MOLECULAR PAIN

RESEARCH   **Open Access**

Serotonin receptors are involved in the spinal mediation of descending facilitation of surgical incision-induced increase of Fos-like immunoreactivity in rats

João Walter S Silveira[1], Quintino M Dias[1], Elaine A Del Bel[2] and Wiliam A Prado*[1]

Abstract

Background: Descending pronociceptive pathways may be implicated in states of persistent pain. Paw skin incision is a well-established postoperative pain model that causes behavioral nociceptive responses and enhanced excitability of spinal dorsal horn neurons. The number of spinal c-Fos positive neurons of rats treated intrathecally with serotonin, noradrenaline or acetylcholine antagonists where evaluated to study the descending pathways activated by a surgical paw incision.

Results: The number of c-Fos positive neurons in laminae I/II ipsilateral, lamina V bilateral to the incised paw, and in lamina X significantly increased after the incision. These changes: remained unchanged in phenoxybenzamine-treated rats; were increased in the contralateral lamina V of atropine-treated rats; were inhibited in the ipsilateral lamina I/II by $5\text{-HT}_{1/2B/2C}$ (methysergide), 5-HT_{2A} (ketanserin) or $5\text{-HT}_{1/2A/2C/5/6/7}$ (methiothepin) receptors antagonists, in the ipsilateral lamina V by methysergide or methiothepin, in the contralateral lamina V by all the serotonergic antagonists and in the lamina X by LY 278,584, ketanserin or methiothepin.

Conclusions: We conclude: (1) muscarinic cholinergic mechanisms reduce incision-induced response of spinal neurons inputs from the contralateral paw; (2) $5\text{-HT}_{1/2A/2C/3}$ receptors-mediate mechanisms increase the activity of descending pathways that facilitates the response of spinal neurons to noxious inputs from the contralateral paw; (3) $5\text{-HT}_{1/2A/2C}$ and $5\text{-HT}_{1/2C}$ receptors increases the descending facilitation mechanisms induced by incision in the ipsilateral paw; (4) $5\text{-HT}_{2A/3}$ receptors contribute to descending pronociceptive pathways conveyed by lamina X spinal neurons; (5) α-adrenergic receptors are unlikely to participate in the incision-induced facilitation of the spinal neurons.

Background

Bulbospinal pathways descend to the spinal cord to either inhibit (antinociceptive) or facilitate (pronociceptive) the transmission of nociceptive inputs (for review see [1,2]). The contribution of supraspinal areas in the control of descending pronociceptive pathways was confirmed by several studies. As examples, the lesion or neural block of rostral ventromedial medulla (RVM) or periaqueductal gray (PAG) reduces the hyperalgesia induced by spinal nerve ligature [3,4], or intraplantar injection of formalin [5,6] or mustard oil [7]. Furthermore, low intensity electrical stimulation of, or low dose of glutamate into the RVM facilitates the response of spinal nociceptive neurons to noxious inputs, whereas high intensity electrical stimulation or high dose of glutamate produces the opposite effect [8].

Descending pronociceptive pathways may be implicated in states of persistent pain [9,10] and elucidation of their spinal mediation may be useful for discovery of new antihyperalgesic drugs. Spinal serotonin produces antinociception but may be pronociceptive as well (for review see [11]). Also, spinal activation of α_2-adrenergic receptors is antinociceptive whereas activation of α_1-adrenergic receptors is pronociceptive [12,13]. A spinal

* Correspondence: wadprado@fmrp.usp.br
[1] Department of Pharmacology, Faculty of Medicine of Ribeirão Preto, University of Sao Paulo, Ribeirão Preto, SP 14049-900 Brazil
Full list of author information is available at the end of the article

muscarinic cholinergic mechanism activated by descending noradrenergic inputs has also been proposed and it seems to be linked only with antinociception (for review see [14]).

Surgical incision of a rat paw causes primary and secondary punctate hyperalgesia [15] and increases the number of c-Fos positive neurons in the spinal cord [16], an immunohistochemical method that allows the identification of neurons activated by peripheral noxious stimulation [17]. Although being a poorly understood problem, very little effort has been dedicated toward research on the spinal mediation of descending mechanisms of post-incision pain, a model that may allow us to understand mechanisms of sensitization caused by surgery and investigate new therapies for postoperative pain.

The present study was therefore undertaken to examine the changes in the number of c-Fos positive neurons in the spinal cord of rats treated intrathecally with antagonists of serotonin, noradrenaline or acetylcholine, to evaluate whether they contribute in the spinal mediation of descending pronociceptive pathways activated by a surgical incision. The laminae I/II, V and X were systematically examined, since they are predominantly implicated in the reception, processing and rostral transmission of nociceptive information [11].

Results

Effects of intrathecal muscarinic cholinergic, α-adrenergic and serotonergic receptor antagonists on the number of Fos-immunoreactive neurons in the laminae I/II, and V of the rat spinal cord

The number of Fos-LI neurons was very low bilaterally in laminae I/II (Figure 1) and V (Figure 2) of non-incised and non-catheterized anesthetized rats (group A), and was slightly and non-significantly increased in non-incised anesthetized rats treated intrathecally with saline (group AS). The number of positive neurons was greater bilaterally in laminae I/II and V of incised rats treated intrathecally with saline (group ASI), the effect being significant at the ipsilateral laminae I/II and bilateral lamina V.

The incision-induced increase in the number of Fos-LI neurons in the ipsilateral laminae I/II of rats from the group ASI was significantly less intense following methysergide (30 μg/5 μl), and was not changed significantly by atropine (30 μg/5 μl) or phenoxybenzamine (20 μg/5 μl) (ANOVA: $F_{5,26}$ = 24.64; P < 0.0001) (Figure 1a). Similar results were found in the contralateral laminae I/II, but the differences were not significant (ANOVA: $F_{5,26}$ = 1.74; P > 0.05) (Figure 1b).

The incision-induced increase in the number of Fos-LI neurons in the ipsilateral lamina V of rats from the group ASI was less intense after methysergide (30 μg/5 μl), and was not changed by phenoxybenzamine (20 μg/5 μl) or
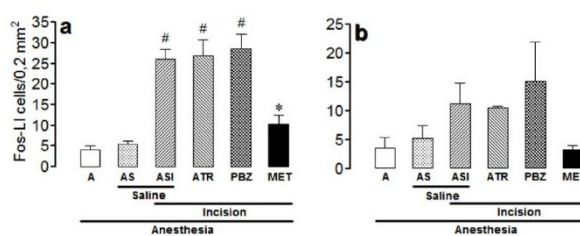

Figure 1 Effects of intrathecal atropine, phenoxybenzamine or methysergide in the incision-induced Fos-like immunoreactivity in lamina I/II. The experiments utilized 4-8 non-incised and non-catheterized anesthetized rats (A), non-incised and catheterized rats treated intrathecally with 5 μl of saline (AS), incised and catheterized rats treated intrathecally with 5 μl of saline (ASI), 30 μg/5 μl of atropine (ATR), 20 μg/5 μl of phenoxybenzamine (PBZ), or 30 μg/5 μl methysergide (MET). Surgical incision of the right hind paw was performed 3 h after PBZ or 15 min after the remaining antagonists. The number of Fos-like immunoreactive (Fos-LI) cells/0.2 mm² are shown for lamina I/II of ipsilateral (a) or contralateral (b) spinal dorsal horn. Bars are mean ± S.E.M. of the number of Fos-LI cells/0.2 mm² found in three sections taken from each rat. P < 0.05 compared to ASI (*) or AS (#).

atropine (30 μg/5 μl) (ANOVA: $F_{5,26}$ = 9.94; P < 0.0001) (Figure 2a). Similar, but less intense effect occurred in the contralateral lamina V of rats treated with methysergide (30 μg/5 μl) or phenoxybenzamine (20 μg/5 μl) as compared with rats from the group ASI, but it was significantly more intense in the contralateral lamina V of rats treated with atropine (30 μg/5 μl) (ANOVA: $F_{5,26}$ = 9.68; P < 0.0001) (Figure 2b).

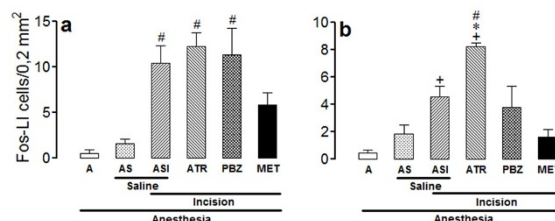Representative photomicrographs taken from sections of the spinal cords of control and test rats are given in Fig-

Figure 2 Effects of intrathecal atropine, phenoxybenzamine or methysergide in the incision-induced Fos-like immunoreactivity in lamina V. The experiments utilized 4-8 non-incised and non-catheterized anesthetized rats (A), non-incised and catheterized rats treated intrathecally with 5 μl of saline (AS), incised and catheterized rats treated intrathecally with 5 μl of saline (ASI), 30 μg/5 μl of atropine (ATR), 20 μg/5 μl of phenoxybenzamine (PBZ), or 30 μg/5 μl methysergide (MET). Surgical incision of the right hind paw was performed 3 h after PBZ or 15 min after the remaining antagonists. The number of Fos-like immunoreactive (Fos-LI) cells/0.2 mm² are shown for lamina V of ipsilateral (a) or contralateral (b) spinal dorsal horn. Bars are mean ± S.E.M. of the number of Fos-LI cells/0.2 mm² found in three sections taken from each rat. P < 0.05 compared to A (+), ASI (*) or AS (#).
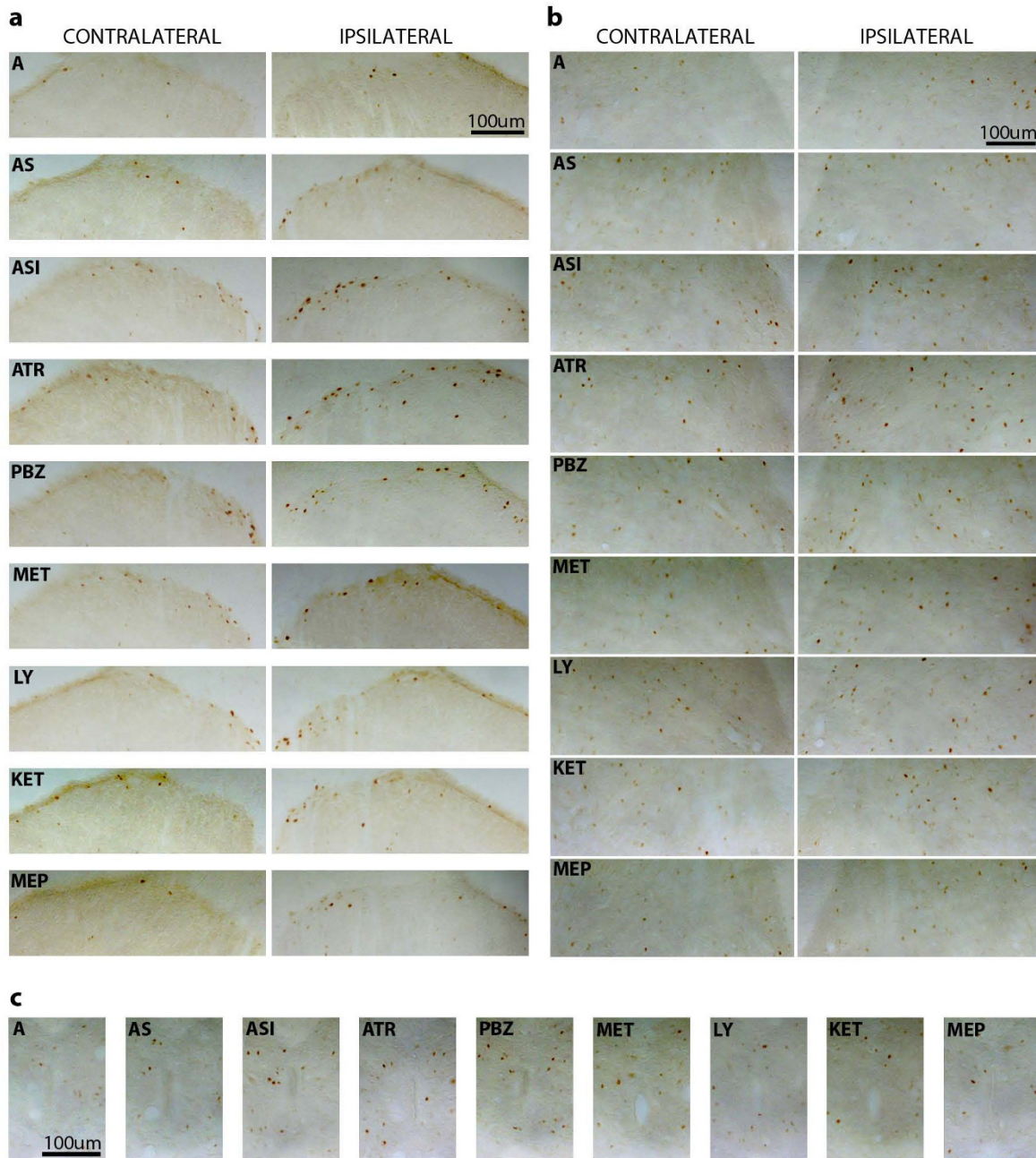
Figure 3 Photomicrographs showing Fos-like immunoreactivity in rat spinal cord. Photomicrographs were taken from 40- μm thick sections and illustrate the expression of Fos-like immunoreactivity in laminae I-II (a), V (b), and X (c) of the spinal cord gray matter (L2/L3 level), 2 h after the plantar incision of the right hind paw. of non-catheterized anesthetized rats (A), non-incised and catheterized rats treated. intrathecally with saline (AS), and incised and catheterized rats treated intrathecally with saline (ASI), atropine (ATR = 30 μg/5 μl), phenoxybenzamine (PBZ = 20 μg/5. μl), methysergide (MET = 30 μg/5 μl), LY 278,584 (LY = 100 μg/5 μl), ketanserin. (KET = 30 μg/5 μl), or methiothepin (MEP = 1.5 μg/5 μl).

ure 3a and 3b for superficial and deep laminae, respectively.

Effects of intrathecal muscarinic cholinergic, α-adrenergic and serotonergic receptor antagonists on the number of Fos-immunoreactive neurons in the lamina X of the rat spinal cord

The number of Fos-LI neurons in lamina X was very small in rats from group A and non-significantly higher in rats from group AS (Figure 4). The hind paw incision also induced a significant increase in the number of positive cells in lamina X, as compared to rats from group AS (ANOVA: $F_{5,24} = 7.06$; $P < 0.0003$). The hind paw incision-induced increase in the number of Fos-LI neurons in lamina X was slightly reduced by methysergide (30 μg/5 μl), and slightly increased by atropine (30 μg/5 μl) or phenoxybenzamine (20 μg/5 μl), but all changes occurred in a non significant manner. Representative photomicrographs taken from sections of the spinal cords of control and test rats are given in Figure 3c.

Effects of intrathecal antagonists of 5-HT receptor subtypes on the number of Fos-immunoreactive neurons in the laminae I, II, and V of the rat spinal cord

The number of Fos-LI neurons was very low in laminae I/II (Figure 5) and V (Figure 6) of rats from group A, and was slightly but non-significantly increased in rats from
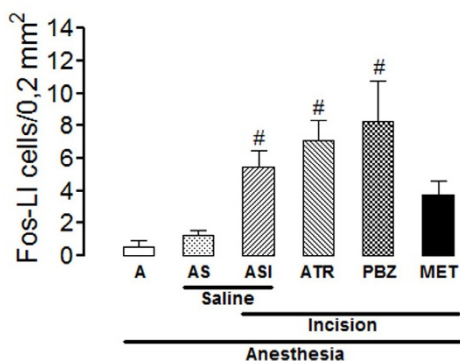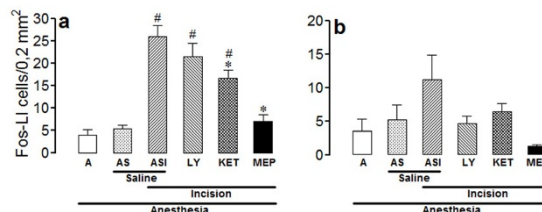
Figure 5 Effects of intrathecal LY278,584, ketanserin or methiothepin in the incision-induced Fos-like immunoreactivity in lamina I/II. The experiments utilized 4-8 non-incised and non-catheterized anesthetized rats (A), non-incised and catheterized rats treated intrathecally with 5 μl of saline (AS), incised and catheterized rats treated intrathecally with 5 μl of saline (ASI), 100 μg/5 μl of LY 278,584 (LY), 30 μg/5 μl of ketanserin (KET) or 1,5 μg/5 μl of methiothepin (MEP). Surgical incision of the right hind paw was performed 15 min after each antagonist. The number of Fos-like immunoreactive (Fos-LI) cells/0.2 mm^2 are shown for lamina I/II of ipsilateral (a) or contralateral (b) spinal dorsal horn. Bars are mean ± S.E.M. of the number of Fos-LI cells/0.2 mm^2 found in three sections taken from each rat. $P < 0.05$ compared to ASI (*) or AS (#).

group AS. The number of positive neurons was higher bilaterally in laminae I/II and V of incised rats treated with saline (group ASI), the effect being significant at the ipsilateral laminae I/II and bilateral lamina V.

The incision-induced increase in the number of Fos-LI neurons in the ipsilateral laminae I/II was significantly reduced by methiothepin (1,5 μg/5 μl) or ketanserin (30 μg/5 μl), but was not changed by LY 278,584 (100 μg/5 μl) (ANOVA: $F_{5,30} = 24.77$; $P < 0.0001$) (Figure 5a). However, no significant difference was demonstrated in the con-

Figure 4 Effects of intrathecal atropine, phenoxybenzamine or methysergide in the incision-induced Fos-like immunoreactivity in lamina X. The experiments utilized 4-8 non-incised and non-catheterized anesthetized rats (A), non-incised and catheterized rats treated intrathecally with 5 μl of saline (AS), incised and catheterized rats treated intrathecally with 5 μl of saline (ASI), 30 μg/5 μl of atropine (ATR), 20 μg/5 μl of phenoxybenzamine (PBZ), or 30 μg/5 μl methysergide (MET). Surgical incision of the right hind paw was performed 3 h after PBZ or 15 min after the remaining antagonists. The number of Fos-like immunoreactive (Fos-LI) cells/0.2 mm^2 are shown for lamina X. Bars are mean ± S.E.M. of the number of Fos-LI cells/0.2 mm^2 found in three sections taken from each rat. $P < 0.05$ compared to AS (#).
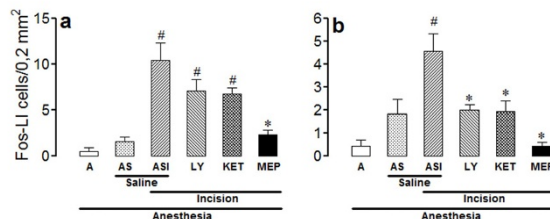
Figure 6 Effects of intrathecal LY278,584, ketanserin or methiothepin in the incision-induced Fos-like immunoreactivity in lamina V. The experiments utilized 4-8 non-incised and non-catheterized anesthetized rats (A), non-incised and catheterized rats treated intrathecally with 5 μl of saline (AS), incised and catheterized rats treated intrathecally with 5 μl of saline (ASI), 100 μg/5 μl of LY 278,584 (LY), 30 μg/5 μl of ketanserin (KET) or 1,5 μg/5 μl of methiothepin (MEP). Surgical incision of the right hind paw was performed 15 min after each antagonist. The number of Fos-like immunoreactive (Fos-LI) cells/0.2 mm^2 is shown for lamina V of ipsilateral (a) or contralateral (b) spinal dorsal horn. Bars are mean ± S.E.M. of the number of Fos-LI cells/0.2 mm^2 found in three sections taken from each rat. $P < 0.05$ compared to ASI (*) or AS (#).

tralateral laminae I and II among the experimental groups (ANOVA: $F_{5,30}$ = 2.02; P > 0.05) (Figure 5b).

The incision-induced increase in the number of Fos-LI neurons in the ipsilateral lamina V was also reduced by methiothepin (1,5 µg/5 µl), ketanserin (30 µg/5 µl) or LY 278,584 (100 µg/5 µl), but only the effect of methiothepin was significant (ANOVA: $F_{5,26}$ = 9.94; P < 0.0001) (Figure 6a). In contrast, ketanserin, LY 278,584 and mainly methiothepin all reduced significantly the incision-induced increase in the number of positive cells in the contralateral lamina V (ANOVA: $F_{5,30}$ = 7.27; P = 0.0001) (Figure 6b).

Representative photomicrographs taken from sections of the spinal cords of control and test rats are given in Figure 3a and 3b for superficial and deep laminae, respectively.

Effects of intrathecal antagonists of 5-HT receptor subtypes on the number of Fos-immunoreactive neurons in the lamina X of the rat spinal cord

The number of Fos-LI neurons in lamina X was very small in rats from group A and was non-significantly superior in rats from group AS (Figure 7). The hind paw incision also induced a significant increase in the number of positive cells in lamina X, as compared to rats from group AS, the effect being significantly reduced by LY

Figure 7 Effects of intrathecal LY278,584, ketanserin or methiothepin in the incision-induced Fos-like immunoreactivity in lamina X. The experiments utilized 4-8 non-incised and non-catheterized anesthetized rats (A), non-incised and catheterized rats treated intrathecally with 5 µl of saline (AS), incised and catheterized rats treated intrathecally with 5 µl of saline (ASI), 100 µg/5 µl of LY 278,584 (LY), 30 µg/5 µl of ketanserin (KET) or 1,5 µg/5 µl of methiothepin (MEP). The number of Fos-like immunoreactive (Fos-LI) cells/0.2 mm² are shown for lamina X. Surgical incision of the right hind paw was performed 15 min after each antagonist. Bars are mean ± S.E.M. of the number of Fos-LI cells/0.2 mm² found in three sections taken from each rat. P < 0.05 compared to ASI (*) or AS (#).

278,584 (100 µg/5 µl), ketanserin (30 µg/5 µl) or methiothepin (1,5 µg/5 µl) (ANOVA: $F_{5,28}$ = 9.26; P < 0.0001). Representative photomicrographs taken from sections of the spinal cords of control and test rats are given in Figure 3c.

Discussion

Fos protein is a product of the proto-oncogene c-Fos, which is expressed in the neuronal nuclei few hours after an appropriate stimulus [18]. The increase in the expression of Fos-LI neurons in spinal cord has been used as a marker of the neuronal activity induced by noxious stimuli in various pain models [17]; for review see [19,20]. Although being a poorly understood problem, very little effort has been dedicated toward research on the spinal mediation of descending mechanisms of post-incision pain, a model that may allow us to understand mechanisms of sensitization caused by surgery and investigate new therapies for postoperative pain.

The present study confirmed that a surgical incision in a rat hind paw increased significantly the number of Fos-LI neurons in the spinal dorsal horn laminae I/II ipsilateral, and lamina V bilateral to the incised paw as shown elsewhere [16,21,22].

Also, a significant increase in the number of Fos-LI neurons were also found in the lamina X of the spinal gray matter following the incision, as described elsewhere subsequent to peripheral or visceral nociceptive inputs [23].

Incision of a hind paw increases both the response of spinal wide dynamic range cells to mechanical stimulation [15] and the spontaneous activity of nociceptive primary afferents [24]. The model of incision pain differs from inflammatory pain models [25,26], but the tissue reactions to incision are likely to involve some inflammatory responses to local injury [27].

Evidence that descending influences can affect spinal phenomena induced by a paw incision includes the demonstration that incision pain is further increased in rats with lesion of dorsolateral funiculus [28], that conveys descending pain inhibitory pathways [11], or electrolytic lesion of the anterior pretectal nucleus [29], that participates in the activation of descending mechanisms of pain control [30]; in the ipsilateral spinal cord, the incision-induced increase in the number of Fos-LI neurons was significantly reduced in the superficial lamina and significantly increased in the deep lamina of animals previously treated with bupivacaine in the contralateral anterior pretectal nucleus [16]; finally, descending pathways are known as the only sources of serotonin in the spinal cord [2,11,31].

Acetylcholine, noradrenaline and serotonin modulate noxious inputs processing in the spinal cord (for review see [11]. Atropine, a non selective muscarinic receptor
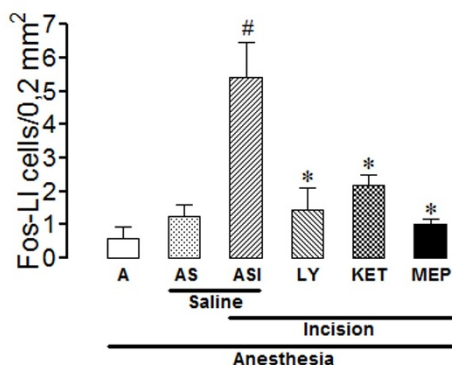
antagonist was used here at 30 μg/rat, 3 fold superior to the dose known to be effective against the neostigmine-induced antinociception in rats [32].

Phenoxybenzamine, a non selective α-adrenergic receptor antagonist was used here at 20 μg/rat, 2.5 fold superior to the dose early shown to be effective against glutamate-induced analgesia from the RVM of rats [33]. Nonetheless, neither the atropine nor the phenoxybenzamine changed the incision-induced increase in the number of Fos-LI neurons in the laminae I/II, bilaterally, ipsilateral lamina V, or in lamina X. Consequently, muscarinic and α-adrenergic receptors are unlikely to participate in mechanisms that facilitate the response of spinal neurons to noxious inputs evoked by the incision. On the contrary, the increase in the number of Fos-LI neurons in the lamina V contralateral to the incised paw was significantly greater in atropine- than in saline-treated rats. In agreement to earlier studies [34-36], we interpret the result as indirect evidence that a muscarinic cholinergic mechanism responds to a surgical incision reducing the response of spinal neurons to noxious inputs from the contralateral paw. A possibility remains that the depression of c-Fos labeling is due to toxic effects of the antagonists used in the study. However, as far as we know, there is no report showing that at the doses used in the study, the intrathecal antagonists induces toxic effects against spinal dorsal horn cells in rats.

Among the seven classes of serotonin receptors ($5\text{-HT}_{1\text{-}7}$) currently known (for review see [37]), at least three ($5\text{-HT}_{1/2/3/}$) were found in the spinal cord [38,39] and are implicated in spinal pain processing [40-43]. The antinociceptive effect of 5-HT may also occur via interaction with spinal 5-HT_4 receptors [44,45], but experiments involving pharmacological blockade of these receptors were not conducted in this study.

The increase in the number of Fos-LI neurons in the lamina I/II ipsilateral to the incised paw was significantly inhibited by antagonists for $5\text{-HT}_{1/2B/2C}$ (methysergide), 5-HT_{2A} (ketanserin) or $5\text{-HT}_{1/2A/2C/5/6/7}$ (methiothepin) receptors, but was not changed by a 5-HT_3 antagonist (LY 278,584). The same effect was also significantly inhibited in the lamina V ipsilateral to the incised paw by methysergide or methiothepin, but remained unchanged following ketanserin or LY 278,584. Therefore, $5\text{-HT}_{1/2A/2C}$ receptors in laminae I/II and $5\text{-HT}_{1/2C}$ receptors in lamina V, but not 5-HT_3 receptors in either laminae I/II or V, are involved in the activity of a descending pathway that facilitates the response of spinal neurons to noxious inputs induced by a surgical incision in the ipsilateral paw.

The significant increase in the number of Fos-LI neurons in the contralateral lamina V was inhibited by all the serotonergic antagonists used in the study. Thus, $5\text{-HT}_{1/2A/2C/3}$ receptors contribute to the effect of serotonin in the activity of a descending pathway that facilitates the response of spinal neurons to noxious inputs induced by a surgical incision in the contralateral paw. Finally, the increase in the number of Fos-LI neurons in lamina X was significantly inhibited by LY 278,584, ketanserin or methiothepin, but not by methysergide. We then conclude that $5\text{-HT}_{2A/3}$ receptors contribute to the effects of serotonin in a descending pronociceptive pathway conveyed by lamina X spinal neurons.

Altogether, these results support the involvement of serotonin in descending mechanisms that facilitate the response of spinal neurons to nociceptive inputs in the spinal cord. Serotonergic nerve terminals found in the spinal cord originate from supraspinal sources [2,11,31] and, therefore, descending pathways utilizing serotonin somehow excite nociceptive cells in the spinal cord while a postoperative pain is in course. Bulbospinal influences from the RVM contribute to facilitation of noxious inputs and development of secondary hyperalgesia in persistent inflammatory, neuropathic, and visceral pain models[46]. Primary and secondary hyperalgesia observed after a surgical incision do not appear to be modulated by descending influences from the RVM, thus supporting the view that incision pain involves different mechanisms compared with inflammatory and neuropathic pain[47]. However, in this study Pogatzki *et al.*, utilized rats 5 days after RVM lesion and, therefore, it is possible that a structure located more rostrally assumes the control of the primary and secondary hyperalgesia observed after a surgical incision. In fact, several other studies have shown the involvement of descending pain pathways in this model [29,30,48-51].

The spinal actions of serotonin has long been associated to suppression of the responses to nociceptive inputs [52-54], but evidence has accumulated questioning whether descending serotonergic pathways play an exclusive spinal suppressive effect against nociceptive inputs [53,55]. The activation of spinal serotonin receptors has been associated with both pronociceptive and antinociceptive effects depending on algesimetric test, drug dosage, duration of the treatment and pathophysiological condition [56-58].

The spinal dorsal horn contains high concentrations of 5-HT_{1A}, and 5-HT_{1B} receptors [39,59], but the occurrence of 5-HT_{1D} receptors is possible [60,61]. The activation of spinal 5-HT_{1A} may result analgesia [62,63] (see also [64]), or hyperalgesia [65,66], while the activation of spinal 5-HT_{1B} receptors produces antinociception [67,68].

The presence of spinal 5-HT_{2A} [69,70], and 5-HT_{2C} receptors [71,72] has already been demonstrated. Stimulation of spinal 5-HT_{2A} receptors is pronociceptive

[53,73], but the presence of 5-HT$_{2A}$ receptors on spinal inhibitory interneurons supports an antinociceptive role for 5-HT [74,75]. The activation of spinal 5-HT$_{2C}$ receptors excites neurons [55,76,77], and its distribution in the spinal cord is compatible with a pronociceptive role of 5-HT in the dorsal horn [12,78]. Conversely, 5-HT$_{2C}$ sites on spinal inhibitory interneurons allow a potential antinociceptive role for serotonin [79].

Studies have demonstrated that 5-HT$_3$ receptors are concentrated in superficial layers of the dorsal horn [59,80] and a significant proportion is located on the terminals of C fibres [12,81]. The activation of the 5-HT$_3$ receptors depolarizes neurons in dorsal root ganglions and, therefore, is expected to increase the transmitter release from the primary afferent terminals into the spinal cord (see [64]). In line with the present results, the activation of spinal 5-HT$_3$ receptors increases nociception [62,82,83], and blockade of spinal 5-HT$_3$ receptor reduces the hypersensitivity of spinal dorsal horn neurons of nerve-ligated rats [84]. A pronociceptive role for 5-HT$_3$ receptors in the spinal cord following activation of descending pronociceptive pathways has already been proposed [84,85].

It is also noteworthy, that a direct facilitation by serotonin of glutamatergic synapses has been demonstrated in the spinal cord [86,87] and, therefore, this mechanism may account for some of the excitatory effects of serotonin found here.

There is evidence that a peripheral damage simultaneously triggers both descending inhibition and facilitation onto both primary and secondary spinal hyperalgesic mechanisms, but the balance for primary hyperalgesia is different from the balance for secondary hyperalgesia (for review see [48]).

Behavioral nociceptive responses of our animals were not evaluated during this study but it may be assumed that the increased number of Fos-LI neurons in the dorsal horn ipsilateral to the incised paw essentially reflects primary hyperalgesia. By extension, a secondary hyperalgesia evoked by central sensitization may be assumed to occur when the effect was observed in the contralateral dorsal horn (see [88]). Therefore, our results show that incision-induced primary and secondary hyperalgesias seem to be spinally mediated by 5-HT$_{1/2A/2C}$ and 5-HT$_{1/2A/2C/3}$ receptors, respectively.

Few studies have accessed which neurotransmitters are involved in the spinal processing of hyperalgesia in the mode of post-incision pain. They have shown that noradrenergic receptors are involved in the spinal mediation of descending inhibitory pathways in the primary hyperalgesia of postoperative pain [29,49,51]. Few reports are also available regarding secondary hyperalgesia after post-incision pain [47,89-94] but, as far we know, none of them have studied spinal neurotransmitters involved in descending mechanisms.

Conclusions

In conclusion (Table 1), 5-HT$_{1/2A/2C}$ receptors in laminae I/II and 5-HT$_{1/2C}$ receptors in lamina V contribute to the effects of serotonin in descending pathway that facilitates the response of spinal neurons to noxious inputs from the ipsilateral paw (primary hyperalgesia?); 5-HT$_{1/2A/2C/3}$ receptors mediate the effects of a descending pathway that facilitates the response of spinal neurons to noxious

Table 1: Effects of antagonists in the incision-induced increase of Fos-like immunoreactivity in the rat spinal cord

Antagonists	LI/LII		LV		LX
	ipsi	contra	ipsi	contra	
Atropine (muscarinic)	-	-	-	9*	-
Phenoxybenzamine (α-adrenergic)	-	-	-	-	-
Methysergide (5-HT$_{1/2B/2C}$)	|*	-	|#	|#	|#
LY 278,584 (5-HT$_3$)	-	-	-	|*	|*
Ketanserin (5-HT$_{2A}$)	|*	-	-	|*	|*
Methiothepin (5-HT$_{1/2A/2C/5/6/7}$)	|*	-	|*	|*	|*

Antagonists were injected intrathecally. Abbreviations: ipsi = ipsilateral and contra = contralateral to the incised hind paw; | = decrease and 9 = increase of the effect produced by the incision on the Fos-like immunoreactivity. (*) Significantly different from intrathecal saline-treated incised rats. (#) Abolished the significance of the incision-induced increase of Fos-like immunoreactivity but did not differ significantly from intrathecal saline-treated incised rats.

inputs from the contralateral paw (secondary hyperalgesia?); and 5-HT$_{2A/3}$ receptors contribute to the effects of serotonin in a descending pronociceptive pathway conveyed to lamina X spinal neurons. Finally, the study confirms that spinal muscarinic cholinergic mechanism responds to a surgical incision reducing the response of spinal neurons to noxious inputs from the contralateral paw.

Methods

Subjects

Male Wistar rats (200-250 g) were used in this study. Animals were housed two to a cage under controlled temperature (22 ± 1°C) and on a 12-h light-dark cycle, with the dark cycle beginning at 07:00 h, and had free access to food and water. The experiments were approved by the Commission of Ethics in Animal Research, Faculty of Medicine of Ribeirão Preto, University of São Paulo (Number 009/2004). The guidelines of the Committee for Research and Ethical Issues of IASP [95] were followed throughout the experiments.

Surgery

Each rat was anesthetized with halothane via a loose-fitting, cone-shaped mask, and catheterization of the spinal subarachnoid space was performed as described elsewhere [96]. Briefly, a 20-gauge Weiss needle was introduced through the skin into the L5-L6 intervertebral space. The correct positioning of the needle was assured by a typical flick of the tail or hind paw. A 12-mm length of polyethylene tubing (PE tubing, o.d. = 0.4 mm, dead space = 10 μl) was then introduced through the needle to protrude 2.0 cm into the subarachnoid space in a cranial direction. The needle was then carefully removed and the tubing anchored to the back skin with a cotton thread suture. Drug or saline was injected intrathecally soon after the catheterization in a volume of 5 μl over a period of 60 s, followed by 5 μl of sterile saline at the same rate to flush the catheter. The plantar side of the right hind paw was prepared with a 10% povidone-iodine solution 15-min later. A 1-cm longitudinal incision was made with a surgical blade, through the skin and fascia of the plantar region, starting 0.5 cm from the proximal edge of the heel, as described elsewhere [97]. The plantaris muscle was elevated, but its origin and insertion were left intact. After hemostasia, the skin was apposed with one single suture of 5-0 nylon, and the animal was allowed to recover in the home cage for a period of 2 h. The positioning of the intrathecal catheter was verified when the spinal cord was removed for Fos immunohistochemistry.

Fos immunohistochemistry

The animals were sacrificed with an intraperitoneal overdose of sodium thiopental performed 2 h after the plantar incision, and perfused transcardially with saline followed by 4% paraformaldehyde in 0.1 M PBS, pH 7.4. The spinal cord was removed, fixed for 2 h in paraformaldehyde and stored for at least 48 h in 30% sucrose. The side ipsilateral to the incised paw was marked with a little knife cut. The samples were then frozen in Tissue Teck (Sakura°). Fos immunohistochemistry was processed, as described elsewhere [98], on 40 μm transverse sections obtained with a cryostat (Leica CM 1850) from L2-L3 spinal cord segments. The tissue sections were successively washed and incubated for 1 h in goat anti-rabbit biotinylated antibody (1:400 in PBS; Vector Laboratories, Burlingame, CA). They were then processed by the avidin-biotin immunoperoxidase method (Vectastain ABC kit, Vector Lab, Burlingame, CA, U.S.A.), and then Fos-like immunoreactivity (FLI) was revealed by the addition of chromogen diaminobenzidin (Sigma).

All reactions were performed at room temperature. Fos-like immunoreactivity was quantified using an image analysis system (Leica, Quantimet 500, Leica Microsystems Inc. Cambridge, UK) that identified and counted immunostained neurons according to a gray level that was empirically determined prior to analysis. The number of Fos-like immunoreactive (Fos-LI) neurons/section was calculated as the mean of the three sections examined for each rat. For analysis of the laminar distribution of Fos-LI neurons, the spinal cord gray matter was divided into three regions (laminae I-II, V, and X). Assessment of Fos-like immunoreactivity was conducted in a blind manner.

The number of Fos-LI neurons per region on both the ipsilateral and contralateral slides was counted in fixed area sizes (0.2 mm² for spinal cord laminae I/II, V or X), using a software 9.0 image analysis system (W. Rasband, National Institute of Health). Only rats showing catheter tip positioned at the dorsal spinal cord were considered for data analysis.

Drugs

Atropine sulfate, a non selective muscarinic cholinergic antagonist, phenoxybenzamine hydrochloride, a non selective α-receptor antagonist, methysergide, a 5-HT$_{1/2B/2C}$ antagonist [99], ketanserin, a 5-HT$_{2A}$ antagonist [100], methiothepin, a 5-HT$_{1/2A/2C/5/6/7}$ antagonist [101], and LY 278,584, a 5-HT$_3$ antagonist [102,103], were purchased from Sigma (St Louis, MO, USA) and diluted in sterile isotonic saline at the moment of the injection.

Experimental design

All animals were anesthetized and allocated to one of four experimental groups. A group for the overall control of the experiment had non-incised and non-catheterized anesthetized rats (group A), which were sacrificed 2 h after the beginning of anesthesia. A group for control of

the effects of intrathecal catheterization had non-incised rats treated intrathecally with saline (5 μl) and were sacrificed 2 h later (group AS). A group for control of the effects of the hind paw incision had rats treated intrathecally with saline and submitted to the surgical incision of the right hind paw performed 15-min later (group ASI). Test groups had rats submitted to paw incision carried out 3 h after intrathecal phenoxybenzamine or 15-min after methysergide, atropine, LY 278,584, ketanserin or methiothepin. Animals from group ASI and test groups were sacrificed 2 h after the incision.

Statistics

The mean (± SEM) of the number Fos-LI cells/0.2 mm² of 3 sections from the spinal cord of 4 - 8 animals per group was taken to allow comparisons among the different treatments. Comparisons of groups were made using one-way ANOVA followed by the Tukey's Multiple Comparison test. The level of significance was set at $P < 0.05$ in all cases.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

JWSS performed a large portion of the experiments and analyzed data; QMD performed a portion of experiments; EAD helped with the experimental design and analysis of Fos immunohistochemistry photographs; WAP supervised the study and helped with the writing of the manuscript. All authors read and approved the final manuscript.

Author Details

¹Department of Pharmacology, Faculty of Medicine of Ribeirão Preto, University of Sao Paulo, Ribeirão Preto, SP 14049-900 Brazil and ²Department of Morphology, Estomatology and Physiology, Faculty of Odontology of Ribeirão Preto, University of Sao Paulo, Ribeirão Preto, SP 14040-900 Brazil

References

1. Gebhart GF: **Descending modulation of pain.** *Neurosci Biobehav Rev* 2004, **27**:729-737.
2. Fields HL, Heinricher MM, Mason P: **Neurotransmitters in nociceptive modulatory circuits.** *Annu Rev Neurosci* 1991, **14**:219-245.
3. Kovelowski CJ, Ossipov MH, Sun H, Lai J, Malan TP, Porreca F: **Supraspinal cholecystokinin may drive tonic descending facilitation mechanisms to maintain neuropathic pain in the rat.** *Pain* 2000, **87**:265-273.
4. Pertovaara A, Wei H, Hamalainen MM: **Lidocaine in the rostroventromedial medulla and the periaqueductal gray attenuates allodynia in neuropathic rats.** *Neurosci Lett* 1996, **218**:127-130.
5. Vaccarino AL, Melzack R: **Temporal processes of formalin pain: differential role of the cingulum bundle, fornix pathway and medial bulboreticular formation.** *Pain* 1992, **49**:257-271.
6. Wiertelak EP, Roemer B, Maier SF, Watkins LR: **Comparison of the effects of nucleus tractus solitarius and ventral medial medulla lesions on illness-induced and subcutaneous formalin-induced hyperalgesias.** *Brain Res* 1997, **748**:143-150.
7. Pertovaara A: **A neuronal correlate of secondary hyperalgesia in the rat spinal dorsal horn is submodality selective and facilitated by supraspinal influence.** *Exp Neurol* 1998, **149**:193-202.
8. Zhuo M, Gebhart GF: **Biphasic modulation of spinal nociceptive transmission from the medullary raphe nuclei in the rat.** *J Neurophysiol* 1997, **78**:746-758.
9. Calejesan AA, Ch'ang MH, Zhuo M: **Spinal serotonergic receptors mediate facilitation of a nociceptive reflex by subcutaneous formalin injection into the hindpaw in rats.** *Brain Res* 1998, **798**:46-54.
10. Wei F, Dubner R, Ren K: **Nucleus reticularis gigantocellularis and nucleus raphe magnus in the brain stem exert opposite effects on behavioral hyperalgesia and spinal Fos protein expression after peripheral inflammation.** *Pain* 1999, **80**:127-141.
11. Millan MJ: **Descending control of pain.** *Prog Neurobiol* 2002, **66**:355-474.
12. Millan MJ, Girardon S, Bervoets K: **8-OH-DPAT-induced spontaneous tail-flicks in the rat are facilitated by the selective serotonin (5-HT)2C agonist, RO 60-0175: blockade of its actions by the novel 5-HT2C receptor antagonist SB 206,553.** *Neuropharmacology* 1997, **36**:743-745.
13. Stanfa LC, Dickenson AH: **Enhanced alpha-2 adrenergic controls and spinal morphine potency in inflammation.** *Neuroreport* 1994, **5**:469-472.
14. Eisenach JC: **Muscarinic-mediated analgesia.** *Life Sci* 1999, **64**:549-554.
15. Zahn PK, Brennan TJ: **Incision-induced changes in receptive field properties of rat dorsal horn neurons.** *Anesthesiology* 1999, **91**:772-785.
16. Villarreal CF, Del Bel EA, Prado WA: **Involvement of the anterior pretectal nucleus in the control of persistent pain: a behavioral and c-Fos expression study in the rat.** *Pain* 2003, **103**:163-174.
17. Hunt SP, Pini A, Evan G: **Induction of c-fos-like protein in spinal cord neurons following sensory stimulation.** *Nature* 1987, **328**:632-634.
18. Hughes P, Dragunow M: **Induction of immediate-early genes and the control of neurotransmitter-regulated gene expression within the nervous system.** *Pharmacol Rev* 1995, **47**:133-178.
19. Coggeshall RE: **Fos, nociception and the dorsal horn.** *Prog Neurobiol* 2005, **77**:299-352.
20. Sandkuhler J: **The organization and function of endogenous antinociceptive systems.** *Prog Neurobiol* 1996, **50**:49-81.
21. Sun X, Yokoyama M, Mizobuchi S, Kaku R, Nakatsuka H, Takahashi T, Morita K: **The effects of pretreatment with lidocaine or bupivacaine on the spatial and temporal expression of c-Fos protein in the spinal cord caused by plantar incision in the rat.** *Anesth Analg* 2004, **98**:1093-1098.
22. Tsuda M, Koizumi S, Inoue K: **Role of endogenous ATP at the incision area in a rat model of postoperative pain.** *Neuroreport* 2001, **12**:1701-1704.
23. Nicholas AP, Zhang X, Hokfelt T: **An immunohistochemical investigation of the opioid cell column in lamina X of the male rat lumbosacral spinal cord.** *Neurosci Lett* 1999, **270**:9-12.
24. Hamalainen MM, Gebhart GF, Brennan TJ: **Acute effect of an incision on mechanosensitive afferents in the plantar rat hindpaw.** *J Neurophysiol* 2002, **87**:712-720.
25. Leonard PA, Arunkumar R, Brennan TJ: **Bradykinin antagonists have no analgesic effect on incisional pain.** *Anesth Analg* 2004, **99**:1166-1172.
26. Pogatzki-Zahn EM, Shimizu I, Caterina M, Raja SN: **Heat hyperalgesia after incision requires TRPV1 and is distinct from pure inflammatory pain.** *Pain* 2005, **115**:296-307.
27. Wieseler-Frank J, Maier SF, Watkins LR: **Central proinflammatory cytokines and pain enhancement.** *Neurosignals* 2005, **14**:166-174.
28. Kina VA, Villarreal CF, Prado WA: **The effects of intraspinal L-NOARG or SIN-1 on the control by descending pathways of incisional pain in rats.** *Life Sci* 2005, **76**:1939-1951.
29. Villarreal CF, Kina VA, Prado WA: **Participation of brainstem nuclei in the pronociceptive effect of lesion or neural block of the anterior pretectal nucleus in a rat model of incisional pain.** *Neuropharmacology* 2004, **47**:117-127.
30. Villarreal CF, Prado WA: **Modulation of persistent nociceptive inputs in the anterior pretectal nucleus of the rat.** *Pain* 2007, **132**:42-52.
31. Stamford JA: **Descending control of pain.** *Br J Anaesth* 1995, **75**:217-227.
32. Lavand'homme PM, Eisenach JC: **Sex differences in cholinergic analgesia II: differing mechanisms in two models of allodynia.** *Anesthesiology* 1999, **91**:1455-1461.
33. Satoh M, Oku R, Akaike A: **Analgesia produced by microinjection of L-glutamate into the rostral ventromedial bulbar nuclei of the rat and its inhibition by intrathecal alpha-adrenergic blocking agents.** *Brain Res* 1983, **261**:361-364.

34. Iwamoto ET, Marion L: **Characterization of the antinociception produced by intrathecally administered muscarinic agonists in rats.** *J Pharmacol Exp Ther* 1993, **266:**329-338.

35. Li DP, Chen SR, Pan YZ, Levey AI, Pan HL: **Role of presynaptic muscarinic and GABA(B) receptors in spinal glutamate release and cholinergic analgesia in rats.** *J Physiol* 2002, **543:**807-818.

36. Zhuo M, Gebhart GF: **Tonic cholinergic inhibition of spinal mechanical transmission.** *Pain* 1991, **46:**211-222.

37. Hoyer D, Hannon JP, Martin GR: **Molecular, pharmacological and functional diversity of 5-HT receptors.** *Pharmacol Biochem Behav* 2002, **71:**533-554.

38. Hamon M, Gallissot MC, Menard F, Gozlan H, Bourgoin S, Verge D: **5-HT3 receptor binding sites are on capsaicin-sensitive fibres in the rat spinal cord.** *Eur J Pharmacol* 1989, **164:**315-322.

39. Thor KB, Nickolaus S, Helke CJ: **Autoradiographic localization of 5-hydroxytryptamine1A, 5-hydroxytryptamine1B and 5-hydroxytryptamine1C/2 binding sites in the rat spinal cord.** *Neuroscience* 1993, **55:**235-252.

40. Danzebrink RM, Gebhart GF: **Evidence that spinal 5-HT1, 5-HT2 and 5-HT3 receptor subtypes modulate responses to noxious colorectal distension in the rat.** *Brain Res* 1991, **538:**64-75.

41. Giordano J: **Analgesic profile of centrally administered 2-methylserotonin against acute pain in rats.** *Eur J Pharmacol* 1991, **199:**233-236.

42. Radhakrishnan R, King EW, Dickman JK, Herold CA, Johnston NF, Spurgin ML, Sluka KA: **Spinal 5-HT(2) and 5-HT(3) receptors mediate low, but not high, frequency TENS-induced antihyperalgesia in rats.** *Pain* 2003, **105:**205-213.

43. Zhuo M, Gebhart GF: **Spinal serotonin receptors mediate descending facilitation of a nociceptive reflex from the nuclei reticularis gigantocellularis and gigantocellularis pars alpha in the rat.** *Brain Res* 1991, **550:**35-48.

44. Jeong CY, Choi JI, Yoon MH: **Roles of serotonin receptor subtypes for the antinociception of 5-HT in the spinal cord of rats.** *Eur J Pharmacol* 2004, **502:**205-211.

45. Liu FY, Xing GG, Qu XX, Xu IS, Han JS, Wan Y: **Roles of 5-hydroxytryptamine (5-HT) receptor subtypes in the inhibitory effects of 5-HT on C-fiber responses of spinal wide dynamic range neurons in rats.** *J Pharmacol Exp Ther* 2007, **321:**1046-1053.

46. Urban MO, Gebhart GF: **Supraspinal contributions to hyperalgesia.** *Proc Natl Acad Sci USA* 1999, **96:**7687-7692.

47. Pogatzki EM, Urban MO, Brennan TJ, Gebhart GF: **Role of the rostral medial medulla in the development of primary and secondary hyperalgesia after incision in the rat.** *Anesthesiology* 2002, **96:**1153-1160.

48. Vanegas H, Schaible HG: **Descending control of persistent pain: inhibitory or facilitatory?** *Brain Res Brain Res Rev* 2004, **46:**295-309.

49. Wang Y, Feng C, Wu Z, Wu A, Yue Y: **Activity of the descending noradrenergic pathway after surgery in rats.** *Acta Anaesthesiol Scand* 2008, **52:**1336-1341.

50. Villarreal CF, Kina VA, Prado WA: **Antinociception induced by stimulating the anterior pretectal nucleus in two models of pain in rats.** *Clin Exp Pharmacol Physiol* 2004, **31:**608-613.

51. Hayashida K, DeGoes S, Curry R, Eisenach JC: **Gabapentin activates spinal noradrenergic activity in rats and humans and reduces hypersensitivity after surgery.** *Anesthesiology* 2007, **106:**557-562.

52. Basbaum AI, Fields HL: **Endogenous pain control mechanisms: review and hypothesis.** *Ann Neurol* 1978, **4:**451-462.

53. Eide PK, Hole K: **The role of 5-hydroxytryptamine (5-HT) receptor subtypes and plasticity in the 5-HT systems in the regulation of nociceptive sensitivity.** *Cephalalgia* 1993, **13:**75-85.

54. Millan MJ: **Endorphins and nociception: an overview.** *Methods Find Exp Clin Pharmacol* 1982, **4:**445-462.

55. Millan MJ: **The induction of pain: an integrative review.** *Prog Neurobiol* 1999, **57:**1-164.

56. Colpaert FC, Tarayre JP, Koek W, Pauwels PJ, Bardin L, Xu XJ, Wiesenfeld-Hallin Z, Cosi C, Carilla-Durand E, Assie MB, Vacher B: **Large-amplitude 5-HT1A receptor activation: a new mechanism of profound, central analgesia.** *Neuropharmacology* 2002, **43:**945-958.

57. Fasmer OB, Berge OG, Hole K: **Changes in nociception after lesions of descending serotonergic pathways induced with 5,6-dihydroxytryptamine. Different effects in the formalin and tail-flick tests.** *Neuropharmacology* 1985, **24:**729-734.

58. Millan MJ, Seguin L, Honore P, Girardon S, Bervoets K: **Pro- and antinociceptive actions of serotonin (5-HT)1A agonists and antagonists in rodents: relationship to algesiometric paradigm.** *Behav Brain Res* 1996, **73:**69-77.

59. Laporte AM, Doyen C, Nevo IT, Chauveau J, Hauw JJ, Hamon M: **Autoradiographic mapping of serotonin 5-HT1A, 5-HT1D, 5-HT2A and 5-HT3 receptors in the aged human spinal cord.** *J Chem Neuroanat* 1996, **11:**67-75.

60. Bonaventure P, Voorn P, Luyten WH, Jurzak M, Schotte A, Leysen JE: **Detailed mapping of serotonin 5-HT1B and 5-HT1D receptor messenger RNA and ligand binding sites in guinea-pig brain and trigeminal ganglion: clues for function.** *Neuroscience* 1998, **82:**469-484.

61. Waeber C, Moskowitz MA: **[3H]sumatriptan labels both 5-HT1D and 5-HT1F receptor binding sites in the guinea pig brain: an autoradiographic study.** *Naunyn Schmiedebergs Arch Pharmacol* 1995, **352:**263-275.

62. Oyama T, Ueda M, Kuraishi Y, Akaike A, Satoh M: **Dual effect of serotonin on formalin-induced nociception in the rat spinal cord.** *Neurosci Res* 1996, **25:**129-135.

63. Robles LI, Barrios M, Del Pozo E, Dordal A, Baeyens JM: **Effects of K+ channel blockers and openers on antinociception induced by agonists of 5-HT1A receptors.** *Eur J Pharmacol* 1996, **295:**181-188.

64. Yoshimura M, Furue H: **Mechanisms for the anti-nociceptive actions of the descending noradrenergic and serotonergic systems in the spinal cord.** *J Pharmacol Sci* 2006, **101:**107-117.

65. Ali Z, Wu G, Kozlov A, Barasi S: **The actions of 5-HT1 agonists and antagonists on nociceptive processing in the rat spinal cord: results from behavioural and electrophysiological studies.** *Brain Res* 1994, **661:**83-90.

66. Zhang Y, Yang Z, Gao X, Wu G: **The role of 5-hydroxytryptamine1A and 5-hydroxytryptamine1B receptors in modulating spinal nociceptive transmission in normal and carrageenan-injected rats.** *Pain* 2001, **92:**201-211.

67. Alhaider AA, Wilcox GL: **Differential roles of 5-hydroxytryptamine1A and 5-hydroxytryptamine1B receptor subtypes in modulating spinal nociceptive transmission in mice.** *J Pharmacol Exp Ther* 1993, **265:**378-385.

68. Xu W, Qiu XC, Han JS: **Serotonin receptor subtypes in spinal antinociception in the rat.** *J Pharmacol Exp Ther* 1994, **269:**1182-1189.

69. Cornea-Hebert V, Riad M, Wu C, Singh SK, Descarries L: **Cellular and subcellular distribution of the serotonin 5-HT2A receptor in the central nervous system of adult rat.** *J Comp Neurol* 1999, **409:**187-209.

70. Maeshima T, Ito R, Hamada S, Senzaki K, Hamaguchi-Hamada K, Shutoh F, Okado N: **The cellular localization of 5-HT2A receptors in the spinal cord and spinal ganglia of the adult rat.** *Brain Res* 1998, **797:**118-124.

71. Fonseca MI, Ni YG, Dunning DD, Miledi R: **Distribution of serotonin 2A, 2C and 3 receptor mRNA in spinal cord and medulla oblongata.** *Brain Res Mol Brain Res* 2001, **89:**11-19.

72. Wu S, Zhu M, Wang W, Wang Y, Li Y, Yew DT: **Changes of the expression of 5-HT receptor subtype mRNAs in rat dorsal root ganglion by complete Freund's adjuvant-induced inflammation.** *Neurosci Lett* 2001, **307:**183-186.

73. Kjorsvik A, Tjolsen A, Hole K: **Activation of spinal serotonin(2A/2C) receptors augments nociceptive responses in the rat.** *Brain Res* 2001, **910:**179-181.

74. Obata H, Saito S, Sasaki M, Ishizaki K, Goto F: **Antiallodynic effect of intrathecally administered 5-HT(2) agonists in rats with nerve ligation.** *Pain* 2001, **90:**173-179.

75. Sasaki M, Ishizaki K, Obata H, Goto F: **Effects of 5-HT2 and 5-HT3 receptors on the modulation of nociceptive transmission in rat spinal cord according to the formalin test.** *Eur J Pharmacol* 2001, **424:**45-52.

76. Cardenas CG, Del Mar LP, Scroggs RS: **Two parallel signaling pathways couple 5HT1A receptors to N- and L-type calcium channels in C-like rat dorsal root ganglion cells.** *J Neurophysiol* 1997, **77:**3284-3296.

77. Cardenas CG, Mar LP, Vysokanov AV, Arnold PB, Cardenas LM, Surmeier DJ, Scroggs RS: **Serotonergic modulation of hyperpolarization-activated current in acutely isolated rat dorsal root ganglion neurons.** *J Physiol* 1999, **518(Pt 2):**507-523.

78. Bervoets K, Millan MJ, Colpaert FC: **Agonist action at 5-HT1C receptors facilitates 5-HT1A receptor-mediated spontaneous tail-flicks in the rat.** *Eur J Pharmacol* 1990, **191:**185-195.

79. Bardin L, Schmidt J, Alloui A, Eschalier A: **Effect of intrathecal administration of serotonin in chronic pain models in rats.** *Eur J Pharmacol* 2000, **409**:37-43.

80. Laporte AM, Koscielniak T, Ponchant M, Verge D, Hamon M, Gozlan H: **Quantitative autoradiographic mapping of 5-HT3 receptors in the rat CNS using [125I]iodo-zacopride and [3H]zacopride as radioligands.** *Synapse* 1992, **10**:271-281.

81. Morales M, McCollum N, Kirkness EF: **5-HT(3)-receptor subunits A and B are co-expressed in neurons of the dorsal root ganglion.** *J Comp Neurol* 2001, **438**:163-172.

82. Ali Z, Wu G, Kozlov A, Barasi S: **The role of 5HT3 in nociceptive processing in the rat spinal cord: results from behavioural and electrophysiological studies.** *Neurosci Lett* 1996, **208**:203-207.

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

84. Suzuki R, Rahman W, Hunt SP, Dickenson AH: **Descending facilitatory control of mechanically evoked responses is enhanced in deep dorsal horn neurones following peripheral nerve injury.** *Brain Res* 2004, **1019**:68-76.

85. Green GM, Scarth J, Dickenson A: **An excitatory role for 5-HT in spinal inflammatory nociceptive transmission; state-dependent actions via dorsal horn 5-HT(3) receptors in the anaesthetized rat.** *Pain* 2000, **89**:81-88.

86. Li P, Kerchner GA, Sala C, Wei F, Huettner JE, Sheng M, Zhuo M: **AMPA receptor-PDZ interactions in facilitation of spinal sensory synapses.** *Nat Neurosci* 1999, **2**:972-977.

87. Li P, Zhuo M: **Silent glutamatergic synapses and nociception in mammalian spinal cord.** *Nature* 1998, **393**:695-698.

88. Fu KY, Light AR, Maixner W: **Long-lasting inflammation and long-term hyperalgesia after subcutaneous formalin injection into the rat hindpaw.** *J Pain* 2001, **2**:2-11.

89. Curtin LI, Grakowsky JA, Suarez M, Thompson AC, DiPirro JM, Martin LB, Kristal MB: **Evaluation of buprenorphine in a postoperative pain model in rats.** *Comp Med* 2009, **59**:60-71.

90. Pogatzki EM, Niemeier JS, Brennan TJ: **Persistent secondary hyperalgesia after gastrocnemius incision in the rat.** *Eur J Pain* 2002, **6**:295-305.

91. Zahn PK, Brennan TJ: **Primary and secondary hyperalgesia in a rat model for human postoperative pain.** *Anesthesiology* 1999, **90**:863-872.

92. Jones TL, Lustig AC, Sorkin LS: **Secondary hyperalgesia in the postoperative pain model is dependent on spinal calcium/calmodulin-dependent protein kinase II alpha activation.** *Anesth Analg* 2007, **105**:1650-1656.

93. Pogatzki EM, Niemeier JS, Sorkin LS, Brennan TJ: **Spinal glutamate receptor antagonists differentiate primary and secondary mechanical hyperalgesia caused by incision.** *Pain* 2003, **105**:97-107.

94. Pogatzki-Zahn EM, Niemeier JS, Sorkin LS, Brennan TJ: **[Spinal glutamate receptor antagonists differentiate primary and secondary mechanical hyperalgesia caused by incision].** *Schmerz* 2006, **20**:245-253.

95. Zimmermann M: **Ethical guidelines for investigations of experimental pain in conscious animals.** *Pain* 1983, **16**:109-110.

96. Prado WA: **Antinociceptive potency of intrathecal morphine in the rat tail flick test: a comparative study using acute lumbar catheter in rats with or without a chronic atlanto-occipital catheter.** *J Neurosci Methods* 2003, **129**:33-39.

97. Brennan TJ, Vandermeulen EP, Gebhart GF: **Characterization of a rat model of incisional pain.** *Pain* 1996, **64**:493-501.

98. Lino-de-Oliveira C, Sales AJ, Del Bel EA, Silveira MC, Guimaraes FS: **Effects of acute and chronic fluoxetine treatments on restraint stress-induced Fos expression.** *Brain Res Bull* 2001, **55**:747-754.

99. Silberstein SD: **Methysergide.** *Cephalalgia* 1998, **18**:421-435.

100. Glennon RA, Metwally K, Dukat M, Ismaiel AM, De los Angeles J, Herndon J, Teitler M, Khorana N: **Ketanserin and spiperone as templates for novel serotonin 5-HT(2A) antagonists.** *Curr Top Med Chem* 2002, **2**:539-558.

101. Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ, Saxena PR, Humphrey PP: **International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin).** *Pharmacol Rev* 1994, **46**:157-203.

102. Miller K, Weisberg E, Fletcher PW, Teitler M: **Membrane-bound and solubilized brain 5HT3 receptors: improved radioligand binding assays using bovine area postrema or rat cortex and the radioligands 3H-GR65630, 3H-BRL43694, and 3H-LY278584.** *Synapse* 1992, **11**:58-66.

103. Wong DT, Robertson DW, Reid LR: **Specific [3H]LY278584 binding to 5-HT3 recognition sites in rat cerebral cortex.** *Eur J Pharmacol* 1989, **166**:107-110.