CHAPTER 7:

ROLE OF MICROGLIA IN
ACUTE AND NEUROPATHIC PAIN
7.1. INTRODUCTION

Peripheral nerve injury and inflammation often induce exaggerated pain states characterized by sensitization of peripheral and central primary afferent neurons. Recently, investigators have placed emphasis on the role of non-neuronal cells, such as supporting and immune cells of the spinal cord, in the exaggerated pain states. Garrison et al (1991) first reported that nerve injury that produces neuropathic pain also activates spinal cord glia. Growing body of evidence indicates that glial cells, particularly astroglial and microglial cells, in addition to their classical role as supporting and nutrition sources for neurons, have been implicated in the nociceptive processing. The glial cells are activated following peripheral and central noxious insult and their activation is thought to play an important role in central sensitization (Meller et al., 1994; Colburn et al., 1997; Watkins et al., 1997; Fu et al., 2000; Sweitzer et al., 2001). Moreover, activated glia increases the release of various proinflammatory cytokines and further both the glia and neurons express receptors for various neurotransmitters and neuromodulators as well (Kreutzberg, 1996; Koistinaho and Koistinaho, 2002). The recognition of glia as powerful modulator of nociception stimulated the search for agents that specifically inhibit the activation and metabolism of glial cells leading to the discovery of suramin, flurocitrate and propentophylline as glial modulators, which showed antiallodynic and antihyperalgesic properties in various models of experimental pain (Meller et al., 1994; Sweitzer et al., 2001; Wu et al., 2004).

Minocycline is a semisynthetic second-generation tetracycline that exerts anti-inflammatory effect that is completely separate and distinct from its antimicrobial action (Yrjanheikki et al., 1998, 1999; Tikka et al., 2001). It is a lipophilic molecule absorbed rapidly and readily crosses the blood brain barrier (Aronson, 1980). It selectively disrupts the activation of microglia without directly affecting neurons or astroglia (Tikka et al., 2001; Tikka and Koistinho, 2001). Inhibition of microglial activation has also been demonstrated in vitro (Tikka et al., 2001) and in experimental models of acute and chronic brain insults (Chen et al., 2000; Tikka et al., 2001; Wu et al., 2002). In the brain, it shows neuroprotection by inhibiting inflammation, decreases free radical formation by inhibiting iNOS, COX-2, and inhibits the caspase-1 in experimental model of Parkinson’s and Huntington’s diseases, and prevents NMDA mediated neurotoxicity (Yrjanheikki et al., 1998, 1999; Chen et al., 2000; Tikka et al., 2001; Wu et al., 2002). Recently, its antihyperlagesic and antiallodynic
effects have been demonstrated in spinal nerve transection model of neuropathic pain (Raghavendra et al., 2003a).

A number of animal models of neuropathic pain have been developed and characterized over the past two decades. There are a number of shortcomings with these animal models, which provide important clues in understating the underlying pathophysiology of neuropathic pain in humans. The CCI model involves some kind of injury to the sciatic nerve, however, leave some degree of intact peripheral nerve fibers (Bennett and Xie 1988; Hao et al., 2000) whereas the SNI model involves axotomy and ligation of the tibial and common peroneal nerves leaving the sural nerve intact, which enables investigation of the changes in both injured primary sensory neurons and neighboring sensory neurons, and their contribution to the pathophysiology of neuropathic pain (Decosterd and Woolf, 2000; Erichson and Blackburn-Munro, 2002). Thus, we investigated the effect of inhibition of microglial activation in these two experimental models of neuropathic pain. To date, there are no studies that evaluated the effects of minocycline on acute nociception as well as persistent and long lasting hyperalgesia. In addition, studies on its long lasting post-drug treatment effects in attenuating hypersensitivity may be beneficial in understanding the inhibition of microglial activation on ongoing pain related behaviors. Thus, there are not only mechanistic reasons to examine the inhibition of microglial activation, but also practical approaches to evaluate long lasting effects in attenuating hypersensitivity.

In the present study, we examined the effects of acute as well as chronic systemic administration of minocycline on development and maintenance of hypersensitivity following formalin injection and in CCI of the sciatic nerve and the SNI models of neuropathic pain in rats.

### 7.2. MATERIALS AND METHODS

#### 7.2.1. Experimental animals

As per 1.2.1.

Male Wistar rats weighing 150-180 g at the start of the surgery were randomly divided into various groups consisting of 6 - 8 animals per group. Following surgery, the animals were housed in groups of 3 per cage and provided with food and water *ad libitum*.

#### 7.2.2. Behavioral test paradigms
7.2.2.1. Formalin test
As per 5.2.3.1.
The procedure as described by Dirig et al. (1997) was followed for the rat formalin test. Animals were gently restrained and injected with 50 μl of 5% formalin solution in normal saline subcutaneously into the plantar surface of the right hind paw with a 26-guage needle fitted to a microsyringe. Two phases of spontaneous flinching was observed after formalin injection. The interval from 0 – 5 min was defined as the first phase and the interval between 10 – 60 min as the second phase, respectively.

7.2.2.2. Chronic constriction nerve injury (CCI)
As per 3.1.2.2.

7.2.2.3. Spared nerve injury (SNI)
As per 4.2.2.

7.2.3. Behavioral assessment of nociception
Allodynia (heightened response to normally non-noxious stimuli) and hyperalgesia (decreased threshold to noxious stimuli) were evaluated in sham (both CCI and SNI), CCI and SNI rats.

7.2.3.1. Formalin-induced thermal hyperalgesia
As per 1.2.2.3a.
The decrease in the mean paw withdrawal latency after formalin injection was considered as hyperalgesia.

7.2.3.2. Cold allodynia
As per 4.2.3.1.
The duration of time in seconds that the animal spent lifting, shaking, or licking the acetone applied hind paw was recorded during a 2 min period that started immediately after acetone application.

7.2.3.3. Mechanical hyperalgesia
As per 1.2.2.3b.
The withdrawal threshold was evaluated using an analgesymeter (Ugo Basile, Italy) by applying noxious pressure to hind paw and expressed in grams.

7.2.4. Drugs and treatment schedule
Minocycline (Wyeth-Lederle Pharmaceuticals, India) and formalin (37% formaldehyde; SD Fine Chemicals, India) were used in this study. The drug solutions for intraperitoneal administration were freshly prepared by suspending them in ne or...
two drops of Tween 80 in normal saline and administered 1 ml/100 g body weight. Formalin was diluted in normal saline.

**7.2.5. Treatment schedule**

All animals were acclimatized to laboratory environment for at least 2 h before testing. The paw withdrawal responses to thermal and mechanical stimulation were measured on day 0 before injecting formalin or performing surgery. To investigate whether microglia participate in the acute nociception, rats were administered intraperitoneally (i.p.) with vehicle or minocycline (10 or 30 mg/kg,) 30 min before formalin injection and immediately observed for nociceptive behavior for one hour. In the same set of animals, paw withdrawal responses to thermal stimulus (47 °C) was also measured at 1 h before, 3, 6, and 24 h after formalin injection subsequent to acute nociceptive paradigm.

To investigate whether inhibition of microglial activation able to prevent the development of hyperalgesia following formalin-induced nociception, rats were pretreated with saline vehicle or minocycline (10 or 30 mg/kg, i.p.) administered 30 min before formalin injection and continued once daily for 7 days following formalin injection. The paw withdrawal response to thermal stimulation was tested on day 0, 4, 7, 10, 14, and once a week thereafter for 4 weeks after formalin injection. Further, saline vehicle or minocycline (10 or 30 mg/kg, i.p.) were administered to separate group of rats after behavioral assessment on day 7 following formalin injection and continued once daily for 7 days to determine the effect of inhibition of microglial activation on the existing hypersensitivity. Thermal hyperalgesia was tested on day 0, 7 (2 h before), 10, 14, 17, 21, and 28 after formalin injection.

To investigate whether inhibition of microglial activation able to prevent the development of hyperalgesia following nerve injury, rats were pretreated with saline vehicle or minocycline (10 or 30 mg/kg, i.p.) administered 2 h before surgery and continued once daily for 7 days following nerve injury in both models of neuropathic pain. The paw withdrawal response to thermal and mechanical stimulation was tested on day 0, 4, 7, 10, 14, and once a week thereafter for 4 weeks after nerve injury. To investigate whether inhibition of microglial activation affects the existing hypersensitivity, minocycline was administered during the development and after the hypersensitivity was fully established. A single dose of normal saline or minocycline (10 or 30 mg/kg, i.p.) was administered to CCI and SNI rats on day 4 and the paw
withdrawal responses to cold and mechanical stimulation was tested 2h before, 0.5, 1, 2, 4, and 24 h after treatment. In another set of animals Further, to determine the effect of inhibition of microglial activation on the maintenance of hypersensitivity, saline vehicle or minocycline (10 or 30 mg/kg, i.p.) were administered to separate group of rats after behavioral assessment on day 7 following nerve injury and continued once daily for 7 days. The paw withdrawal response to thermal and mechanical stimulation was tested on day 0, 7 (2 h before), 10, 14, 17, 21, and 28 after nerve injury.

7.2.6. Statistical analysis
As per 3.1.2.5.

7.3. RESULTS

7.3.1. Effect of minocycline in the formalin test
In rats administered with vehicle, subsequent injection of formalin into hind paw resulted in a biphasic nociceptive response characterized by robust flinching of the affected paw. Administration of minocycline (10 or 30 mg/kg, i.p.), 30 min before injection of formalin, had no effect on flinching behavior in both the phases of the formalin test as compared to vehicle treated rats (Fig. 7.1).

7.3.2. Effect of minocycline on formalin-induced hyperalgesia
In rats injected with formalin into hind paws, the mean paw withdrawal responses at 1 and 3 h were not significantly different from that of baseline levels, however, there was a significant decrease in the mean paw withdrawal responses to thermal stimulation at 6 and 24 h after formalin injection indicating the development of thermal hyperalgesia. Pre-treatment with a single dose of minocycline (10 or 30 mg/kg, i.p.) significantly reduced thermal hyperalgesia at 6 h following formalin injection. A higher dose of minocycline (30 mg/kg) almost completely attenuated the hyperalgesia at one day later (Fig. 7.2).

7.3.3. Effect of minocycline on prevention and reversal of formalin-induced long lasting hyperalgesia
The paw formalin injection produced a significant decrease in the mean paw withdrawal responses as compared to baseline levels throughout the observation period. Chronic treatment with minocycline (10 or 30 mg/kg, i.p., 30 min before and once daily for 7 days following formalin injection) in paw formalin-injected rats significantly attenuated and fully abolished the development of thermal
Chapter 7

hypersensitivity as compared with vehicle-treated paw formalin-injected rats (Fig. 7.3A). In contrast, administration of minocycline (10 or 30 mg/kg, i.p., initiated from day 7 to day 14 post-formalin injection) did not show any significant difference in the thermal hyperalgesia in paw formalin-injected rats compared with vehicle-treated group (Fig. 7.3B).

7.3.4. General behavior of sham-operated, CCI and SNI animals

In the present series of experiments, the baseline paw withdrawal response in each test obtained on day 0 for each rat was relatively stable and showed no significant variation. The mean paw withdrawal latency to thermal (47 °C) stimulation was 8.21 ± 0.87 s on the left and 8.02 ± 0.66 s on the right hind paws on day 0 before formalin injection. Before the CCI or SNI, application of an innocuous cold stimulus (acetone drop) to the left or right hind paw evoked no flexor response. The mean paw withdrawal threshold to pressure was 173.66 ± 11.33 g and 179.37 ± 13.42 g, respectively, on day 0 before performing surgery in rats. Following surgery, the animals kept their nerve injured paw elevated above the cage floor, but otherwise appeared healthy, exhibited normal grooming and feeding behavior, and gained weight normally. The paw withdrawal responses to thermal and mechanical stimulation in sham-operated (both CCI and SNI) rats remained unchanged from baseline values throughout the entire observation period. The ipsilateral paw withdrawal responses of all the vehicle-treated nerve injured rats were significantly less than that of sham-operated rats on day 4 onwards and reached steady state between days 7 and 28 after surgery indicating the development of allodynia (Fig. 7.4A and 7.4B) and hyperalgesia (Fig. 7.4C and 7.4D) in a time-dependent manner.

7.3.5. Effect of minocycline on development of CCI- and SNI-induced allodynia and hyperalgesia

Chronic systemic administration of minocycline (10 or 30 mg/kg, i.p., 2 h before and once daily for 7 days post-nerve injury) in nerve injured rats significantly attenuated the development of hypersensitivity in the ipsilateral paw as compared with vehicle-treated SNI rats during the treatment period (Fig. 7.4A and 4B). Similarly, the paw withdrawal responses to cold and mechanical stimulation of the ipsilateral hind paw were significantly attenuated by chronic administration of minocycline (10 or 30 mg/kg, i.p., 2 h before and once daily for 7 days post-nerve injury) in the CCI rats as compared to vehicle-treated CCI rats (Fig. 7.5A and 5B). In addition, pretreatment

273
with minocycline further delayed the development of hypersensitivity in ipsilateral paw on day 10 and 14, but not on day 21 and 28 as compared with vehicle-treated SNI rats (Fig. 7.4A and 4B). Following the termination of treatment, the ipsilateral paw withdrawal responses to cold and mechanical stimulation were significantly different from that of vehicle-treated CCI rats and both the doses of minocycline delayed the development of hypersensitivity in CCI rats on 10, 14 and 21, but not on day 28 as compared with vehicle-treated CCI rats (Fig. 7.5A and 5B).

7.3.6. Effect of minocycline on maintenance of CCI- and SNI-induced allodynia and hyperalgesia

Although, the development of hypersensitivity was robust in SNI rats, single dose of minocycline (10 or 30 mg/kg, i.p.) administered on day 4 failed to reverse the hypersensitivity in ipsilateral paw (Fig. 7.6A and 6B). Similarly, a single dose of minocycline (10 or 30 mg/kg, i.p.) administered on day 4 (during the development of hypersensitivity) after surgery had no effect on the ipsilateral paw withdrawal responses to cold (Fig. 7.7A) and mechanical (Fig. 7.7B) stimulation in CCI rats.

Chronic treatment with minocycline (10 or 30 mg/kg, i.p., initiated on day 7 and continued once daily till day 14 post-nerve injury) had no effect on the hypersensitivity in ipsilateral paw of the SNI rats throughout the observation period (Fig. 7.8A and 8B). Similarly, administration of minocycline (10 or 30 mg/kg, i.p., initiated on day 7 and continued once daily till day 14 post-nerve injury) in CCI rats did not produce any significant difference in the ipsilateral paw withdrawal responses to cold (Fig. 7.9A) and mechanical (Fig. 7.9B) stimulation throughout the observation period as compared with vehicle-treated SNI rats.

7.4. DISCUSSION

The nonneuronal cells, glial cells, have become the focus of much behavioral nociceptive research. The present study systematically investigated the role of microglia in formalin-induced acute nociception as well as persistent and long lasting hyperalgesia. It is observed that minocycline, an inhibitor of microglial activation, which is a second-generation tetracycline, can attenuate the development of neuropathic pain. Further, the present study also investigated the long lasting antiallodynic and antihyperalgesic effects of minocycline in attenuating hypersensitivity.
Fig. 7.1. Time course of formalin responses of rats after administration with vehicle or minocycline (Min; 10 or 30 mg/kg). The data represent the mean ± S.E.M. of formalin-induced flinching responses per minute during 5 min intervals observed for a period of 60 min. (n = 6-8 in each group).

Fig. 7.2. Effect of minocycline (Min; 10 or 30 mg/kg, i.p.) on thermal hyperalgesia in paw formalin-injected rats. Responses before formalin injection represent baseline paw withdrawal responses. Values are mean ± S.E.M. *P < 0.05 vs baseline levels, aP < 0.05 as compared to vehicle treated group. (n = 6-8 in each group).
Fig. 7.3. Effect of minocycline (Min; 10 or 30 mg/kg, i.p.) on (A) development and (B) established hypersensitivity in paw formalin-injected rats. The paw withdrawal response (in seconds) to thermal stimulation was assessed before (baseline) and for 4 weeks after formalin injection. Values are mean ± S.E.M. *P < 0.05 vs baseline levels, aP < 0.05 as compared to vehicle. (n = 6-8 in each group).
Fig. 7.4. Effect of chronic administration of minocycline (Min; 10 or 30 mg/kg, i.p.) on prevention of (A) cold allodynia and (B) mechanical hyperalgesia in spared nerve injury (SNI) rats. Responses on day 0 represent baseline paw withdrawal responses. Values are mean ± S.E.M. *P < 0.05 vs sham-operated and #P < 0.05 vs saline-treated nerve injured animals (one-way ANOVA followed by Dunnett’s test). (n = 6-8 in each group).
Fig. 7.5. Effect of chronic administration of minocycline (Min; 10 or 30 mg/kg, i.p.) on prevention of (A) cold alldynia and (B) mechanical hyperalgesia in chronic constriction injury (CCI) rats. Responses on day 0 represent baseline paw withdrawal responses. Values are mean ± S.E.M. *P < 0.05 vs sham-operated and aP < 0.05 vs saline-treated nerve injured animals (one-way ANOVA followed by Dunnett’s test), (n = 6-8 in each group).
Fig. 7.6. Effect of acute administration of minocycline (Min; 10 or 30 mg/kg, i.p.) on (A) cold allodynia and (B) mechanical hyperalgesia in spared nerve injury (SNI) rats. Responses on day 0 represent baseline paw withdrawal responses. Arrow indicates time of minocycline administration on day 4 following SNI in rats. Values are mean ± S.E.M. *P < 0.05 vs sham-operated (one-way ANOVA followed by Dunnett’s test). (n = 6-8 in each group).
Fig. 7.7. Effect of acute administration of minocycline (Min; 10 or 30 mg/kg, i.p.) on (A) cold allodynia and (B) mechanical hyperalgesia in chronic constriction injury (CCI) rats. Responses on day 0 represent baseline paw withdrawal responses. Arrow indicates time of minocycline administration on day 4 following CCI in rats. Values are mean ± S.E.M. *P < 0.05 vs sham-operated (one-way ANOVA followed by Dunnett’s test). (n = 6-8 in each group).
Fig. 7.8. Effect of chronic administration of minocycline (Min; 10 or 30 mg/kg, i.p.) on reversal of (A) cold allodynia and (B) mechanical hyperalgesia in spared nerve injury (SNI) rats. Responses on day 0 represent baseline paw withdrawal responses. Values are mean ± S.E.M. *P < 0.05 vs sham-operated (one-way ANOVA followed by Dunnett’s test). (n = 6-8 in each group).
Chapter 7

Fig. 7.9. Effect of chronic administration of minocycline (Min; 10 or 30 mg/kg, i.p.) on reversal of (A) cold allodynia and (B) mechanical hyperalgesia in chronic constriction injury (CCI) rats. Responses on day 0 represent baseline paw withdrawal responses. Values are mean ± S.E.M. *P < 0.05 vs sham-operated (one-way ANOVA followed by Dunnett’s test). (n = 6-8 in each group).
The second generation tetracycline minocycline, when administered prior to formalin injection did not affect biphasic nociceptive behavior. Interestingly, systemic acute administration of minocycline reduced formalin-induced hyperalgesia response at later time points. It is unlikely that minocycline fail to reach threshold levels in spinal cord to alleviate sufficient nociceptive response, because minocycline is known to readily cross the blood-brain barrier and significant neuroprotection was achieved after systemic administration (Aronson, 1980; Yrjanheikki et al., 1998, 1999). One possibility is that microglia might not be activated in response to acute noxious stimuli and involved in acute nociception within the time frame of the formalin test. Previous studies have shown that paw formalin injection produces long lasting hyperalgesia and is related to gradual persistent activation of glia, particularly spinal microglia, which was attenuated by glial modulators (Watkins et al., 1997; Fu et al., 1999, 2000; Wu et al., 2004). Further, spinal microglial activation increased from 6 h to day 1 onwards, peaked at week 1, and persisted to week 3 following formalin injection (Fu et al., 1999; 2001). This is particularly important in that minocycline reduced formalin-induced hyperalgesia. Moreover, minocycline is known to selectively inhibit microglial activation (Tikka et al., 2001; Tikka and Koistinho, 2001). The lack of effect of minocycline against an acute and noxious chemical stimulus despite its anti-hyperalgesic effect at later time points indicates a selective interaction of microglial activation on central nociceptive processing associated with pathophysiological events rather than with normal nociceptive function.

In the present study, minocycline was administered to address the contribution of microglial activation to the development and maintenance of hypersensitivity. The systemic chronic administration of minocycline following formalin injection and nerve injury significantly attenuated the hypersensitivity. It is important to note that similar, but acute, treatment with minocycline during the development of hypersensitivity, did not alter neuropathic pain. In addition, chronic administration of minocycline also failed to reverse existing hypersensitivity suggesting the differential role of microglia in the development and maintenance of hypersensitivity. It has been hypothesized that microglial and astrocytes have distinct role in hypersensitivity and microglial activation was observed in the induction phase with delayed activation of astrocytes (Colburn et al., 1997, Coyle, 1998). Recently, this differential pattern of glial activation has been demonstrated in various models of neuropathic pain (Coyle, 1998; Colburn et al., 1999; Winkelstein and Deleo 2002). Further, it has been reported
that there is a sequential activation of extracellular regulated kinases (ERK) in the spinal cord after nerve injury first in neurons, but only for a short period, then in microglia for many days, and finally with a delay in several weeks, in astrocytes (Zhuang et al., 2005). One possibility is that activated microglia contributes to the induction and development of exaggerated pain states and further activates various other cells, particularly astrocytes that are likely to contribute to maintaining hypersensitivity. Of particular relevant to this, it has been previously shown that inhibition of microglial activation attenuated the development, however, it had no effect on maintenance of hypersensitivity (Raghavendra et al., 2003a). Because, minocycline is without any effect on neurons and astrocytes (Tikka et al., 2001; Tikka and Koistinho, 2001), it seems likely that the attenuation of hypersensitivity could be due to selective inhibition of microglial activation. These data along with the present results support the proposal that activation of microglia is involved in the induction and development, but not in the maintenance of exaggerated pain.

The most striking findings of the study were those revealing that chronic administration of minocycline started prior to noxious chemical stimuli or nerve injury delayed the development of hypersensitivity with more marked effect was observed in formalin-induced hyperalgesia and CCI-induced neuropathic pain even after termination of drug administration. Recently, it has been reported that there are phenotypic changes, differential activation of MAPK in injured and uninjured neurons and satellite glial cells (Fukuoka and Noguchi, 2002; Li et al., 2000; Obata et al., 2004). Further, development of ectopic activity and upregulation of TNF-α have been described in injured and uninjured dorsal root ganglia neurons after peripheral nerve injury (Schaefers et al., 2003; Obata et al., 2004). Thus, the results of the present study along with others demonstrating that the differential sensitivity of pain behavior to minocycline in these two animal models of nerve injury could be due to differences in the type nerve injury, differential contribution of injured as well as uninjured and intact neurons to neuronal excitation, microglial activation and subsequent development of hypersensitivity in these nerve injury models.

It is well known that the diluted formalin when injected into hind paw of rats shows characteristic biphasic flinching behavior. It is generally agreed that the first phase results at least in part from direct activation of primary afferent fibers, both low-threshold mechanoreceptive and nociceptive types whereas the second phase
Chapter 7

reflects a facilitated state of central sensitization driven by the persistent primary afferent inputs and this ongoing activity releases excitatory amino acids (glutamate) and neuropeptides (SP) that are necessary for the development of the second phase (Puig and Sorkin 1996; Abbadie et al., 1997). It is generally agreed that both peripheral and central mechanisms have been involved in the pathogenesis of neuropathic pain (Zimmerman 2001). Further, direct administration of glutamate or SP into spinal cord activates glia and increases p38 MAPK expression in spinal microglia leading to hyperalgesia and allodynia, which were significantly attenuated by MAPK inhibitors (Garrison et al., 1991; Svensson et al., 2003; Zhuang et al., 2005). In addition, activated glia also enhances the release of excitatory amino acids and SP from nerve terminals including primary afferents in the spinal cord (Malcangio et al., 1996; Inoue et al., 1999). Recent studies reported that the activation of ERK and p38 MAPK both in neurons and glial cells in the spinal cord contribute to persistent inflammatory and neuropathic pain (Svensson et al., 2003; Zhuang et al., 2005). Indeed, p38 MAPK is a key player in the intracellular signaling cascade leading to the production of proinflammatory cytokines in glia and immune cells (Koistinaho and Koistinaho, 2002; Shi and Gaestel, 2002).

Abundant evidence has shown that both astrocytes and microglial cells activation synthesize a variety of neuroexcitatory substances such as cytokines proinflammatory cytokines IL-1β, IL-6, and TNF-α, and increases the expression of COX-2 and iNOS leading to the synthesis of PGs and NO that potentiate pain transmission by neurons (Minami et al., 1994; Kreutzberg, 1996; Weiseler-Frank et al., 2004). These proinflammatory cytokines directly sensitize neurons and act indirectly in an autocrine or paracrine fashion to induce the synthesis and secretion of neurotransmitters that act on local neurons lead to hyperexcitable sensory states, which promote the development of hypersensitivity (DeLeo and Yezierski, 2001; Watkins et al., 2001). Although we did not quantify microglial activation and proinflammatory mediators, previous studies demonstrate that minocycline markedly inhibited p38 MAPK activity and decreased spinal IL-1β and TNF-α expression, both in vitro and in vivo (Yrjanheikki et al., 1998; 1999; Du et al., 2001; Tikka and Koistinaho, 2001). Taken together, these present findings suggest that microglial cells might be continuously activated and releases excitatory pain neurotransmitters/neuromodulators that leads to the development of hypersensitivity.
Inhibition of microglial activation early in the neuronal excitation and central sensitization to occur could prevent exaggerated pain states.

In summary, the results demonstrate that there is no role for microglia in acute pain. Further, the observed minocycline attenuation of development of hypersensitivity due to formalin and nerve injury is likely associated with its ability to inhibit activated microglia. The present results suggest that chronic treatment with minocycline started early before noxious chemical stimuli and nerve injury could prevent or at least delays the development of hypersensitivity.