Available online at www.sciencedirect.com

SCIENCE DIRECT

European Journal of Pharmacology 497 (2004) 29–33

www.elsevier.com/locate/ejphar

High-efficacy 5-HT_{1A} receptor activation causes a curative-like action on allodynia in rats with spinal cord injury

Francis C. Colpaert[a,*], Wei-Ping Wu[b], Jing-Xia Hao[b], Isabelle Royer[c], François Sautel[c], Zsuzsanna Wiesenfeld-Hallin[b], Xiao-Jun Xu[b]

[a]*Centre de Recherche Pierre Fabre, 17 Avenue Jean Moulin, 81100 Castres, France*
[b]*Department of Laboratory Medicine, Division of Clinical Neurophysiology, Karolinska Institutet, SE-141 86 Stockholm, Sweden*
[c]*Centre Expérimental et Pharmacocinétique de Campans, 81106 Castres Cedex, France*

Received 19 February 2004; received in revised form 24 May 2004; accepted 17 June 2004
Available online 20 July 2004

Abstract

The selective, high-efficacy 5-HT_{1A} receptor agonist, (3-chloro-4-fluoro-phenyl)-[4-fluoro-4-{[(5-methyl-pyridin-2-ylmethyl)-amino]-methyl}piperidin-1-yl]-methanone (F 13640) has been reported to produce long-term analgesia in rodent models of chronic nociceptive and neuropathic pain; it also preempts allodynia following spinal cord injury. Here, rats underwent spinal cord injury, fully developed allodynia, and were infused with saline or 0.63 mg/day of F 13640 for 56 days. Infusion was then discontinued, and further assessments of allodynia (vocalization threshold to von Frey filament stimulation, responses to brush and cold) were conducted for another 70 days. F 13640-induced analgesia persisted during this post-treatment period. The data offer initial evidence that high-efficacy 5-HT_{1A} receptor activation produces an unprecedented curative-like action on pathological pain.

Keywords: Serotonin; Curative analgesia; Neuropathic pain; F 13640

1. Introduction

The recently discovered centrally-acting, broad-spectrum analgesic, F 13640, demonstrates high affinity for 5-HT_{1A} receptors and activates those receptors to an extent that is unique among selective 5-HT_{1A} receptor ligands (Colpaert et al., 2002). These molecular features make F 13640 induce novel neuroadaptive mechanisms whereby, in rodents, can be achieved an analgesia that grows rather than decays with chronicity and that is enhanced by the presence of nociceptive input. With chronic as well as acute nociceptive pains, these mechanisms cause an analgesia that is rivaled only by opioids (Colpaert et al., 2002; Bardin et al., 2003). With chronic neuropathic pains, continuous F 13640 infusion causes inverse tolerance and, hence, a lasting analgesia that cannot be achieved with opioids or analgesics exemplifying other molecular mechanisms of central action (Colpaert et al., 2002).

Current concepts distinguish between two, overlapping, types of pain (Ji and Woolf, 2001; Melzack et al., 2001). Physiological (e.g., nociceptive) pain refers to transient sensations that occur in response to noxious stimulation; the sensory apparatus bears similarity with other physiological sensations and produces a sensation that has adaptive significance. Pathological (e.g., neuropathic) pain develops progressively following nerve injury and loss of sensory input (Jensen, 2002); it may persist for a long time and is maladaptive (Ji and Woolf, 2001; Bruce et al., 2002; Siddall et al., 2002).

In a model of spinal cord injury-induced, central neuropathic pain, rats sustain a photochemical, ischaemic lesion of spinal cord dorsal horn segments L3–L5 and develop allodynic responses to cutaneous stimulations such as von

* Corresponding author. Tel.: +33 563 71 42 71; fax.: +33 563 71 43 79.
E-mail address: francis.colpaert@pierre-fabre.com (F.C. Colpaert).

0014-2999/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2004.06.026

Frey filament application, a gentle brush, or a cold spray (Hao et al., 1998). In this model, F 13640 infusion causes analgesia in a classic or "symptomatic" manner, alleviating these responses while the agent is being administered. Remarkably, the agent produces this analgesia in an incremental fashion, thus demonstrating inverse tolerance (Colpaert et al., 2002; see also Deseure et al., 2003). The infusion of morphine and of other agents exemplifying further molecular mechanisms of central analgesia (i.e., imipramine, ketamine, gabapentin) was ineffective (Colpaert et al., 2002). Equally remarkably, F 13640 "preempts" spinal cord injury-induced allodynia; F 13640 infusion from the time of injury onward inhibits the development of allodynia and allodynia remains at this reduced level upon the discontinuation of infusion (Wu et al., 2003; see also Deseure et al., 2003). Here, we determined whether in addition to its symptomatic and preemptive actions, F 13640 can also produce a "curative-like" action on spinal cord injury-induced neuropathic pain. In particular, spinal injured rats that had fully developed allodynia were infused with F 13640 or saline; we determined whether in these conditions F 13640's analgesic action would persist upon the discontinuation of F 13640 infusion.

2. Material and methods

2.1. Study design

In general conditions that have been detailed elsewhere (Wu et al., 2003), 32 female Sprague–Dawley rats (180–210 g; B&K Universal, Sollentuna, Sweden) underwent spinal cord injury and their responses to sensory stimulation were determined once weekly (see below). Four weeks after injury, 20 rats having demonstrated marked allodynia-like responses were selected for the study and assigned randomly to one of two groups (n=10/group) that were to be treated with either F 13640 or saline. For this, a first 28-day pump was implanted (day 0), and replaced by a second pump on day 28 that was removed on day 56. During this treatment period, allodynia was assessed twice weekly; these assessments continued, once weekly, for 10 weeks after treatment discontinuation. One animal in each group died in the course of the study.

The European Community guidelines for the use of experimental animals have been adhered to; the protocol complies with these guidelines and was approved by the institutional Ethics Committee.

2.2. Spinal cord injury

Ischaemic spinal cord injury was produced with a photochemical technique as detailed elsewhere (Hao et al., 1998); rats were anesthetized, one jugular vein canulated and vertebrae T12-L1 exposed. The animals were positioned beneath a tuneable argon ion laser and irradiated for 10 min with a knife edge beam covering the single T13 vertebra. Before as well as once during the irradiation, 32.5 mg/kg of Erythrosin B was injected i.v.

2.3. Assessment of allodynia

To assess allodynia, as elsewhere (Hao et al., 1998; Wu et al., 2003), a set of calibrated von Frey filaments was used to determine the vocalization threshold to graded mechanical touch/pressure ranging from 0.021 to 410 g; normal rats exhibit a threshold of around 70 g. Responses to brush were determined by having the blunt point of a pencil gently brushing the skin in rostro-caudal direction. Responses to cold were determined by spraying ethyl chloride on the area exhibiting mechanical allodynia. Responses to both brush and cold were assigned a 0–3 score; normal animals typically obtain a score of 0 or 1, respectively.

2.4. Osmotic pump implantation

As elsewhere (Wu et al., 2003), rats were implanted subcutaneously, and once re-implanted, with an osmotic pump (Alzet 2 ML4) releasing (nominal rate: 2.5 μl/h for 28 days) saline or F 13640 (synthesized in-house) at a dose (0.63 mg/day; expressed as free base) that produces symptomatic (Colpaert et al., 2002) and preemptive effects (Wu et al., 2003) in similarly lesioned SCI rats.

2.5. Pharmacokinetics

In a parallel pharmacokinetic study, rats were similarly implanted and re-implanted with a pump releasing 0.63 mg/day of F 13640 for 56 days. In different groups of rats (n=6/group), a blood sample from the abdominal aorta was collected under isoflurane anesthesia 1 day and then daily for up to 5 days after pump explantation on day 56. F 13640 was assayed using a validated LC/ESI-MS/MS method, the quantification limit being 0.1 ng/ml.

3. Results

Repeated-measurement, two-factor analysis of variance (ANOVA; followed where appropriate by Fisher contrast analysis; Myers and Well, 1995; alpha=0.05) of data (Fig. 1) obtained during treatment indicated a significant effect of F 13640 with the von Frey threshold as well as with responses to brush and cold [$F(1,15)$=5.72, 6.29 and 7.78, respectively; P<0.05 in either case]. The effect of time was significant with brush and cold [$F(15,240)$=2.49; P<0.01 and F=1.74; P<0.05, respectively], but not with von Frey (F=1.19; P>0.05).

Importantly, after treatment was discontinued, its effect persisted with each parameter [$F(1,15)$=25.05, 5.72 and 4.78 with von Frey, brush and cold, respectively; P<0.01

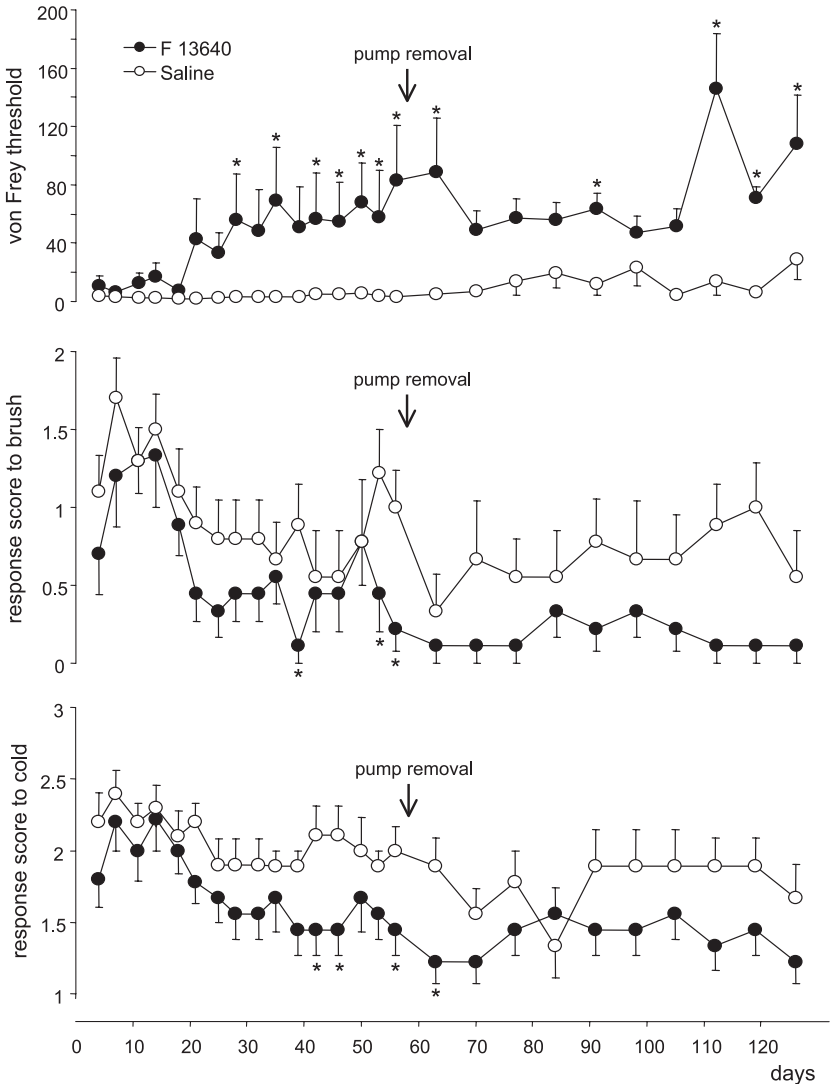

Fig. 1. Curative action of F 13640 on SCI-induced allodynia. Rats received a spinal cord injury, were allowed to develop allodynia and were then treated from day 0 onward with either 0.63 mg/day of F 13640 or saline released by an osmotic pump. Pumps were explanted on day 56, and assessments of allodynia (vocalization threshold to von Frey filament stimulation, response to a gentle brush or cold spray; ordinates) were continued for another 10 weeks. Data points are mean±1 S.E.M. of n=9. ANOVA analysis (see "Results") of data obtained after pump explantation indicated a main treatment effect on the von Frey threshold (P<0.01) as well as with the responses to brush and cold (P<0.05 in either case). Asterisks indicate P<0.05 (post-hoc Fisher contrast test).

with von Frey; P<0.05 with brush and cold]. During this period, the effect of time was significant with von Frey [F(9,144)=3.34; P=0.001], but not with brush and cold (F=0.82 and 1.09, respectively; P>0.05 in either case).

In a previous pharmacokinetic study (see Deseure et al., 2003) pumps releasing 0.63 mg/day of F 13640 generated a steady-state plasma level of about 80 ng/ml. Here, 24 h after pump explantation, the F 13640 plasma concentration was below the 0.1 ng/ml detection limit in four rats and was 0.12 and 0.21 ng/ml in two further animals. The mean concentration remained just above the limit on days 2–4 after explantation [mean (±S.E.M.): 0.27 (±0.07), 0.17 (±0.02) and 0.20 (±0.04) ng/ml, respectively]; the plasma concentration was under the limit in all but one (0.11 ng/ml) of six rats on day 5.

4. Discussion

The present data indicate that following F 13640 infusion in spinal cord-injured rats in which allodynia had been well established, analgesia persists for 10 weeks after the infusion was discontinued. This persistent analgesia is unlikely to be due to a persistent, molecular drug action; F 13640 was undetectable in arterial plasma 5 days after pump explantation. Also, the injection of the selective 5-HT_{1A} receptor antagonist (*N*-4{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl) cyclohexanecarboxamide trihydrochloride) (WAY 100635; Forster et al., 1995) in normal rats having been infused for 2 weeks with the same F 13640 dose, caused a stark analgesia in the Randall-Selitto assay (Colpaert et al., 2002). To our knowledge,

drug-produced analgesia has never before been reported under the present conditions that differ operationally from those in which "symptomatic" and "preemptive" actions are studied (Introduction). Here, treatment was instituted only after allodynia had well developed, and the treatment effect persisted long after the treatment had been discontinued. In other areas of pharmacology, the qualification "curative" is used for the long-term, though not necessarily infinite persistence of a beneficial effect on an established pathology after treatment is discontinued (e.g., Dryver et al., 2003); we here adopt this qualification by analogy. To consider this analogy with regard to pain (see also: Jensen and Baron, 2003; Sah et al., 2003) may be all the more appropriate in as much as neuropathic allodynia constitutes a disease in its own right. Indeed, while the classical notion of "physiological" (e.g., nociceptive, "adaptive") pain refers to that which is associated in time with imminent or actual tissue damage, the recent notion of "pathological" (e.g., neuropathic, "maladaptive") pain refers to that which arises progressively, presumably involves central sensitization, and is long lasting (Ji and Woolf, 2001; Melzack et al., 2001; Scholz and Woolf, 2002; Woolf and Salter, 2000; Sah et al., 2003). However, the diagnostic criteria of pathological pain (Jensen and Baron, 2003) and the therapeutic criteria of this pain's relief remain to be specified in order for "curative" actions on pain to be defined in operational terms.

F 13640's "curative" action can perhaps be understood in the terms of a formal concept of signal transduction in nociceptive systems; the concept proposes that any input to such systems causes not one effect but two effects that are opposite in sign (Colpaert, 1996). Thus, opioids not only cause analgesia as a "1st order" effect, but also "2nd order" hyperalgesia, and this as the consequence of what can be referred to as a downward shift of the baseline. While the 1st order effect results directly from opioid receptor activation, the 2nd order effect is induced by the 1st order effect and outlasts the receptor activation. With chronicity, the 2nd order hyperalgesia amplifies at the expense of 1st order analgesia, thus accounting for opioid analgesic tolerance. F 13640 was designed to mimic the central effects of nociceptive stimulation and produce the mirror inverse of opioid actions; it should and effectively does produce hyperalgesia and analgesia as 1st and 2nd order effects, respectively, and causes an upward baseline shift and inverse tolerance (Colpaert et al., 2002). Opioid-induced 2nd order hyperalgesia and tolerance are theoretically expected to, and do outlast the 1st order analgesia (and the molecular action of opioids) by a period of time that may be long but should be finite (Colpaert, 1996; Bruins Slot and Colpaert, 1999). Indeed, ample evidence indicates that opioid analgesic tolerance in rats, while reversible, persists for long periods of time (Cox, 1990); in a parametric study using the hot plate, Cochin and Kornetsky (1964) found the effects of a previous injection of 20 mg/kg of morphine to continue to be detectable 1 year later. In a similar manner, then, 5-HT_{1A} receptor agonist-induced 2nd order analgesia can be expected to outlast 5-HT_{1A} receptor activation by a period of time that is long though finite. Much as with the agent's two-month-long preemptive action (Wu et al., 2003), there was no evidence here for any decay in F 13640's curative action in the course of the 10-week post-discontinuation period; longer study periods are required to determine whether and when these actions decay. Provided that such a decay would eventually occur, the present account, however formal, may parsimoniously identify F 13640's 1st order effect as the single cause of the operationally different actions that the agent produces (i.e., symptomatic, preemptive, curative).

The physiological and pharmacological specificity of F 13640's actions on neuropathic allodynia in spinal cord-injured rats remains to be documented. However, in rats with an unilateral constriction injury of the infraorbital nerve, F 13640 suppressed ipsilateral allodynic responses to von Frey filament stimulation, but did not lower the (ipsilateral, contralateral) response beyond the level observed in non-injured animals (Deseure et al., 2002). Similarly, reflecting its analgesic action on the chronic nociceptive pain of adjuvant arthritis (Colpaert et al., 2001), F 13640 in arthritic rats suppressed the oral consumption of a fentanyl solution without affecting overall fluid consumption (Colpaert et al., 2002). There is little doubt that F 13640 exerts its actions specifically by 5-HT_{1A} receptor activation; the compound possesses nanomolar affinity for 5-HT_{1A} receptors while being devoid of any interactions with a host of other target proteins at 1000-fold higher concentrations (Colpaert et al., 2002). The extent to which 5-HT_{1A} receptor ligands exert analgesic effects (in the formalin model of tonic nociceptive pain) correlates very highly with the extent to which the ligands activate the 5-HT_{1A} receptor (Colpaert et al., 2002), leaving little possibility for any such additional actions as the ligands may exert, to account for this analgesia. Indeed, using the selective 5-HT_{1A} receptor antagonist WAY 100635, F 13640's actions have consistently been found to involve 5-HT_{1A} receptors (Colpaert et al., 2002; Bardin et al., 2003; Buritova et al., 2003). It would be of interest, however, to pharmacologically analyze the molecular systems that mediate F 13640's "curative" action after it has been established by 5-HT_{1A} receptor activation.

Descending serotonergic fibers are thought to inhibit spinal second-order and polymodal wide-dynamic-range neurons (Garraway and Hochman, 2001), and their disruption may well be involved in spinal cord injury-induced allodynia (for recent discussion, see Bruce et al., 2002). The particular role here of 5-HT_{1A} receptors and the cellular mechanisms and neurophysiological pathways that mediate F 13640's actions remain to be specified. However, and supporting the compound's ability to mimic the central effects of nociceptive stimulation, F 13640, like nociception (Munglani and Hunt, 1995; Harris, 1998), induces the expression of c-Fos protein in rat spinal cord dorsal horn

neurons that are involved in pain processing (Buritova et al., 2003). As it can be blocked by WAY 100635, this F 13640-induced c-Fos protein expression is mediated by 5-HT_{1A} receptors (Buritova et al., 2003). Interestingly, much like F 13640's analgesic action outlasts the agent's 5-HT_{1A} receptor activation, noxious stimulation-induced c-Fos protein expression in dorsal horn neurons also outlasts the activation of peripheral nociceptors (Williams et al., 1990; Presley et al., 1990). Further research will examine the possible role of spinal c-Fos protein expression in the curative action of F 13640 with neuropathic allodynia. It would also be of interest to characterize the relationship between the duration of 5-HT_{1A} receptor activation and that of the ensuing curative action. Indeed, to the extent that neuropathic pain becomes established by such neuroplastic processes as the wind-up of spinal cord dorsal horn wide-dynamic-range neurons (Melzack et al., 2001), F 13640 on theoretical grounds (Colpaert, 1996) can perhaps be expected to reverse or un-wind such processes in a manner that is also time-dependent. Equally, serotonergic projections from the raphe magnus express brain-derived neurotrophic factor (BDNF)-responsive tyrosine kinase B receptors; after spinal cord injury, BDNF immunoreactivity increases near the injury site, and BDNF can stimulate sprouting of serotonergic fibers, potentially replacing degenerated axons (see Bruce et al., 2002). It remains to be examined whether 5-HT_{1A} receptors are involved in this potential, time-dependent replacement.

Acknowledgements

The authors thank E. Carilla-Durand for data management, S. Bréand for statistical analyses and C. Catala for preparing the paper.

References

Bardin, L., Tarayre, J.P., Malfetes, N., Koek, W., Colpaert, F.C., 2003. Profound, non-opioid analgesia produced by the high-efficacy 5-HT_{1A} agonist, F 13640, in the formalin model of tonic nociceptive pain. Pharmacology 67, 182–194.

Bruce, J.C., Oatway, M.A., Weaver, L.C., 2002. Chronic pain after clip-compression injury of the rat spinal cord. Exp. Neurol. 178, 33–48.

Bruins Slot, L.A., Colpaert, F.C., 1999. Experimental realization of a signal transduction algorithm. J. Theor. Biol. 200, 39–48.

Buritova, J., Tarayre, J.P., Besson, J.-M., Colpaert, F.C., 2003. The novel analgesic and high-efficacy 5-HT_{1A} receptor agonist, F 13640 induces c-Fos protein expression in spinal cord dorsal horn neurons. Brain Res. 974, 212–221.

Cochin, J., Kornetsky, C., 1964. Development and loss of tolerance to morphine in the rat after single and multiple injections. J. Pharmacol. Exp. Ther. 145, 1–10.

Colpaert, F.C., 1996. System theory of pain and of opiate analgesia: no tolerance to opiates. Pharmacol. Rev. 48, 355–402.

Colpaert, F.C., Tarayre, J.P., Alliaga, M., Bruins Slot, L.A., Attal, N., Koek, W., 2001. Opiate self-administration as a measure of chronic nociceptive pain in arthritic rats. Pain 91, 33–45.

Colpaert, F.C., Tarayre, J.P., Koek, W., Pauwels, P.J., Bardin, L., Xu, X.J., Wiesenfeld-Hallin, Z., Cosi, C., Carilla-Durand, E., Assié, M.B., Vacher, B., 2002. Large-amplitude 5-HT_{1A} receptor activation: a new mechanism of profound central analgesia. Neuropharmacology 43, 945–958.

Cox, B.M., 1990. Drug tolerance and physical dependence. In: Pratt, W.L., Taylor, P. (Eds.), Principles of Drug Action. The Basis of Pharmacology. Chruchill Livingstone, New York, pp. 639–690.

Deseure, K., Koek, W., Colpaert, F.C., Adriaensen, H., 2002. The 5-HT_{1A} receptor agonist F 13640 attenuates mechanical allodynia in a rat model of trigeminal neuropathic pain. Eur. J. Pharmacol. 456, 51–57.

Deseure, K., Koek, W., Adriaensen, H., Colpaert, F.C., 2003. Continuous administration of the 5-hydroxytryptamine$_{1A}$ agonist (3-chloro-4-fluoro-phenyl)-[4-fluoro-4-{[(5-methyl-pyridin-2-ylmethyl)-amino]-methyl}piperidin-1-yl]-methadone (F 13640) attenuates allodynia-like behavior in a rat model of trigeminal neuropathic pain. J. Pharmacol. Exp. Ther. 306, 505–514.

Dryver, E.T., Jerström, H., Tompkins, K., Buckstein, R., Imric, K.R., 2003. Follow-up of patients with Hodgkin's disease following curative treatment: the routine CT scan is of little value. Br. J. Cancer 89, 482–486.

Forster, E.A., Cliffe, I.A., Bill, D.J., Dover, G.M., Jones, D., Reilly, Y., Fletcher, A., 1995. A pharmacological profile of the selective silent 5-HT_{1A} receptor antagonist, WAY-100635. Eur. J. Pharmacol. 281, 81–88.

Garraway, S.M., Hochman, S., 2001. Serotonin increases the incidence of primary afferent-evoked long-term depression in rat deep dorsal horn neurons. J. Neurophysiol. 85, 1864–1872.

Hao, J.-X., Wiesenfeld-Hallin, Z., Xu, X.-J., 1998. Treatment of chronic allodynia in spinally injured rats: effects of intrathecal selective opioid receptor agonists. Pain 75, 209–217.

Harris, J.A., 1998. Using c-fos as a neural marker of pain. Brain Res. Bull. 45, 1–8.

Jensen, T.S., 2002. An improved understanding of neuropathic pain. Eur. J. Pain 6, 3–11.

Jensen, T.S., Baron, R., 2003. Translation of symptoms and signs into mechanisms in neuropathic pain. Pain 102, 1–8.

Ji, R.R., Woolf, C.J., 2001. Neuronal plasticity and signal transduction in nociceptive neurons: implications for the initiation and maintenance of pathological pain. Neurobiol. Dis. 8, 1–10.

Melzack, R., Coderre, T.J., Katz, J., Vaccarino, A.L., 2001. Central neuroplasticity and pathological pain. Ann. N.Y. Acad. Sci. 933, 157–174.

Munglani, R., Hunt, S.P., 1995. Molecular biology of pain. Br. J. Anaesth. 75, 186–192.

Myers, J.L., Well, A.D., 1995. Research Design and Statistical Analysis. Lawrence Erlbaum Associates, Broadway, Hilsdale, New Jersey.

Presley, R.W., Menétrey, D., Levine, J.D., Basbaum, A.I., 1990. Systemic morphine suppresses noxious stimulus-evoked Fos protein-like immunoreactivity in the rat spinal cord. J. Neurosci. 10, 323–335.

Sah, D.W.Y., Ossipov, M.H., Porreca, F., 2003. Neurotrophic factors as novel therapeutics for neuropathic pain. Nat. Rev., Drug Discov. 2, 470–472.

Scholz, J., Woolf, C.J., 2002. Can we conquer pain? Nat. Neurosci. 5, 1062–1067.

Siddall, P.J., Yezierski, R.P., Loeser, J.D., 2002. Taxonomy and epidemiology of spinal cord injury pain. In: Yezierski, R.P., Burchiel, K.J. (Eds.), Spinal Cord Injury Pain: Assessment, Mechanisms, Management. IASP Press, Seattle, pp. 9–24.

Williams, S., Evans, G.I., Hunt, S.P., 1990. Changing patterns of c-Fos induction in spinal neurons following thermal cutaneous stimulation in the rat. Neuroscience 36, 73–81.

Woolf, C.J., Salter, M.W., 2000. Neuronal plasticity: increasing the gain in pain. Science 288, 1765–1768.

Wu, W.P., Hao, J.X., Xu, X.J., Wiesenfeld-Hallin, Z., Koek, W., Colpaert, F.C., 2003. The very-high-efficacy 5-HT_{1A} receptor agonist, F 13640, preempts the development of allodynia-like behaviors in rats with spinal cord injury. Eur. J. Pharmacol. 478, 131–137.