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Peripheral immune contributions to the maintenance of central glial activation underlying neuropathic pain

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ABSTRACT

Recent evidence implicates an adaptive immune response in the central nervous system (CNS) mechanisms of neuropathic pain. This review identifies how neuropathic pain alters CNS immune privilege to facilitate T cell infiltration. Once in the CNS, T cells may interact with the local antigen presenting cells, microglia, via the major histocompatibility complex and the costimulatory molecules CD40 and B7. In this way, T cells may contribute to the maintenance of neuropathic pain through pro-inflammatory interactions with microglia and by facilitating the activation of astrocytes in the spinal dorsal horn. Based on the evidence presented in this review, we suggest that this bidirectional, pro-inflammatory system of neurons, glia and T cells in neuropathic pain should be renamed the pentapartite synapse, and identifies the latest member as a potential disease-modifying therapeutic target.

1. Introduction

Neuropathic pain is a chronic pain condition that may arise as a consequence of a lesion or disease affecting the somatosensory system (Treede et al., 2008). Such pain may be due to a primary lesion or dysfunction to either the peripheral or the central nervous systems (PNS; CNS), afflicts up to 8% of the general European population (Torrance et al., 2006; Bouhassira et al., 2008) and may be associated with hypersensitivity to noxious (hyperalgesia) and non-noxious (allodynia) stimuli. In addition to an accepted role for central immune signalling (Milligan and Watkins, 2009), a role for the peripheral immune system in the pathophysiology of neuropathic pain has been recognised for over a decade, and has recently been the subject of several excellent reviews (Thacker et al., 2007; Austin and Moalem-Taylor, 2010; Ren and Dubner, 2010). For example, many components of the innate immune system, including mast cells (Metcalfe et al., 1997; Zuo et al., 2003; Galli et al., 2005), neutrophils (Perkins and Tracey, 2000; Morin et al., 2007; Shaw et al., 2008), macrophages (Liu et al., 2000; Barclay et al., 2007; Hu et al., 2007; Vega-Avelaira et al., 2009) and complement proteins (Twining et al., 2004; Li et al., 2007; Levin et al., 2008; Vega-Avelaira et al., 2009), contribute to nociceptive hypersensitivity at the site of peripheral nerve injury and the dorsal root ganglia. Key studies have also implicated a PNS adaptive immune response in animal models of nerve injury (Cui et al., 2000; Hu and McLachlan, 2002; Moalem et al., 2004; Kleinschnitz et al., 2006; Hu et al., 2007; Li et al., 2007; Jung and Miller, 2008; Kim and Moalem-Taylor, 2011). Whilst T lymphocyte-microglial interactions (immunological synapses (Fig. 1)) have been explored in many other disease models, most notably in multiple sclerosis (Wilson et al., 2010; Chastain et al., 2011), they have only been inferred, but not directly demonstrated in the context of neuropathic pain. To a certain degree this is not surprising, given the subtle neuroinflammatory processes that are associated with neuropathic pain, in comparison to the gross, and rather obvious, pathology of diseases such as multiple sclerosis. As such, this review will cover the current evidence for the types of peripheral immune cells that infiltrate the CNS, the conditions under which these cells infiltrate, the potential interactions of these cell types with microglia, and will conclude by highlighting the exciting scope for treatment opportunities and further research in this particular field.

2. Cell types that infiltrate the CNS following nerve injury

A number of studies have examined the affected portions of the lumbar spinal cord following peripheral nerve injury for evidence of peripheral immune cell infiltration (Sweitzer et al., 2002a; Hu et al., 2007; Cao and DeLeo, 2008; Costigan et al., 2009; Grace et al., 2011). Sweitzer et al. (2002a) used rat chimeras to show that peripheral immune cells traffic to the ipsilateral dorsal and ventral horns of the lumbar spinal cord in response to L5 spinal nerve

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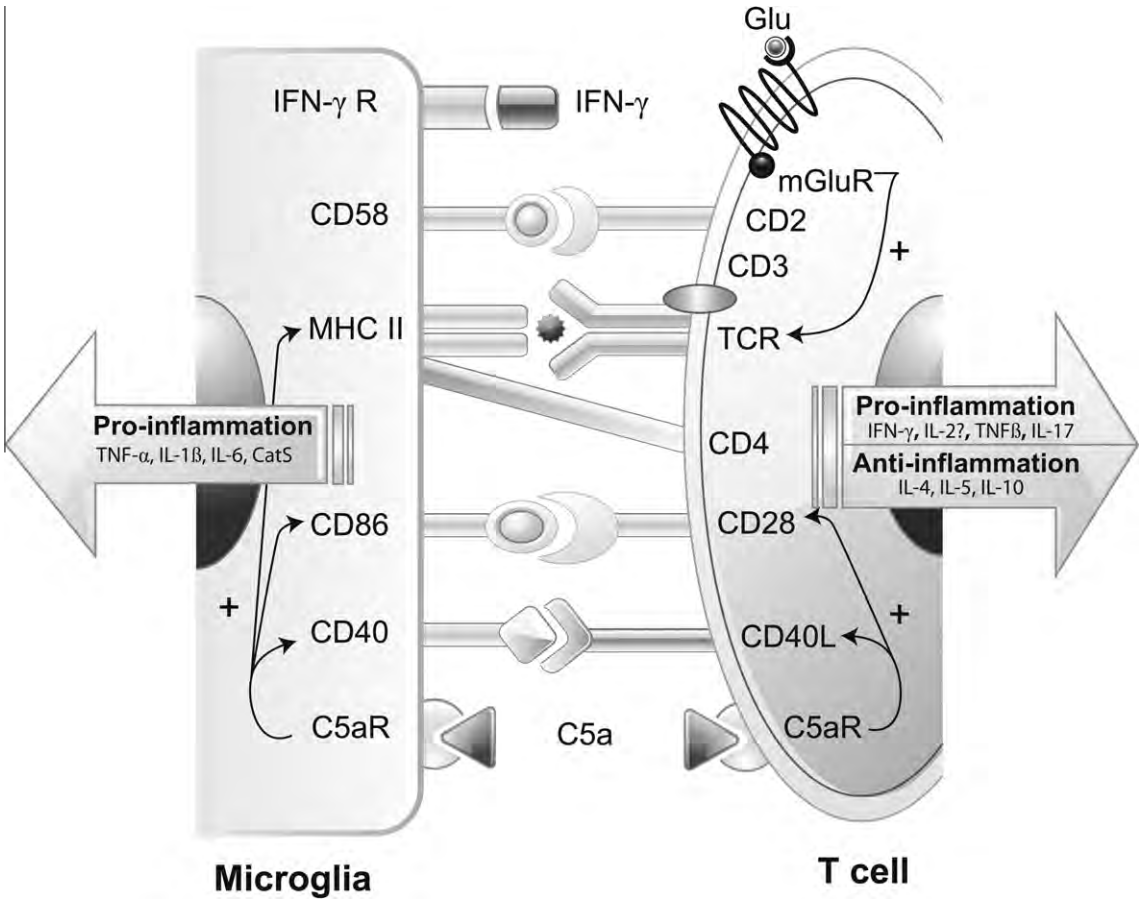

Fig. 1. The CNS immunological synapse in neuropathic pain. The antigen presenting cells of the CNS, microglia, present antigen via the major histocompatibility complex (MHC) class II. The antigen is recognised via the T cell receptor (TCR)–CD3 complex and the co-receptor CD4 (signal 1). This is interaction is assisted and stabilised by adhesion molecules, such as CD2, which attach the microglial ligand LFA-3/CD58. Following the activation signal received via the TCR–CD3 complex, the signal is verified by costimulatory molecules, of which CD40 and CD86 have been identified in neuropathic pain (signal 2). Constitutive or inducible T cell metabotropic glutamate receptors (mGluRs) and microglial and T cell complement component 5a receptors (C5aR) may respectively direct an immune response against local antigens and enhance expression of MHC class II and costimulatory molecules. The result of this interaction is secretion of pro-inflammatory or anti-inflammatory products by both cell types depending on the helper T cell subset (T_H1 vs. T_H2 vs. T_H17). CatS, cathepsin S; Glu, glutamate; IFN, interferon; IL, interleukin; TNF, tumour necrosis factor.

transection (L5Tx) and were shown to colocalise with the macrophage marker CD163 (ED2) at early timepoints (days 1 and 3 post injury), rather than the neuronal marker NeuN. The morphology of the infiltrating immune cells was similar to that of macrophages and T cells, with the presence of the latter confirmed by $CD3^+$ immunoreactivity in the ipsilateral dorsal and ventral horns of the lumbar spinal cord (Sweitzer et al., 2002a). However, a subsequent study from the same group used flow cytometry to show that $CD3^-CD11b^+I\text{-}A^{d+}$ macrophages did not infiltrate the lumbar spinal cords of L5Tx-operated mice (Cao and DeLeo, 2008). This finding does not preclude the presence of infiltrating macrophages expressing other surface activation markers. Moreover, rat chimeras have been used to show that bone marrow-derived monocytes infiltrated the ipsilateral dorsal and ventral horns of the lumbar spinal cord at 14 days after nerve injury (Zhang et al., 2007). Virtually all of the infiltrating bone marrow-derived monocytes resembled ramified microglia and expressed the microglial activation marker Iba1 (Zhang et al., 2007). It has been confirmed that B cells and Natural Killer cells do not infiltrate the lumbar spinal cords of mice under nerve-injured conditions (Cao and DeLeo, 2008) and no behavioural alterations were observed in nerve injured B cell deficient mice (Costigan et al., 2009). Hu et al. (2007) showed that chronic constriction injury (CCI) of the sciatic nerve in rats increased the numbers of α/β TCR^+ cells in the ipsilateral lumbar spinal cord compared to sham controls and that the majority of these cells were $CD8^+$, leaving only a smaller population of $CD4^+$ cells. These findings are in contrast to a subsequent study in which $CD4^+$ T cells ($CD3^+CD4^+$) and not $CD8^+$ T cells ($CD3^+CD8^+$) were consistently identified in the lumbar spinal cords of L5Tx-operated mice (Cao and DeLeo, 2008). A specific role for $CD4^+$ T cells has been corroborated by another study showing expression and a pronociceptive function of cytokines associated with $CD4^+$ T cell signalling in the ipsilateral lumbar dorsal horns of nerve-injured adult rats (Costigan et al., 2009).

In summary, it is evident that under nerve-injured conditions, B cells and Natural Killer cells do not infiltrate the lumbar spinal cord (Cao and DeLeo, 2008; Costigan et al., 2009), but the presence of infiltrating macrophages requires further investigation (Sweitzer et al., 2002a; Zhang et al., 2007; Cao and DeLeo, 2008). All reports agree that T cells infiltrate the CNS of nerve-injured rodents and while there is conflicting data on the subsets responsible, the weight of evidence supports $CD4^+$ T cell infiltration (Sweitzer et al., 2002a; Moalem et al., 2004; Hu et al., 2007; Cao and DeLeo, 2008; Costigan et al., 2009). Neuropathic-like nerve injuries, despite inducing less pronounced inflammation than acute inflammatory models (Sweitzer et al., 2002b) and less overt neuronal damage than axotomy (Hu et al., 2007), nonetheless have the peculiar ability to induce an adaptive immune response within the CNS. This raises the question as to how and why T cells in particular infiltrate the CNS following nerve injury, and whether this is beneficial or harmful in regard to the development of neuropathic pain.

3. Nerve injury-induced alterations to CNS immune privilege that facilitate T cell infiltration

The seminal observation by Shirai (1921) established the common view that the CNS is a site of immune privilege, which is supported by the physiological/anatomical, immunological and biochemical isolation of the parenchyma, in that it is:

1. protected from invading pathogens by the blood–brain barrier/blood–spinal cord barrier (BSCB) (there is considerable anatomical and pathophysiological heterogeneity associated with these endothelial cell tight junction barriers, and so the more general term "blood–CNS barriers" will also be used), and the epithelial blood–cerebrospinal fluid barrier (Reese and Karnovsky, 1967; Noble and Wrathall, 1989; Gordh et al., 2006; Johanson et al., 2011), and typical lymphatic drainage is absent (Barker and Billingham, 1977);
2. devoid of classical antigen presenting cells (APCs; e.g. dendritic cells) (Wucherpfennig, 1994) and constitutive expression of major histocompatibility complex (MHC) class I and class II antigen (Matsumoto et al., 1986; Wucherpfennig, 1994);
3. an immunosuppressive microenvironment that contains, for example, astrocytes that suppress or anergize infiltrating T cells (Sun et al., 1997; Hailer et al., 1998), and locally produces factors that suppress and regulate the production of immune responses in the CNS (Wilbanks and Streilein, 1992; Streilein, 1993).

Therefore, immune privilege was interpreted as immune ignorance, a lack of CNS immunosurveillance, and thought to be an evolutionary adaptation to protect against the potentially devastating effects of inflammation on the dynamic and complex neural networks of the brain and spinal cord (Streilein, 1995). However, accumulated evidence has shown that the CNS is not an entirely immunologically privileged organ, as it is now well established that lymphocytes continually traffic through the healthy CNS, albeit in restricted areas. Under certain conditions where T cells are activated, due to local trauma for example, greater CNS infiltration is observed (Mason et al., 1986; Hickey et al., 1991; Hirschberg et al., 1998; Furtado et al., 2008; Wilson et al., 2010), although to a lesser extent than that of the PNS, despite comparable insult (Moalem et al., 1999). As identified above, animal models of neuropathic pain are one such condition whereby activated T cells infiltrate the CNS. Therefore, the following section will identify how nerve injury alters each of these aspects of immune privilege (physiological/anatomical, immunological and biochemical isolation) in order to facilitate T cell infiltration of the CNS.

3.1. Nerve injury-induced modifications to the blood–CNS barriers

The first modification to CNS immune privilege is at the level of the blood–CNS barriers (Fig. 2). Significant albumin immunoreactivity was found in the lumbar spinal cord of rats following L4 nerve lesion (Gordh et al., 2006). These data indicate that there is an increase in BSCB permeability, for which both neuronal and immune mechanisms have been identified. Following CCI or spared nerve injury (SNI) in rats, the presence of albumin was also observed in the lumbar spinal cord after a 24 h delay (Beggs et al., 2010). Interestingly, blood–CNS barrier permeability following nerve injury was not localised to the affected portion of the spinal cord, but was also found in sacral, thoracic and cervical spinal regions and in the brainstem, where progressively more rostral brain areas showed less albumin accumulation (Beggs et al., 2010). The influence of CCI on BSCB permeability was mitigated by the application of lidocaine directly to the sciatic nerve prior to, but not after,

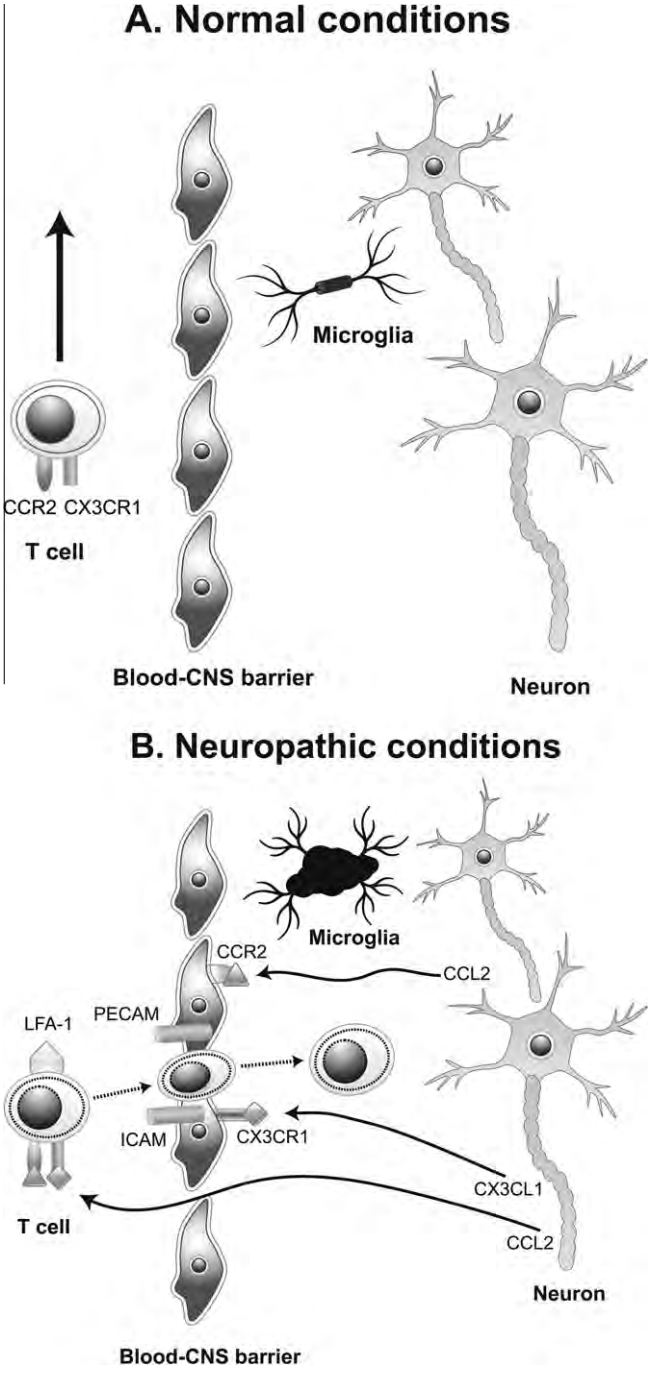

Fig. 2. T cells infiltrate the CNS under neuropathic pain conditions. (A) Under normal conditions, T cells do not infiltrate the CNS as blood–CNS barrier endothelial cells and T cells do not express the appropriate adhesion molecules, blood–CNS barrier tight junction proteins are intact, with spinal dorsal horn microglia in a ramified/'quiescent' phenotype and neurons are functioning normally. (B) Following a primary lesion or dysfunction to either the peripheral or the central nervous systems, primary afferents express many proinflammatory mediators including the chemokines CCL2 and CX_3CL1, which activate microglia, resulting in an amoeboid phenotype. Endothelial cells express cell adhesion molecules (ICAM and PECAM), which would be expected to facilitate the transendothelial migration of activated T cells (expressing LFA-1) into the CNS, which is aided by CCL2 and CX_3CL1. CCL2 also alters expression of tight junction proteins, which may increase blood–CNS barrier permeability to centrally secreted T cell chemotactic molecules, such as CCL2 and CX_3CL1.

surgery (Beggs et al., 2010). Short duration electrical stimulation of, or capsaicin application to, sciatic C-fibres also increased BSCB permeability from 24 h post stimulation, which was attenuated by

pre-treatment with lidocaine (Beggs et al., 2010). Beggs et al. (2010) outline potential mechanisms for the nociceptive action potential-induced increase in BSCB permeability. However, the action of the chemokine CCL2 (formerly MCP-1) should also be noted. CCL2 is secreted from the terminals of primary afferents following nerve injury (Strack et al., 2002; Dansereau et al., 2008; Abbadie et al., 2009), such as those described above, and acts on its blood–CNS barrier-bound cognate receptor (Andjelkovic and Pachter, 2000) to alter expression of tight junction associated proteins, leading to an increase in the permeability of blood–CNS barriers.

Nerve injury also induces several alterations to the blood–CNS barrier that allow activated T cells to infiltrate the permeable barrier. The DeLeo group has shown that adhesion molecules PECAM-1 and ICAM-1 are expressed by the blood–spinal cord barrier of rats in the lumbar region in response to nerve injury (Rutkowski et al., 2002; Sweitzer et al., 2002b). These molecules would be expected to facilitate transendothelial migration of activated T cells and monocytes/macrophages (Engelhardt, 2006; Muller, 2009). Spinal interleukin (IL)-1β has been shown to precede expression of these cell adhesion molecules (Sweitzer et al., 2002b), suggesting that glial activation is a prerequisite to this alteration of CNS immune privilege. In addition, CCL2, as well as CX_3CL1 (formerly fractalkine), directly facilitate T cell trafficking along the blood–CNS barriers (Prinz and Priller, 2010). These changes selectively facilitate transendothelial migration of activated T cells (Hickey et al., 1991), as these cells express higher levels of integrins, such as LFA-1, than resting T cells (Dustin and Springer, 1991), though this has yet to be directly confirmed in neuropathic pain.

3.2. Nerve injury-induced recruitment of antigen presenting cells

The second modification to CNS immune privilege is of the recruitment of CNS APCs, and expression of MHC molecules and the necessary costimulatory molecules. It is now well established that microglia become activated under neuropathic conditions by ATP, excitatory amino acids, substance P, CX_3CL1, CCL2, matrix metalloproteinases, pro-inflammatory cytokines etc. (Milligan and Watkins, 2009), resulting in expression of immunoregulatory molecules such as cellular adhesion molecules, MHC class II and costimulatory molecules that regulate leukocyte infiltration and engagement with APCs (Vass and Lassmann, 1990; Gehrmann et al., 1995; Kreutzberg, 1996; Aloisi, 2001; McCluskey and Lampson, 2001; Yang et al., 2010). These molecules often serve as activation markers for microglia (Bradesi, 2010), but their functional role in allowing activated microglia to operate as APCs in the CNS should not be overlooked (Hickey and Kimura, 1988; Hart and Fabry, 1995; Shrikant and Benveniste, 1996). The implication of MHC expression by microglia is that antigen is being presented in the cleft and is recognised by the TCR/CD3 complex. MHC class I expression denotes presentation of antigen to $CD8^+$ T cells, while class II expression indicates presentation of a antigen to $CD4^+$ T cells. T cell recognition of antigen is controlled by MHC molecules, in that T cells are specific to the antigen being presented and the self antigen associated with the MHC molecule (MHC restriction) (Kazansky, 2008). The source of the antigen presented by microglia following nerve injury through MHC class II expression remains to be addressed, as it may be derived from the injury site due to prolonged inflammation, or conversely, may be of central origin.

Reports of expression of specific MHC classes in the lumbar spinal cord of nerve-injured rats are mixed. Hu et al. (2007) observed minimal MHC class II^+ microglia in the ipsilateral dorsal horn, but rather observed extensive microglial MHC class I immunoreactivity in the CCI model, which was supported by corresponding predominance of $CD8^+$ T cell infiltration in the ipsilateral dorsal horn of the lumbar spinal cord. However, the DeLeo group has observed expression of MHC class II by microglia at time points correlating with nociceptive hypersensitivity in other models of neuropathic pain and radiculopathy, which bears some similarity to the CCI model (Hashizume et al., 2000; Rutkowski et al., 2002; Sweitzer and DeLeo, 2002; Sweitzer et al., 2002b). These studies did not quantify MHC class I expression, but the prominence of MHC class II expression in the spinal cord dorsal horn of the nerve-injured rodent is further supported by studies demonstrating an MHC class II/$CD4^+$ T cell ('signal 1': recognition; Fig. 1) mediated immune response in animals models of neuropathic pain (Sweitzer et al., 2002b; Moalem et al., 2004; Cao and DeLeo, 2008; Costigan et al., 2009). In addition to MHC expression, APC-bound costimulatory molecules ('signal 2': verification), such as CD40 (ligated by T cell CD40L) and B7 (ligated by T cell CD28), were upregulated (Fig. 1) in the ipsilateral lumbar dorsal horns of nerve-injured rodents (Rutkowski et al., 2004; Cao et al., 2009).

The term 'immunological synapse' was coined to describe the area of contact between T cells and the cells they are recognising (Paul and Seder, 1994; Monks et al., 1998; Grakoui et al., 1999). In the context of T cell engagement with microglia in the CNS, the immunological synapse incorporates the structure in which T cell receptors and their ligands, antigen-MHC complexes, are concentrated in the centre of the cell–cell contact, with a ring of adhesion molecules surrounding this central region (Monks et al., 1998; Grakoui et al., 1999). The evidence presented above infers that these CNS immunological synapses are formed following nerve injury. However, the presence of these structures requires confirmation, as do other aspects, such as MHC restriction.

3.3. Nerve injury-induced changes to the CNS microenvironment

The third nerve injury-induced alteration to CNS immune privilege is a change from an immunosuppressive microenvironment to one that is conducive to the recruitment and activation of immune cells. One such change may be through the production of complement, which was implicated in the pathophysiology of neuropathic pain when it was shown that intrathecal soluble complement receptor 1, which blocks the production of C3a, C5a and Membrane Attack Complex, prevented development of, and reversed established allodynia in three rat models of neuropathic pain (Twining et al., 2005). These findings were advanced by Griffin et al. (2007) who used *in situ* hybridization to show colocalisation of the complement proteins C1q, C4, and C3 with the microglial activation marker Iba1. Through a series of experiments that excluded the C3a and Membrane Attack Complex pathways, C5a was left as the only remaining pain effector (Griffin et al., 2007). The role of C5a in neuropathic pain was supported by the finding that C5a mRNA was upregulated in the dorsal horn after SNI, and confirmed by the observation that intrathecal administration of C5a peptide in naïve rats increased nerve injury-enhanced cold pain, which was reciprocally attenuated by intrathecal administration of a C5aR antagonist (Griffin et al., 2007). While Griffin et al. (2007) discuss potential mechanisms of complement induction, they do not discuss the pronociceptive mechanisms of C5a, for which subsequent studies may provide some insight. Traditionally, the complement system was considered integral to the innate immune system and to function only in the humoral responses of adaptive immunity. However, recent data demonstrates that complement locally produced by both T cells and microglia at the immunological synapse influences the strength of the $CD4^+$ T cell response following receptor activation by the engaged cells (Strainic et al., 2008). This is due to the role of complement signalling in positively regulating expression of MHC class II and the costimulatory molecules CD40 and B7 by the APC, and of the respective costimulatory molecules, CD40L and CD28, by T cells (Fig. 1) (Strainic et al., 2008). In addition, it has been shown that complement signalling is necessary to sustain naïve T cells (Strainic et al.,

2008), to direct induction of specific T cell subsets (Weaver et al., 2010), and to enhance T cell expansion by inhibiting apoptosis (Lalli et al., 2008). These studies suggest that complement induced by nerve injury may contribute to nociceptive hypersensitivity by supporting the activation and survival of T cells within the CNS, however this hypothesis requires confirmation. In addition to complement, the excess glutamate present at the dorsal horn under neuropathic-like conditions (Milligan and Watkins, 2009) may support the immunological synapse. Activation of constitutive and inducible metabotropic glutamate receptors on T cells may direct a T cell-mediated immune response against the dominant local self-antigens (Fig. 1) (Pacheco et al., 2007).

As well as increased expression of immunoregulatory and costimulatory molecules, activated glia and depolarised neurons differentially express cytokines and chemokines that are T cell chemoattractants. For example, it has been demonstrated that T cell infiltration is impaired in *IL-15* or *IL-15 receptor* -/- mice following trigeminal nerve injury (Huang et al., 2007), a cytokine also upregulated in the dorsal horn and colocalised with microglial and astrocyte activation markers following CCI (Gomez-Nicola et al., 2008). Cao and DeLeo (2008) report unpublished data from their laboratory showing increased lumbar spinal cord expression of proteins that, in addition to other cell types, are chemoattractant to T cells, including CCL2, CCL3 (formerly MIP-1α), CCL4 (formerly MIP-1β), CCL5 (formerly RANTES) and CXCL10 (formerly IP-10) at days 3, 7 and 14 following L5Tx. Furthermore, CX_3CL1, a chemokine posited to have a causative role in the central mechanisms of neuropathic pain (Milligan et al., 2008), is also a chemoattractant (Umehara et al., 2004), acting via its cognate receptor on T cells (Combadiere et al., 1998). The increased permeability of blood–CNS barrier tight junctions under neuropathic conditions (see above; Fig. 2) may allow diffusion of chemoattractants into the periphery (Song and Pachter, 2004).

T cells may also be activated by the same signals that activate glia, as they express functional TLR2, TLR3 and TLR4 (Kabelitz, 2007; Zanin-Zhorov et al., 2007; Kulkarni et al., 2011), innate immune pattern recognition receptors that have been implicated in neuropathic pain (Tanga et al., 2005; Kim et al., 2007; Obata et al., 2008).

An adoptive transfer paradigm developed in our laboratory supports the evidence presented above. We induced low or high pain in rats by placing either 1 (+3 equal pieces of subcutaneous chromic gut) or 4 chromic gut sutures around the sciatic nerve, respectively (Grace et al., 2010). Splenocytes were then harvested from high pain donors and intraperitoneally transferred to syngeneic low pain recipients, resulting in long-lasting potentiation of allodynia (Grace et al., 2011). Repetition of this experiment with peripheral blood mononuclear cells resulted in similar observations (Grace et al., 2011). However, when high pain cells were systemically transferred to recipients without underlying nerve injury, the effect was not observed (Grace et al., 2011), which indicates that immune cells are incapable of initiating exaggerated nociception in the absence of the neuropathic-induced changes to CNS immune privilege described above. These data also suggest that, in addition to central neuroimmune activation, these central neuroinflammatory processes may contribute to the pathology of persistent pain.

4. T cell contributions to the CNS mechanisms of neuropathic pain

The preceding discussion has established that T cells (most likely $CD4^+$) do in fact infiltrate the CNS under neuropathic conditions. However, as alluded to above, a number of studies have also established that T cells critically contribute to the nociceptive hypersensitivity associated with neuropathic pain.

4.1. Evidence that T cells critically contribute to neuropathic pain

Particular support for a role for T cells in neuropathic pain comes from studies that have demonstrated attenuated behavioural hypersensitivity in nerve-injured nude mice and rats (Moalem et al., 2004; Cao and DeLeo, 2008; Costigan et al., 2009) and recombination activating genes-1 ($RAG\text{-}1)^{-/-}$ mice (Kleinschnitz et al., 2006; Costigan et al., 2009), as well as severe combined immunodeficiency (SCID) mice (Labuz et al., 2010), compared to the respective nerve-injured wild-type littermates. Other studies have also observed that neonatal rats, which are essentially T cell deficient, fail to develop significant nociceptive hypersensitivity following nerve injury (Costigan et al., 2009; Vega-Avelaira et al., 2009). Further evidence has been obtained from our laboratory, where we showed that direct intrathecal transfer of $CD45^+$ cells isolated from the spinal cords of high pain donors to low pain syngeneic recipients was sufficient to potentiate allodynia, demonstrating that infiltrating cells are not passive bystanders but actively contribute to nociceptive hypersensitivity in the lumbar spinal cord (Grace et al., 2011). A specific role for $CD4^+$ T cells has been shown by Cao and DeLeo (2008), who demonstrated attenuated allodynia in $CD4^{-/-}$ mice, which was restored by systemic adoptive transfer of $CD4^+$ T cells, excluding any major contribution from $CD4^+$ microglia and/or macrophages. The presence of $CD4^+$ T cells in the mouse lumbar dorsal horn was transitory, rising from baseline levels at day 3, peaking at day 7, and back to baseline levels by day 14 post L5Tx (Cao and DeLeo, 2008). This temporal infiltration profile is similar to the transitory permeability of the BSCB following CCI and SNI in rats (Beggs et al., 2010). However, other studies in rats have demonstrated a longer infiltration period, that also began on day 3, but continued past days 14 post L5Tx (Sweitzer et al., 2002a) and 21 post SNI (Costigan et al., 2009), which more closely agrees with another study examining the temporal profile of BSCB permeability following L4 nerve lesion in rats (Gordh et al., 2006). A predominant role for $CD4^+$ T cells in models of neuropathic pain is supported by findings from Moalem et al. (2004) who demonstrated that systemic adoptive transfer of type 1 helper T cells (T_H1) cells to nerve-injured nude rats reinstated allodynia. Kim and Moalem-Taylor (2011) found that allodynia was significantly attenuated in nerve-injured $IL\text{-}17^{-/-}$ mice, which was accompanied by attenuated glial activation in the ipsilateral L3–L5 spinal cord, suggesting a role for the T_H17 subset in the central mechanisms of neuropathic pain. Despite demonstrating that IL-17 positively regulated T cell recruitment to the site of injury (Kim and Moalem-Taylor, 2011), the CNS mechanisms of IL-17 and T_H17 cells in neuropathic pain require further investigation, as the same study did not examine CNS infiltration by T_H17 cells.

Hu et al. (2007) and Cao and DeLeo (2008) have shown that the infiltrating immune cells accumulate in regions of glial activation, the superficial laminae of the dorsal horn, thus implicating the CNS immunological synapse in the pathology of neuropathic pain (Fig. 1). Findings from the DeLeo group support this hypothesis by demonstrating that allodynia is attenuated in nerve-injured MHC class $II^{-/-}$ mice (Sweitzer et al., 2002b) and microglial $CD40^{-/-}$ mice (a peripheral contribution was excluded by the use of chimeras) (Cao et al., 2009), compared to wild-type littermates. Furthermore, the trait of neuropathic pain has significant linkage to certain MHC class II polymorphisms in rats (Dominguez et al., 2008). The CNS immunological synapse may contribute to the maintenance of neuropathic pain in two ways: by expressing

pro-inflammatory mediators and by facilitating astrocyte activation and hence the persistence of neuropathic pain.

4.2. T cells express pro-inflammatory mediators

The recursive interactions between T cells and local microglia could result in secretion of pro-inflammatory cytokines. For example, recent studies have demonstrated a key role in neuropathic pain for interferon (IFN)-γ, the pro-inflammatory T_H1 cytokine, due to action at receptors expressed by pre- and post-synaptic terminals (Vikman et al., 1998) and microglia (Butovsky et al., 2007; Tsuda et al., 2009) in the superficial dorsal horn. IFN-γ is upregulated in the lumbar spinal cord of nerve-injured rodents (Tanga et al., 2005; Costigan et al., 2009), and intrathecal injection of IFN-γ has been shown to induce neuropathic pain behaviours in naïve rodents (Robertson et al., 1997; Tsuda et al., 2009), possibly via induction of CCL2 and inducible nitric oxide synthase (Racz et al., 2008). Recent studies showed that genetic knockout of IFN-γ receptors attenuated pain behaviours in nerve-injured mice (Costigan et al., 2009; Tsuda et al., 2009) and impaired activation of microglia (Tsuda et al., 2009). Other pro-inflammatory T cell cytokines, such as IL-2 (which is upregulated in the nerve-injured adult ipsilateral lumbar dorsal horn (Costigan et al., 2009)) and IL-15, have both been shown to induce allodynia and hyperalgesia when intrathecally injected over the lumbar enlargement of naïve rats (Cata et al., 2008), which may enhance nociceptive hypersensitivity by directly modulating the systems involved in neuropathic pain, and/or indirectly by enhancing proliferation of T cells in the CNS. In addition, IL-15 has been proposed to regulate IL-17 (Kleinschnitz et al., 2006; Huang et al., 2007), for which mRNA has been quantified in the ipsilateral lumbar dorsal horns of nerve-injured adult rats (Costigan et al., 2009). Furthermore, IL-17 has been shown to induce nociceptive hypersensitivity in naïve rats following intrathecal injection (Kim and Moalem-Taylor, 2011), possibly by directly activating glia via their IL-17 receptors (Das Sarma et al., 2009), or indirectly via induction of other pro-inflammatory products, such as IL-6, IL-8, G-CSF, CCL2, PGE_2, NO and matrix-metalloproteinases (Bettelli et al., 2007; Korn et al., 2009). Finally, Cao and DeLeo (2008) note that the peak expression of cathepsin S, a microglia-derived lysosomal cysteine protease that cleaves CX_3CL1 from the neuronal membrane (Clark et al., 2007; Milligan et al., 2008), coincided with the maximal $CD4^+$ T cell infiltration at day 7 post nerve injury, and suggest that the T cell-microglia interaction may result in microglial secretion of cathepsin S.

4.3. T cell interactions in the CNS may underlie the maintenance of neuropathic pain

The CNS immunological synapse may contribute to the persistence of neuropathic pain by facilitating astrocyte activation, as a pattern of transient allodynia has been described for a number of T cell deficient rodent models of neuropathic pain (Cao and DeLeo, 2008; Cao et al., 2009; Costigan et al., 2009). Cao and DeLeo (2008) observed expression of the microglial activation marker CD11b, but not the astrocyte activation marker GFAP, at 7 days following nerve injury in $CD4^{-/-}$ mice. This is important, as it has previously been shown in rats that nerve injury results in initial activation of microglia, which is followed by the activation of astrocytes (Tanga et al., 2004; Romero-Sandoval et al., 2008a) and that pharmacological inhibition of microglial activation by minocycline will attenuate the development of neuropathic-like pain, but is unable to diminish existing hypersensitivity (Raghavendra et al., 2003). Therefore, it seems evident that microglia are involved in the induction of initial nociceptive hypersensitivity and this activation in turn leads to astrocyte activation that maintains persistent pain states (with a contribution from microglia (Tawfik et al., 2007; Romero-Sandoval et al., 2008a)). Moreover, one of the differences between resolving inflammatory pain and persistent neuropathic-like pain, is the expression of cell adhesion molecules (Sweitzer et al., 2002b), which, as described above, facilitate CNS infiltration of activated T cells and monocytes/macrophages. Taken together, the interaction between CNS-infiltrating T cells and microglia may facilitate the activation of astrocytes, and hence T cells may underpin the maintenance of neuropathic pain. The cellular and molecular mechanisms that would support this hypothesis remain to be elucidated.

4.4. The anti-inflammatory role of T cells

The protective role of immune cells, a concept independently advocated by the Popovich and Schwartz groups in models as diverse as spinal cord injury, learning and memory and neurological diseases (Villoslada et al., 2008; Ziv and Schwartz, 2008; Kigerl et al., 2009), cannot be overlooked in the context of neuropathic pain. Moalem et al. (2004) demonstrate that adoptive transfer of T_H2 cells, which secrete an anti-inflammatory cytokine profile, attenuate allodynia in nerve-injured rats. Similarly, Costigan et al. (2009), when comparing the gene expression profile in the ipsilateral spinal cord dorsal horns of nerve-injured adult rats that develop nociceptive hypersensitivity with nerve-injured neonates that do not, observed RNA over-expression of the T_H2 cytokines IL-4 and IL-5 in the adult rat. These cytokines were also significantly overexpressed when compared to the sham adult (Costigan et al., 2009), avoiding some of the difficulties associated neonatal comparisons. Other studies have investigated the expression of the microglial B7 costimulatory molecules, CD80 and CD86, which differentially engage T cell CD28 to stimulate a T_H1 or a T_H2 response, respectively (Fig. 1). Using the L5Tx model, Rutkowski et al. (2004) identified co-expression of CD86, but not CD80, with the microglial activation marker, CD11b, in both the dorsal and ventral horns of the ipsilateral lumbar spinal cord of rats, however colocalisation with T cell markers was not investigated. Any anti-inflammatory effects of T_H2 cell stimulation are likely to be outweighed by the opposing pro-inflammatory processes, as these animals still had marked allodynia compared to sham controls (Rutkowski et al., 2004). Similarly, CD274 (PD-L1; B7-H1), a member of the B7-CD28 family and expressed by APCs, exerts an inhibitory function on T cell proliferation and cytokine production upon ligation with PD-1 on T cells. Whilst the neuroinflammatory consequences of CCI in $CD274^{-/-}$ mice, as well as CD274 expression in the wild-type mice, were only evaluated at the site of injury, it was noted that $CD274^{-/-}$ mice exhibited long-lasting allodynia when compared to the nerve-injured wild-type littermates (Uceyler et al., 2010). Given the data presented above, it seems reasonable to hypothesise that a similar upregulation of CD274 expression may occur within the CNS, in order to regulate local neuroinflammatory processes.

A number of studies have found that IL-2 may have analgesic properties, in contrast to the data presented above. Subcutaneous injection of physiologically relevant concentrations of IL-2 has been shown to increase the pain threshold in naïve rats (Wang et al., 1996; Song et al., 2000), and intrathecal IL-2 gene therapy was shown to dose-dependently attenuate CCI-induced hyperalgesia in the rat (Yao et al., 2002). Studies from the same group have shown that the analgesic effects of IL-2 may be mediated by opioid receptor activity, as the peptide contains 4 opioid receptor binding domains (Wang et al., 1997), was reversible by naloxone, and IL-2 displaced δ opioid receptor-bound diprenorphine in an *in vitro* binding assay (Wang et al., 1996). The potential analgesic contribution of IL-2 under the 'normal' pathological conditions of neuropathic pain, however, remains unclear. Labuz et al. (2009, 2010) have shown that opioid-containing T cells infiltrate the site of nerve injury and may protect against nociceptive hypersensitivity.

Any similar role in the CNS is currently unresolved, however these cells could directly or indirectly alleviate nociceptive transmission by inhibiting presynaptic release of neurotransmitters and excitatory amino acids that would otherwise activate dorsal horn postsynaptic terminals and glia alike.

5. Novel neuropathic pain treatment opportunities

5.1. Evidence that pharmacologically targeting T cells will attenuate nociceptive hypersensitivity

A recent study dosed sirolimus (rapamycin), an immunosuppressant that blocks the intracellular signalling cascade and subsequent T cell proliferation induced by IL-2 receptor activation, 7 times for 14 days during the induction phase of CCI in rats, and resulted in a significant reduction in hyperalgesia and allodynia at 15 days post CCI, compared to controls (Orhan et al., 2010). There was no significant reduction of the cytokines TNF-α, IL-1β or IL-6 in the serum due to sirolimus treatment, however a significant reduction in L4–L5 spinal cord TNF-α levels was observed (Orhan et al., 2010). While the results of this study are promising, further experiments are required in order to identify whether the reduction in nociceptive hypersensitivity and lumbar spinal TNF-α is accompanied by attenuated T cell recruitment to the CNS or site of injury. Other immunosuppressants have been trialled in preclinical neuropathic pain models, and may attenuate the activity of T cells within the CNS. Sweitzer and DeLeo (2002) found that systemic or intrathecal administration of the active metabolite of leflunomide, an immunosuppressant with multiple actions on T cells (Dimitrova et al., 2002), reduced L5Tx-induced allodynia and attenuated glial activation, (reduced MHC class II and CD11b expression) (Sweitzer and DeLeo, 2002). Hashizume et al. (2000) examined the effect of intrathecal methotrexate, a folate antagonist with potent, but selective, immunosuppressive actions on T cells (Johnston et al., 2005), in a model of lumbar radiculopathy, wherein the L5 lumbar spinal root is loosely constricted. It was found that methotrexate attenuated existing allodynia and reduced established allodynia, with effects lasting well beyond the conclusion of dosing. Methotrexate did not alter expression of CD11b or GFAP, but the attenuated allodynia was accompanied by a marked reduction in the spinal expression of MHC class II (Hashizume et al., 2000). These studies demonstrate that the active leflunomide metabolite and methotrexate will disrupt the immunological synapse in the CNS, due to reduced MHC class II expression (Fig. 1). However, future mechanistic studies should investigate the CNS infiltration of T cells in models of neuropathic pain, given that both drugs have suppressive effects on T cells. Furthermore, any glial attenuators, such as minocycline, that reduce expression of immunoregulatory molecules like MHC class II (Nikodemova et al., 2007), may also attenuate neuropathic pain via this mechanism. Recent evidence also suggests that disruption of MHC class II may impair TLR3 and 4 signalling (Liu et al., 2011), which, as mentioned above, have been shown to play a critical role in neuropathic pain. A number of other experimental therapeutics that have been investigated in preclinical models of neuropathic pain may have T cell activity within the CNS. For example, the soluble complement receptor 1 may be well tolerated in man (Lazar et al., 2004; Twining et al., 2005; Li et al., 2007), small molecule TLR4 antagonists, which may also be well tolerated in man (Bettoni et al., 2008; Hutchinson et al., 2008; Tidswell et al., 2010), and CB_2 agonists (Romero-Sandoval et al., 2008b; Luongo et al., 2010).

The prospect of exploiting cells with an anti-inflammatory phenotype is extremely desirable. For example, Hino et al. (2009) intrathecally injected autologous macrophages containing the human proenkephalin gene and observed long-lasting attenuation of CCI-induced nociceptive hypersensitivity in rats, due to production of met-enkephalin at the L4–L5 spinal cord. Similar experimental approaches or pharmacological treatments (Zhang et al., 2009) may be employed to enhance the neuroprotective action of T_H2 cells or regulatory T cells.

5.2. Issues facing the clinical translation of immunosuppressants for neuropathic pain

Despite the convincing demonstration of the role of the peripheral immune system in the maintenance of chronic pain and the tantalising evidence of benefit in the studies described above, there are still many challenges in translating this research in to practical therapy. In this review we have described a number of processes by which activated immune cells amplify the pain process after peripheral nerve injury. However, the bulk of patients with nerve injury will not go on to develop chronic pain. We not only need to understand the signals that generate a pro-inflammatory response, but also those leading to attenuation of this response and a subsequent anti-inflammatory response. It is unappealing to use broad-spectrum immunosuppressive therapy, especially in a prophylactic manner, because of concerns of opportunistic infection and cancer surveillance. However, well-tolerated drugs such as minocycline, which is only effective in animal models when given prior, rather than subsequent to, the injury (Raghavendra et al., 2003), are worthy of evaluation. We are currently investigating the effect of minocycline on the prevention of intercostal neuralgia in patients undergoing open thoractomy, as there is a 50% incidence of this debilitating complication (clincaltrials.gov NCT01314482). For less well-tolerated interventions, convincing demonstration that immune intervention in animal models can either reverse chronic pain, or prevent the transition of acute to chronic pain would be more appealing. Although some of the interventions described in this review are only effective when applied directly to the CNS, and it might be possible to find investigators or patients desperate enough to volunteer for such experiments, a leading journal (PAIN) has recently articulated an editorial policy not to publish such studies on ethical grounds (Eisenach et al., 2010; Rowbotham, 2010).

Hence, we believe progress is most likely be made by teams of integrated preclinical and clinical scientists that use mechanistically similar models and diseases, and who assess interventions that are likely to be safe in clinical practice. The development of biomarkers that can noninvasively track these processes in humans would be of great advantage. Additionally, it is unrealistic to assume that immune interventions alone are likely to be the sole answer in the prevention of chronic pain and approaches which combine immune targeting and other neuronal activation mechanisms (e.g. NMDA receptors) might lead to the best therapies.

6. Concluding remarks

This review has demonstrated that neuropathic pain alters many facets of immune privilege to facilitate entry of T cells into the CNS, and engagement with activated microglia expressing MHC class II and costimulatory molecules (the immunological synapse). T cells serve as an important companion to glial activation and the interaction with microglia leads to an increase in pro-inflammation, and ultimately, enhanced neuropathic pain. Given this growing appreciation for CNS infiltrating T cells to bidirectionally engage with each member of the tetrapartite synapse either directly and indirectly, perhaps it is more appropriate the refer to this complete system as the ‘pentapartite synapse’.

There are, however, still questions that remain to be answered. Evidence for the immunological synapse structures, the nature of

the antigen presented and whether there is MHC restriction are unresolved. Furthermore, while there is a great deal of evidence showing PNS T cell infiltration (Cui et al., 2000; Hu and McLachlan, 2002; Moalem et al., 2004; Kleinschnitz et al., 2006; Hu et al., 2007; Li et al., 2007; Jung and Miller, 2008; Kim and Moalem-Taylor, 2011), there is a paucity of data on the mechanisms by which they may enhance neuropathic pain. Satellite glia and Schwann cells are also activated in neuropathic pain models (Hanani et al., 2002; Campana, 2007) and potential T cell interactions with these glial cells should therefore be investigated, together with the CNS glia described in this review. The hypothesis that T cells facilitate the maintenance of neuropathic pain, as presented in this review, is based on converging lines of evidence, however confirmation and demonstration of a mechanism is required. Beggs et al. (2010) also observed blood–CNS barrier permeability in regions other than the portion of the spinal cord that was directly affected by nerve injury. Since glial activation is not restricted to directly affected spinal sites either (Wei et al., 2008), other regions of the CNS should also be examined for T cell infiltration. From the perspective of pharmacological treatments, very few studies have trialled therapies that directly target T cell mechanisms in neuropathic pain. Moreover, given the predominant role for $CD4^+$ T cells in the current literature, this subset may be specifically targeted to alleviate neuropathic pain.

On a broader note, genetic approaches have been considered in an attempt to understand the constellation of different sensitivities in normal and diseased states that constitute neuropathic pain (Foulkes and Wood, 2008; Mogil, 2009b; Tremblay and Hamet, 2010). These investigations may be complimented by the inclusion of pain immunogenetics, such as those considered by Dominguez et al. (2008).

Besides neuropathic pain, the pentapartite synapse has been associated with preclinical models of Alzheimer's disease, Parkinson's disease, traumatic brain injury, stroke, multiple sclerosis (Amor et al., 2010), autism (Ashwood et al., 2006; Blaylock and Strunecka, 2009), spinal cord injury (Detloff et al., 2008; Ankeny and Popovich, 2009) and schizophrenia (Bernstein et al., 2009; Cardon et al., 2010). However, other conditions with a microglial component, including depression (McNally et al., 2008), epilepsy (Rodgers et al., 2009), drug addictions (Narita et al., 2006), acute opioid analgesia (Hutchinson et al., 2010), naïve tolerance (Shavit et al., 2005), opioid tolerance (Hutchinson et al., 2010), xenobiotic induced hyperalgesia and allodynia (Ledeboer et al., 2006; Hutchinson et al., 2010), should also be investigated for involvement of the pentapartite synapse.

In conclusion, the data presented in this review has been obtained solely from animal models of neuropathic pain. Given the recent discussion on the difficulties associated with translating findings from animal models to the clinic (Rice et al., 2008; Vierck et al., 2008; Craig, 2009; Mao, 2009; Mogil, 2009a; Mogil et al., 2010), it is imperative that T cell mechanisms in neuropathic pain are investigated in clinical populations, as part of the growing movement to replace current symptomatic treatments with disease-modifying therapies.

Conflict of interest statement

All authors declare that there are no financial or commercial conflicts of interest.

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