may point to previously-unexplored mechanisms by which the compromised plasticity which develops with age and in models of Alzheimer's disease, might be reversed or limited.

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