showed that in the *twy/twy* mouse spinal cord, TUNEL-positive oligodendrocytes were immunoreactive to TNF-α, TNFR1, and TNFR2. These findings suggest that TNF-α, and TNFR1 as well as TNFR2 seem to play at least some roles in the demise of glial cells, which probably contribute to axonal degeneration and demyelination in the *twy/twy* mouse spinal cord with severe compression.

■ Key Points

- The number of TUNEL-positive cells increased with the magnitude of compression in the *twy/twy* mouse spinal cord with chronic mechanical compression, and the number of TUNEL-RIP double-positive cells in the white matter appeared to increase with severe cord compression.
- TNF-α, TNFR1 TNFR2, and active-caspase-3 were expressed in correlation with the magnitude of compression, while TNF-α was colocalized in OX-42, GFAP and RIP with severe compression.
- TNF-α, and TNFR1 as well as TNFR2 appear to play some roles in the apoptosis of oligodendrocytes, which contribute to axonal degeneration and demyelination in *twy/twy* mice with severe spinal cord compression.

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Antidepressants inhibit $P2X_4$ receptor function: a possible involvement in neuropathic pain relief

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Abstract

Background: Neuropathic pain is characterized by pain hypersensitivity to innocuous stimuli (tactile allodynia) that is nearly always resistant to known treatments such as non-steroidal anti-inflammatory drugs or even opioids. It has been reported that some antidepressants are effective for treating neuropathic pain. However, the underlying molecular mechanisms are not well understood. We have recently demonstrated that blocking $P2X_4$ receptors in the spinal cord reverses tactile allodynia after peripheral nerve injury in rats, implying that $P2X_4$ receptors are a key molecule in neuropathic pain. We investigated a possible role of antidepressants as inhibitors of $P2X_4$ receptors and analysed their analgesic mechanism using an animal model of neuropathic pain.

Results: Antidepressants strongly inhibited ATP-mediated Ca^{2+} responses in $P2X_4$ receptor-expressing 1321N1 cells, which are known to have no endogenous ATP receptors. Paroxetine exhibited the most powerful inhibition of calcium influx via rat and human $P2X_4$ receptors, with IC_{50} values of 2.45 μM and 1.87 μM, respectively. Intrathecal administration of paroxetine produced a striking antiallodynic effect in an animal model of neuropathic pain. Co-administration of WAY100635, ketanserin or ondansetron with paroxetine induced no significant change in the antiallodynic effect of paroxetine. Furthermore, the antiallodynic effect of paroxetine was observed even in rats that had received intrathecal pretreatment with 5,7-dihydroxytryptamine, which dramatically depletes spinal 5-hydroxytryptamine.

Conclusion: These results suggest that paroxetine acts as a potent analgesic in the spinal cord via a mechanism independent of its inhibitory effect on serotonin transporters. Powerful inhibition on $P2X_4$ receptors may underlie the analgesic effect of paroxetine, and it is possible that some antidepressants clinically used in patients with neuropathic pain show antiallodynic effects, at least in part via their inhibitory effects on $P2X_4$ receptors.

Background

Neuropathic pain is caused by lesions of the central or peripheral nervous system, mainly in patients with diabetes, post-herpetic neuralgia or cancer. Neuropathic pain is especially problematic because of its chronic, severe and intractable pain state, and is characterized by tactile allodynia, which drastically affects the quality of patients' lives. Although a number of patients suffer from neuropathic pain, its pathogenesis is not fully understood. It is widely known that neuropathic pain is nearly always resistant to general analgesics, such as non-steroidal anti-inflammatory drugs or even opioids, but some antidepressants and anticonvulsants have been successful in treating neuropathic pain.

Antidepressants have been used for over 30 years to manage several intractable pain states including chronic headache, low back pain, rheumatoid arthritis and fibromyalgia [1,2]. Accumulated evidence has proved their effectiveness for neuropathic pain states and antidepressants are now considered a mainstay of pharmacological treatment for neuropathic pain, as are anticonvulsants [1]. Tri-cyclic antidepressants (TCAs: amitriptyline, nortriptyline, imipramine, desipramine and clomipramine) have been shown to produce potent analgesic effects in patients with diabetic neuropathy [3-7] and postherpetic neuralgia [8-11]. TCAs achieve analgesic effects at lower doses and with shorter durations of drug exposure than those required to express antidepressive effects [2], indicating putative analgesic mechanisms independent of their antidepressive effect. Among the selective serotonin reuptake inhibitors (SSRIs), it has been shown that fluoxetine and citalopram are less active in treating diabetic neuropathy [12,13]. However, paroxetine (one of SSRIs) has been reported to be effective in patients with diabetic neuropathy [14].

It has been well known that antidepressants induce antidepressive effects via their inhibitory effects on 5-hydroxytryptamine (5-HT) and norepinephrine (NE) transporters in the central nervous system [15]. Monoaminergic neurons descending from the rostral ventral medulla to the spinal cord have been shown to modulate pain transmission, suggesting that inhibition of monoamine transporters may explain the analgesic effects of antidepressants. However, this hypothesis is not fully accepted because antidepressants show non-correlativity between their effectiveness in treating neuropathic pain and their potency of inhibition of monoamine transporters [2,15].

In addition to their inhibitory effects on monoamine transporters, antidepressants have been reported to affect multiple neurotransmitter receptors and ion channels implicated in pain transmission such as NMDA receptors [16,17] and opioid receptors [18]. Recently, it was noted that some antidepressants block several types of sodium channels and calcium channels in recombinant culture [19-22] and neuronal tissue [23]. Although many pharmacological actions of antidepressants have been described, the exact mechanism of action for treating neuropathic pain is not fully understood.

We have recently demonstrated that activating $P2X_4$ receptors in activated microglia plays a key role in the pathogenesis of neuropathic pain. Spinal nerve injury induced upregulation of $P2X_4$ receptors on activated microglia in the spinal cord and spinal blockade of $P2X_4$ receptors induced significant antiallodynic effects [24]. This report strongly suggests that inhibiting $P2X_4$ receptors may be a new therapeutic strategy for patients with neuropathic pain, and it is possible that inhibition of $P2X_4$ receptors may underlie the analgesic effects of the drugs used to treat patients with neuropathic pain. In the present study, we investigated a possible role of antidepressants as inhibitors of $P2X_4$ receptors and analysed their analgesic mechanism using an animal model of neuropathic pain.

Results

Antidepressants inhibit rat and human $P2X_4$ receptor function

To evaluate whether the antidepressants clinically used in patients with neuropathic pain have an influence on $P2X_4$ receptors, we used a real-time calcium imaging system to measure intracellular calcium levels in 1321N1 human astrocytoma cells stably expressing rat or human $P2X_4$ receptors. Native 1321 N1 cells, which are devoid of ATP receptors, showed no $[Ca^{2+}]_i$ response to ATP stimulation (data not shown). 1321N1 cells stably expressing rat $P2X_4$ receptors displayed a reproducible $[Ca^{2+}]_i$ response to ATP stimulation (30 μM, 20 sec) (Figure 1a). The ATP-evoked $[Ca^{2+}]_i$ response disappeared when extracellular calcium was eliminated with EGTA (500 μM) (Figure 1b). Pretreatment of cells with paroxetine (10 μM, 10 min) strongly inhibited the ATP-evoked $[Ca^{2+}]_i$ response via rat $P2X_4$ receptors (Figure 1c). Some antidepressants and anticonvulsants (10 μM, 10 min) showed inhibitory effects on the ATP-evoked $[Ca^{2+}]_i$ response via rat $P2X_4$ receptors (Figure 1d). Paroxetine dose-dependently inhibited the ATP-evoked $[Ca^{2+}]_i$ response via rat $P2X_4$ receptors with an IC_{50} value of 2.45 μM for 30 μM ATP stimulation (Figure 1e). Using 1321N1 cells stably expressing human $P2X_4$ receptors, we next determined whether the antidepressants modulate human $P2X_4$ receptors. Pretreatment of cells with paroxetine, fluoxetine, maprotiline or clomipramine, which potently inhibit rat $P2X_4$ receptors, strongly inhibited the ATP-evoked $[Ca^{2+}]_i$ response via human $P2X_4$ receptors (Figure 2a). Paroxetine dose-dependently inhibited the ATP-evoked $[Ca^{2+}]_i$ response via human $P2X_4$ receptors with an IC_{50} value of 1.87 μM

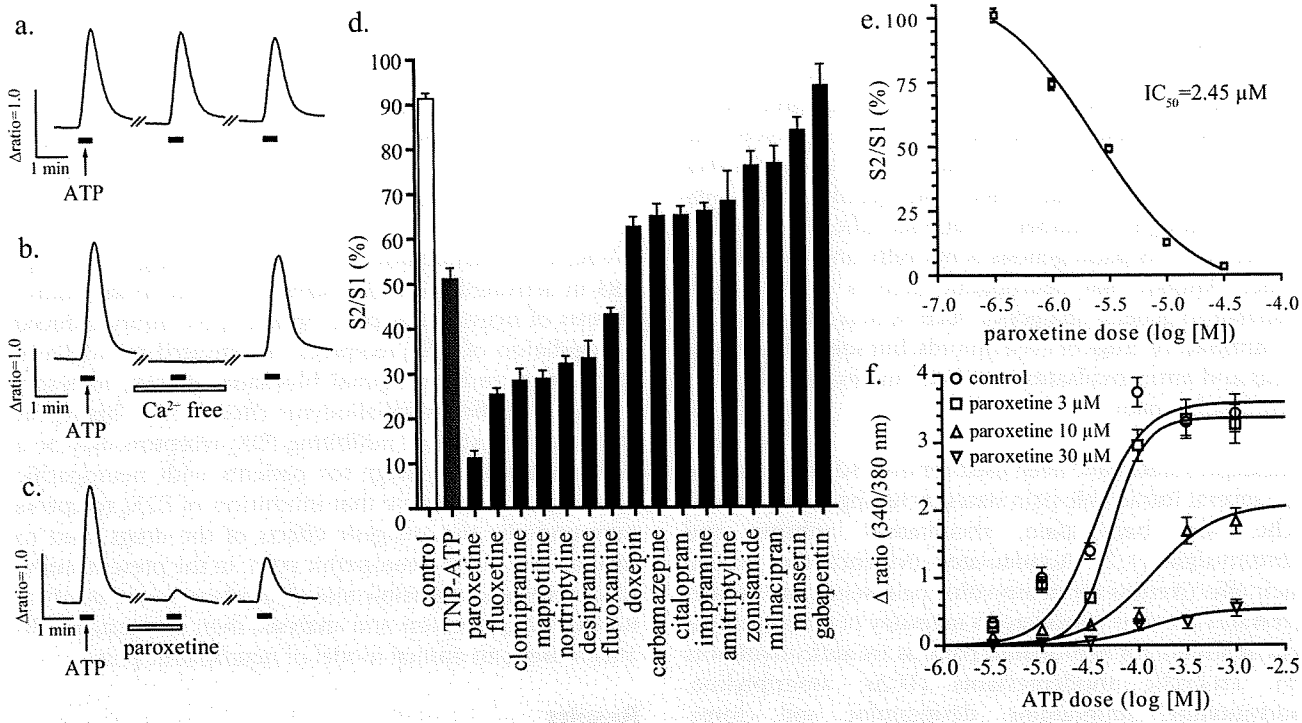

Figure 1
Effect of antidepressants on the ATP-evoked $[Ca^{2+}]_i$ response via rat $P2X_4$ receptors. Three rounds of ATP stimulation (30 μM, 20 sec) induced $[Ca^{2+}]_i$ response in a reproducible fashion (a). Effect of pretreatment of cells with EGTA (500 μM, 10 min) (b) or with paroxetine (10 μM, 10 min) (c) on $[Ca^{2+}]_i$ response evoked by the second ATP stimulation. Traces indicate 340/380 fura-2 emission ratios averaged from (a) 48 cells, (b) 66 cells and (c) 18 cells obtained from each representative experiment. Effect of pretreatment of cells with TNP-ATP, antidepressants and anticonvulsants (10 μM, 10 min) on the ATP-evoked $[Ca^{2+}]_i$ response via rat $P2X_4$ receptors (d). Paroxetine dose-dependently inhibited the $[Ca^{2+}]_i$ response via rat $P2X_4$ receptors with an IC_{50} value of 2.45 μM for 30 μM ATP stimulation (e). ATP dose-response curve was generated in the presence of increasing concentrations of paroxetine (f). Data are means ± SEM of at least 10 cells (d, e and f).

for 30 μM ATP stimulation (Figure 2b). Both in rat and human $P2X_4$ receptor-expressed 1321N1 cells, paroxetine inhibited the maximum response of ATP-evoked $[Ca^{2+}]_i$ increase (Figure 1f and 2c). To elucidate whether paroxetine directly affect recombinant rat $P2X_4$ receptors on 1321N1 cells and native $P2X_4$ receptors on microglia, the electrophysiological experiments were performed. Pretreatment of cells with paroxetine (10 μM, 10 min) significantly inhibited ATP-evoked currents in rat $P2X_4$ receptor-expressed 1321N1 cells (Figure 3a and 3b) and primary micloglia (Figure 3c and 3d).

Paroxetine produces a significant antiallodynic effect in an animal model of neuropathic pain

Next, we investigated whether paroxetine, which showed the strongest inhibitory effect on rat and human $P2X_4$ receptors, has antiallodynic effect, because we have shown that inhibiting $P2X_4$ receptors reversed tactile allodynia in neuropathic rats [24]. A unilateral L5 spinal nerve injury resulted in a marked decrease in the paw withdrawal threshold (PWT) from 15.0 g of pressure (n = 24) before the injury to 3.7 ± 0.2 g (n = 24) at day 7 (Figure 4a) and 3.1 ± 0.8 g (n = 24) at day 14 (Figure 4b) after nerve injury. Intrathecal administration of paroxetine resulted in significant increase in the PWT at doses of 3 nmol (**$p < 0.01$ and *$p < 0.05$; Figure 4a) or 10 nmol (#$p < 0.05$; Figure 4a) at day 7 after nerve injury. After the intrathecal adminis-

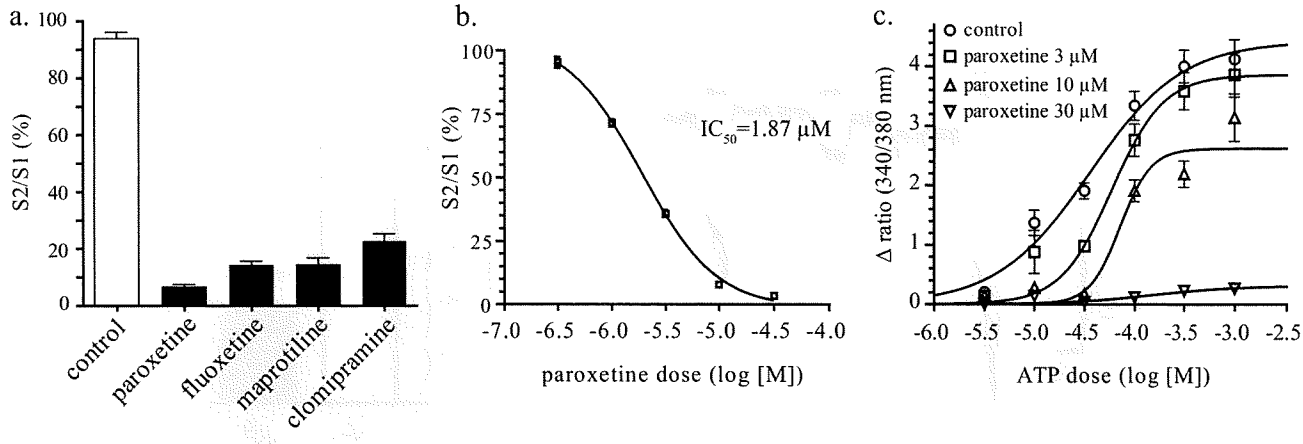

Figure 2
Effect of antidepressants on ATP-evoked $[Ca^{2+}]_i$ response via human $P2X_4$ receptors. Effect of pretreatment of cells with antidepressants (10 μM, 10 min) on the ATP-evoked $[Ca^{2+}]_i$ response via human $P2X_4$ receptors (a). Paroxetine dose-dependently inhibited the ATP-evoked $[Ca^{2+}]_i$ response via human $P2X_4$ receptors with an IC_{50} value of 1.87 μM for 30 μM ATP stimulation (b). ATP dose-response curve was generated in the presence of increasing concentrations of paroxetine (c). Data are means ± SEM of at least 3 cells (a, b and c).

tration of 3 nmol paroxetine, the PWT increased gradually, peaking at about 150 min after the injection, and then returned to the pre-injection level. Intrathecal administration of 3 nmol paroxetine also resulted in a significant increase in the PWT at day 14 after nerve injury ($*p < 0.05$; Figure 4b).

Fluvoxamine but not citalopram produces an antiallodynic effect in an animal model of neuropathic pain

In order to evaluate whether the potencies of inhibition on $P2X_4$ receptors are correlated to the antiallodynic effect, we next investigated other SSRIs, fluvoxamine and citalopram, which have similar pharmacological properties as with paroxetine. Intrathecal administration of 10 nmol fluvoxamine resulted in a moderate increase in the PWT ($**p < 0.01$ and $*p < 0.05$; Figure 5a) at day 7 after nerve injury. Intrathecal administration of 10 nmol citalopram had no effect on the PWT at day 7 after nerve injury ($p > 0.05$; Figure 5b).

Co-administration of 5-HT receptor antagonists did not reverse the antiallodynic effect of paroxetine

We next investigated whether 5-HT upregulation induced by inhibition of 5-HT transporters is involved in the antiallodynic effect of paroxetine using antagonists for three types of 5-HT receptors ($5\text{-}HT_{1A}$, $5\text{-}HT_{2A}$ and $5\text{-}HT_3$ receptors), because their abundant expression in the spinal cord and behavioural studies showing pro- or antinociceptive effects have been reported [25-27]. The $5\text{-}HT_{1A}$ receptor antagonist WAY100635, the $5\text{-}HT_{2A}$ receptor antagonist ketanserin or the $5\text{-}HT_3$ receptor antagonist ondansetron were intrathecally co-administered with paroxetine. No significant change in PWT was observed following co-administration of 100 nmol WAY100635, 30 nmol ketanserin or 30 nmol ondansetron with 3 nmol paroxetine compared with 3 nmol paroxetine alone ($p > 0.05$; Figure 6).

Spinal 5-HT deprivation did not reverse the antiallodynic effect of paroxetine

To further elucidate the interactions between spinal 5-HT system and the antiallodynic effect of paroxetine, we next examined whether pretreatment of cells with 5,7-dihydroxytryptamine (5,7-DHT), which depletes 5-HT in the spinal cord, would affect the antiallodynic effect of paroxetine. Immunohistochemistry revealed that 5-HT immunoreactivity was dramatically reduced throughout the dorsal horn of the spinal cord nine days after 5,7-DHT treatment compared with the saline-treated group (Figure 7a). Double immuno-labelling for $P2X_4$ receptors and OX42, a marker for microglia, showed that L5 spinal nerve injury induced upregulation of $P2X_4$ receptors on hyperactive microglia at the same level in the 5,7-DHT-treated group as in the saline-treated group, seven days after nerve injury (data not shown). 5,7-DHT-treated rats developed tactile allodynia in the same way as saline-treated rats after nerve injury (Figure 7b). No significant change in the antiallodynic effect of paroxetine was observed in 5,7-DHT-treated rats compared with saline-treated rats ($p > 0.05$; Figure 7b).

Discussion

We investigated a possible role of antidepressants as analgesics for neuropathic pain based on their inhibitory

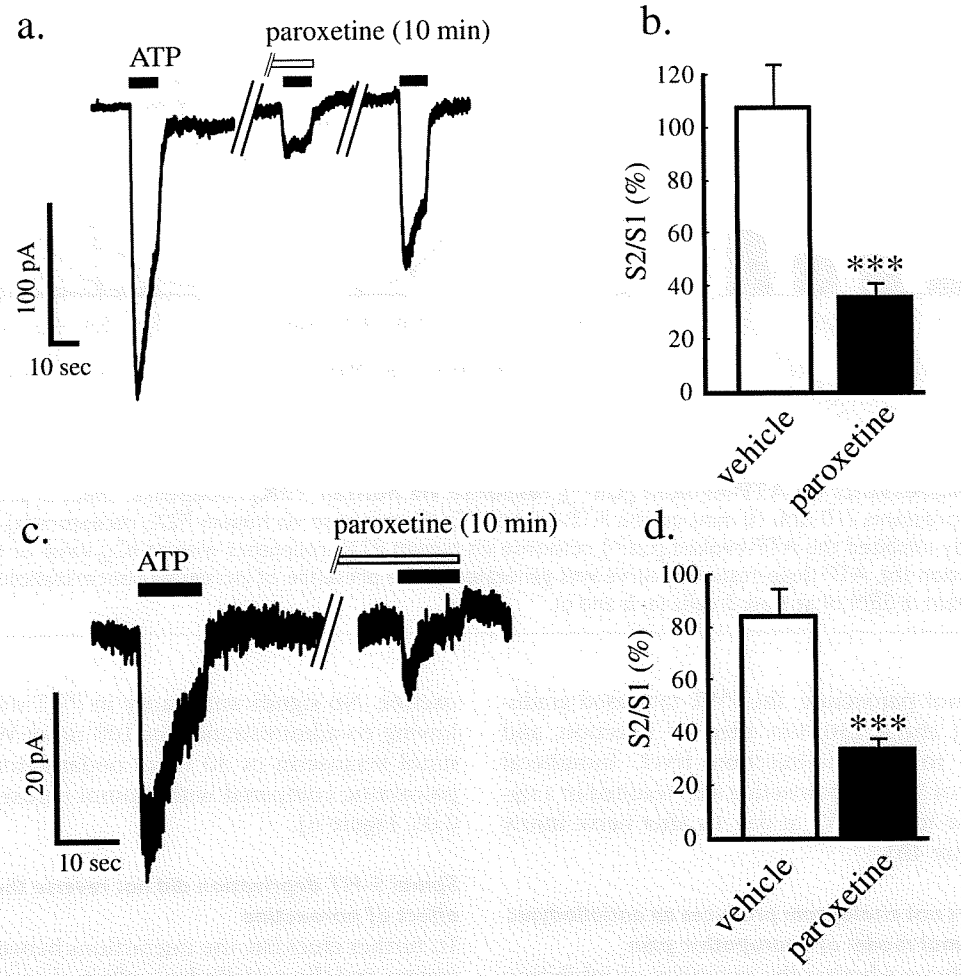

Figure 3
Effect of antidepressants on ATP-evoked currents in 1321N1 cells expressing rat $P2X_4$ receptors and primary microglia. Paroxetine (10 μM, 10 min) inhibited ATP (30 μM, 10 sec) evoked currents in rat $P2X_4$ receptor-expressed 1321N1 cells (a and b) and primary micloglia (c and d) (***$p < 0.001$; paroxetine vs. vehicle, unpaired t-test). Data are means ± SEM of more than three separate experiments.

effects on $P2X_4$ receptors. The cDNAs for rat or human $P2X_4$ receptors were transfected individually into 1321N1 human astrocytoma cells, which are known to be devoid of endogenous ATP receptor activity and widely used for the analysis of recombinant ATP receptors [28,29]. A lack of responsiveness to ATP stimulation under the calcium-free condition indicates that the ATP-evoked $[Ca^{2+}]_i$ increase in 1321N1 cells was induced by calcium influx from extracellular fluid via $P2X_4$ receptors. Recombinant rat or human $P2X_4$ receptors expressed in 1321N1 cells showed pharmacological properties similar to those previously described [30,31]. TNP-ATP (10 μM, 10 min), a well known non-selective blocker of rat $P2X_4$ receptors, exhibited the same degree of inhibition on ATP-evoked $[Ca^{2+}]_i$ response via rat $P2X_4$ receptors as previously reported (IC_{50}; 15 μM) [32]. Thus the assay system used here is considered to be appropriate for the screening of $P2X_4$ receptor blockers.

For the first time, we found that antidepressants inhibit rat and human $P2X_4$ receptor function. Among the drugs used here, paroxetine showed the strongest inhibition of rat and human $P2X_4$ receptors, with IC_{50} values of 2.45 μM and 1.87 μM respectively.

In rat and human $P2X_4$-expressed 1321N1 cells, the maximum response of ATP-evoked $[Ca^{2+}]_i$ increase was markedly suppressed by paroxetine, suggesting that paroxetine inhibits rat and human $P2X_4$ receptors in a non-competitive manner. Using an electrophysiological technique, we

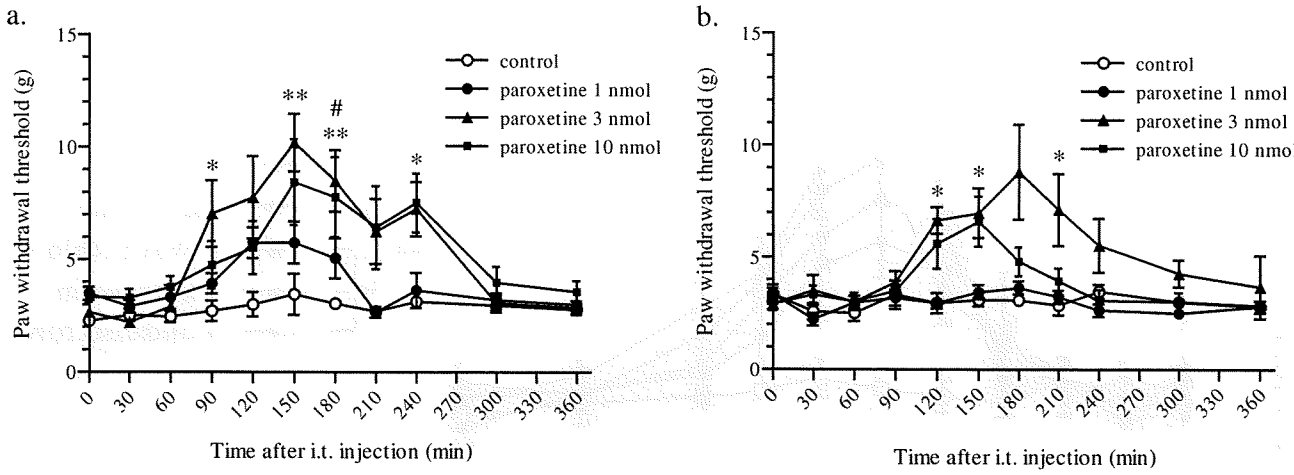

Figure 4
Effect of intrathecal administration of paroxetine on the decrease in the PWT after nerve injury. A significant antiallodynic effect was observed following intrathecal administration of paroxetine at doses of 3 nmol and 10 nmol at day 7 (a), and 3 nmol at day 14 (b) after nerve injury (**$p < 0.01$ and *$p < 0.05$; paroxetine 3 nmol vs. control, #$p < 0.05$; paroxetine 10 nmol vs. control by a Dunn's multiple comparison test after a Kruskal-Wallis test). Data are means ± SEM of 5–8 rats.

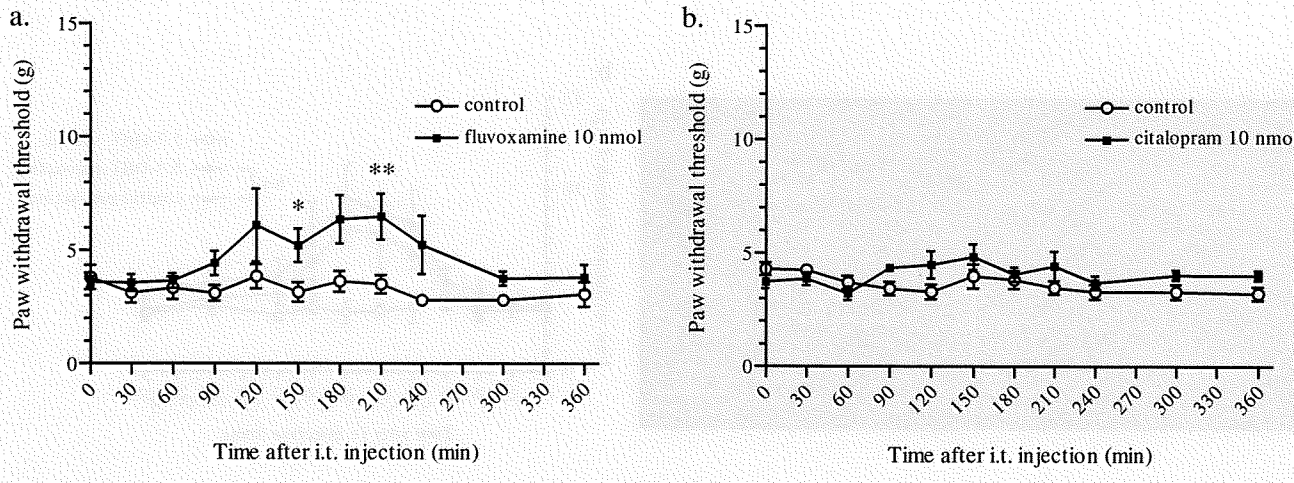

Figure 5
Effect of intrathecal administration of fluvoxamine and citalopram on the decrease in the PWT after nerve injury. A significant antiallodynic effect was observed following intrathecal administration of 10 nmol fluvoxamine at day 7 after nerve injury (a). (**$p < 0.01$ and *$p < 0.05$; fluvoxamine 10 nmol vs. control by a Mann-Whitney U-test.) No significant change was observed following administration of 10 nmol citalopram (b). ($p > 0.05$ by a Mann-Whitney U-test.) Data are means ± SEM of 5–10 rats.

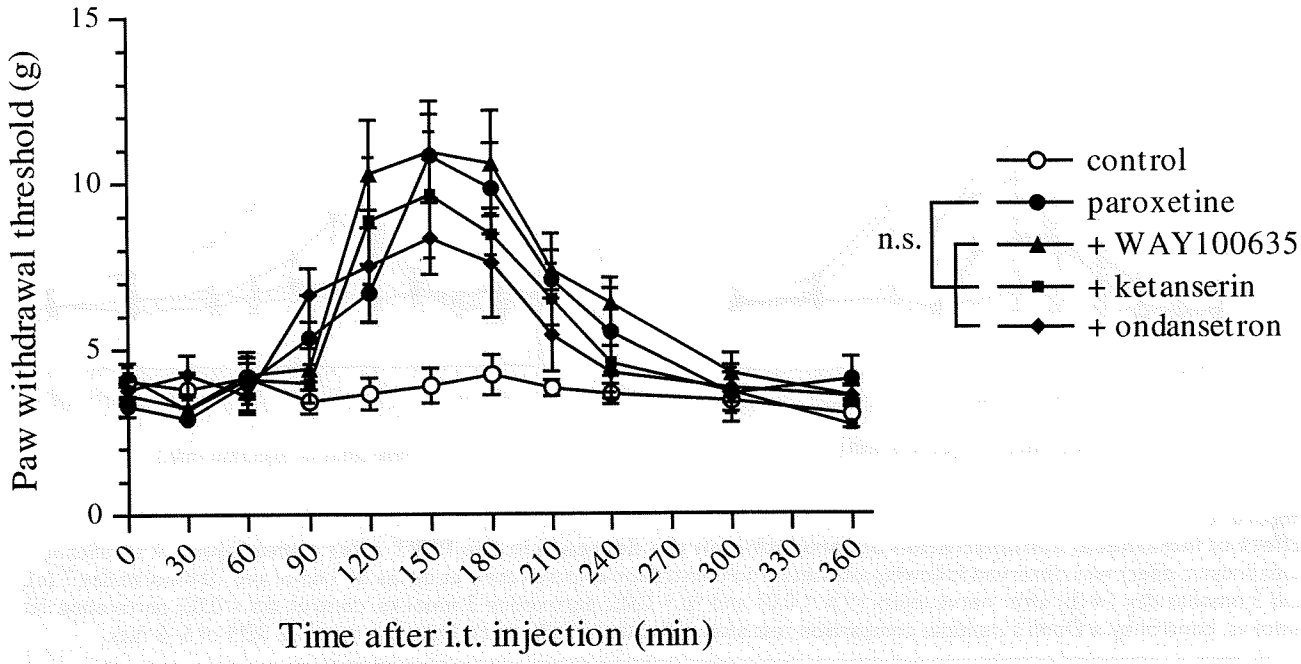

Figure 6
Effect of intrathecal co-administration of 5-HT receptor blockers with paroxetine on the decrease in the PWT after nerve injury. No significant change was observed by intrathecal co-administration of 100 nmol WAY100635, 30 nmol ketanserin or 30 nmol ondansetron with 3 nmol paroxetine compared with 3 nmol paroxetine alone. ($p > 0.05$ by a Dunn's multiple comparison test after a Friedman test.) Data are means ± SEM of 5–6 rats.

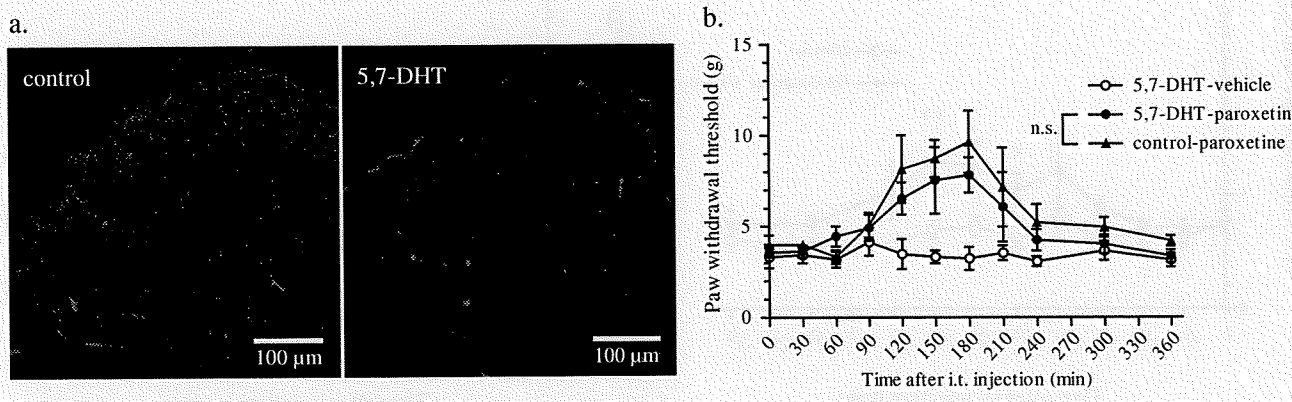

Figure 7
Effect of spinal 5-HT depletion on the antiallodynic effect of paroxetine. 5-HT immunoreactivity in the dorsal horn of the spinal cord nine days after intrathecal injection of either saline or 5,7-dihydroxytryptamine (a). A marked reduction in the number of 5-HT immunoreactive fibres was observed in the dorsal horn of the L5 spinal cord after 5,7-DHT treatment compared with the saline-treated group. No significant change in the antiallodynic effect of paroxetine was observed in the 5,7-DHT-treated group compared with the saline-treated group (b). ($p > 0.05$ by a Dunn's multiple comparison test after a Friedman test.) Data are means ± SEM of 5–6 rats.

found that similar to the results in calcium imaging, the pretreatment of cells with paroxetine strongly inhibited the ATP-induced currents on rat $P2X_4$ receptor-expressed 1321N1 cells. Therefore, it is proposed that paroxetine directly inhibits $P2X_4$ receptors. Furthermore, paroxetine strongly inhibited the ATP-induced currents on primary cultured microglial cells. We have previously shown that an exposure of such concentration of ATP to primary microglia selectively activates $P2X_4$ receptors [33]. These findings indicate that paroxetine inhibits native $P2X_4$ receptors expressed in microglia.

In general, typical serum concentrations of antidepressants range from about 100 to 1000 nM [15]. Antidepressants tend to accumulate in tissues because of their lipophilic nature [34], so in the central nervous system they may reach the effective range for inhibition of $P2X_4$ receptors observed in this experiment. Antidepressants modulate many kinds of ion channels at a wide range of concentrations (0.1 to 1000 μM) *in vitro* [20,35,36], but only the effects observed near the serum concentration are considered to have an influence *in vivo*. Sometimes, the analgesic effect of antidepressants is explained by their inhibitory effects on voltage-dependent sodium channels and calcium channels, that are observed at relatively low concentrations (0.1 to 10 μM) *in vitro* [19]. For example, paroxetine showed inhibitory effects on hNav1.3 (effective range; >2 μM) [21] and hNav1.7 (Ki = 1.45 μM) [20], at concentrations very close to that needed to affect $P2X_4$ receptors in this experiment. These findings indicate that antidepressants may have some influence on spinal $P2X_4$ receptors in patients with neuropathic pain.

Intrathecal administration of paroxetine showed a potent antiallodynic effect at 7 days and 14 days after nerve injury. We have previously shown that intrathecal administration of TNP-ATP induces significant antiallodynic effects at higher doses (10 or 30 nmol) [24] than paroxetine, indicating that there is a correlation between the dose needed to express the antiallodynic effect *in vivo* and the potency of inhibition of $P2X_4$ receptors *in vitro*. The antiallodynic effect of paroxetine was greater at day 7 than day 14 after nerve injury, which is a common feature with the antiallodynic action of intrathecally administered TNP-ATP. In several time points both at day 7 and day 14, paroxetine was more effective at 3 nmol than 10 nmol. It has been reported that paroxetine increases $[Ca^{2+}]_i$ level at high concentrations greater than 50 μM and induces apoptosis in MG63 cells [37]. The weak antiallodynic effect of paroxetine 10 nmol may be due to its cell toxicity.

We also found that fluvoxamine produced a much weaker antiallodynic effect than paroxetine, and citalopram produced no antiallodynic effect, although these SSRIs (paroxetine, fluvoxamine and citalopram) have similar inhibitory action on 5-HT transporters. Citalopram has been reported to be less effective than paroxetine in patients of diabetic neuropathy [14,38]. Interestingly, we found that citalopram (10 μM, 10 min) had no effect on ATP-evoked $[Ca^{2+}]_i$ response mediated by human $P2X_4$ receptors (additional file 1: Effect of citalopram on ATP-evoked $[Ca^{2+}]_i$ response via human $P2X_4$ receptors). These results indicate that the difference in the potency of inhibition on $P2X_4$ receptors may explain the difference in the clinical effectiveness of antidepressants in patients of neuropathy.

It has been well known that microglia express $P2X_7$ receptors as well as $P2X_4$ receptors [39]. We observed that paroxetine (10 μM, 10 min) inhibited BzATP (100 μM, 20 sec) induced $[Ca^{2+}]_i$ response of $P2X_7$ receptor-expressed 1321N1 cells (additional file 2: Effect of paroxetine on BzATP-evoked $[Ca^{2+}]_i$ response via rat $P2X_7$ receptors). Therefore, it is conceivable that intrathecally administered paroxetine may also inhibit $P2X_7$ receptors in the spinal cord. However, we have previously shown that PPADS, a non-selective antagonist for P2X receptors including $P2X_7$, has no effect on mechanical allodynia in neuropathic pain model [24]. Therefore, these results suggest that subtypes of P2X receptors sensitive to PPADS are not involved in the antiallodynic effect of paroxetine under our experimental conditions.

It has been widely accepted that serotonergic neurons descending from the rostral ventral medulla into the spinal cord participate in endogenous antinociceptive mechanisms. Activation of this descending inhibitory pathway or intrathecal administration of 5-HT induced analgesia in several behavioural tests [40-42]. The main pharmacological action of paroxetine is an inhibition of 5-HT transporters, which induces upregulation of 5-HT [15]. Thus we next investigated whether the spinal 5-HT system is involved in the antiallodynic effect of paroxetine. We focused on three subtypes of 5-HT receptors ($5\text{-}HT_{1A}$, $5\text{-}HT_{2A}$ and $5\text{-}HT_3$ receptors) because of their abundant expression in the spinal cord [25-27] and behavioural studies showing pro- or antinociceptive effects induced by intrathecal administration of selective drugs for them. In neuropathic rats, systemic administration of F 13640, a $5\text{-}HT_{1A}$ receptor agonist, attenuated tactile allodynia [43] and intrathecal administration of α-methyl-5-HT maleate, a $5\text{-}HT_{2A}$ receptor agonist, attenuated thermal hyperalgesia, which was abolished by pretreatment with ketanserin [44]. In the spinal cord injury model, a pro-nociceptive effect has been observed following intrathecal administration of m-chlorophenylbiguanide, a $5\text{-}HT_3$ receptor agonist [45]. These reports indicate pain modulation by the spinal 5-HT system in neuropathic rats, but we observed no significant change in the antiallodynic effect of parox-

etine following co-administration with 5-HT receptor blockers.

In neuropathic rats, it has been reported that spinal administration of 5-HT produced only a weak analgesia and needed a 100- to 1000-fold higher dose than that required to achieve the antinociceptive effect in normal rats [40,46]. This indicates some physiological changes in the serotonergic system in the spinal cord of neuropathic rats, leading to less analgesia induced by spinal 5-HT administration. In this report, the 5-HT upregulation induced by spinal-administered paroxetine may not be involved in the antiallodynic effect in the same way as in the previous report.

It has been well established that intrathecal administration of 5,7-DHT depletes spinal 5-HT content [45,47], and we observed a significant reduction of spinal 5-HT immunoreactivity at day 9 after intrathecal administration of 5,7-DHT. Spinal 5-HT depletion induced no significant changes in the degrees of tactile allodynia and immunoreactivity for OX42 and $P2X_4$ receptors at day 7 after nerve injury. No significant change in the antiallodynic effect of paroxetine in 5,7-DHT-treated rats supports a putative antiallodynic mechanism independent of the spinal 5-HT system.

Conclusion

In this study, we found that some antidepressants and anticonvulsants clinically used in patients with neuropathic pain have inhibitory effects on rat and human $P2X_4$ receptor function. Among the drugs used, paroxetine showed the strongest inhibition on rat and human $P2X_4$ receptor function. Intrathecal administration of paroxetine and fluvoxamine, but not citalopram, resulted in an antiallodynic effect in an animal model of neuropathic pain, which correlates the potency of inhibition of rat $P2X_4$ receptors. Co-administration of 5-HT receptor antagonists (WAY100635, ketanserin or ondansetron) and spinal 5-HT depletion did not reverse the antiallodynic effect of paroxetine, which indicates an antiallodynic mechanism independent of the spinal 5-HT system. Powerful inhibition of $P2X_4$ receptors may be responsible for the analgesic effect of paroxetine and it is possible that some antidepressants clinically used in patients with neuropathic pain produce antiallodynic effects mediated at least in part via their inhibitory effect on $P2X_4$ receptors.

Methods

Culturing 1321N1 cells

The cDNAs encoding rat and human $P2X_4$ receptors [provided by Prof. Susumu Seino (Kobe University Graduate School of Medicine, Hyogo) and Prof. Joji Ando (The University of Tokyo, Japan), respectively] incorporated into pcDNA3.1+ (Clontech Laboratories, Inc., Mountain View, CA) [48] were introduced into 1321N1 human astrocytoma cells (a gift from Dr. Michael W. Salter, University of Toronto, Toronto, Canada) using FuGENE6 transfection reagent (Roche Applied Sciences, Basel, Switzerland). 1321N1 cells stably expressing $P2X_4$ receptors were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C and split 1/6 every three days. For the measurement of $[Ca^{2+}]_i$, the cells were plated onto poly-L-lysine-coated glass coverslips, placed in silicon rubber walls (Flexiperm, Greiner Bio-One GmbH, Frickenhausen, Germany) and maintained for about 48 hr.

Culturing primary microglia

Primary cultured microglia were prepared according to the method described previously [24]. In brief, the mixed glial culture was prepared from brain of neonatal Wistar rats (Kyudo, Saga, Japan) and maintained for 9–15 days in DMEM with 10% fetal bovine serum. Microglia were obtained as floating cells over the mixed glial culture. The floating cells were collected by gentle shaking and transferred to culture dishes and then the microglia were cultured for 1–6 h and used for whole-cell patch clamp. The cultures were of >99% purity, determining by immunostaining for OX-42 and Iba1 [33].

Measurement of $[Ca^{2+}]_i$ in single cells

$[Ca^{2+}]_i$ in single cells was monitored by a fura-2 ratio imaging system. The cells were incubated with 2.5 μM fura-2AM (Wako Pure Chemical Industries, Ltd., Osaka, Japan) for 45 min in a balanced salt solution (BSS; composition in mM: NaCl 150, KCl 5, $CaCl_2$ 1.8, $MgCl_2$ 1.2, D-glucose 10 and HEPES 25; pH 7.4) at room temperature. Then, the cells were washed with BSS and mounted on an inverted fluorescence microscope (ECLIPSE TE2000-U: Nikon, Tokyo, Japan) equipped with a Xenon-lamp (Xe75W; Nikon) and band-pass filters of 340 nm and 380 nm. The emission fluorescence was measured at 510 nm. Image data were detected with Aquacosmos (Hamamatsu Photonics, Hamamatsu, Japan), and $[Ca^{2+}]_i$ was expressed as the ratio of the fluorescence intensities at 340 nm and 380 nm. Applying 30 μM ATP for 20 sec to the 1321N1 human astrocytoma cells expressing rat or human $P2X_4$ receptors, a first $[Ca^{2+}]_i$ response (S1) was measured. Drugs were added to the cells for 10 min, and $[Ca^{2+}]_i$ response (S2) was measured by a second ATP application. Inhibitory effects of the drugs were evaluated by the S2/S1 ratio. After washing out the drugs by BSS, we confirmed recovery of $[Ca^{2+}]_i$ response by a third ATP stimulation.

Whole-cell patch clamp

Whole-cell currents were recorded at a holding potential of – 60 mV with Patch clamp L/M-EPC7 (List Medical-

Electronic). The cells were placed in a recording chamber and continuously superfused at room temperature (22 – 24°C) in an extracellular solution composed of the following: 140 mM NaCl; 5 mM KCl; 2.5 mM $CaCl_2$; 1 mM $MgCl_2$; 10 mM HEPES; and 10 mM D-glucose, and the pH was adjusted to 7.4 with NaOH. Patch pipettes were filled with buffer containing: 130 mM KCl; 1 mM $CaCl_2$; 2 mM $MgCl_2$; 10 mM HEPES and 10 mM EGTA, and the pH was adjusted to 7.2 with CsOH. All experimental parameters were controlled using Clampex software (version 9, Molecular Devices) and analyzed with Clampfit (version 9, Molecular Devices). All solutions were applied using custom made Y-tube apparatus. Applying 30 μM ATP for 10 sec to the cells, a first response (S1) was measured. Drugs were added to the cells for 10 min, and a second response (S2) was measured by an ATP application. Inhibitory effects of the drugs were evaluated by the S2/S1 ratio. After washing out the drugs by external solution, we confirmed recovery of response by a third ATP stimulation.

Animals

Male Wistar rats weighing 250–270 g were used in this study. Rats were housed at a temperature of 22 ± 1°C with a 12-h light/dark cycle (light on 8:30 to 20:30) and were fed food and water ad libitum. All of the animals used in the present study were treated in accordance with the guidelines of Kyushu University.

Neuropathic pain model

We used the spinal nerve injury model [49] with some modifications. A unilateral L5 spinal nerve of rats was tightly ligated and cut just distal to the ligature under isoflurane (2.5%) anesthesia. To assess tactile allodynia, calibrated von Frey filaments (0.4–15.1 g, Stoelting Co., Wood Dale, IL) were applied to the plantar surface of the hindpaw from below the mesh floor. The 50% paw withdrawal threshold was determined by the up-down method [50,51]. Drugs were intrathecally administered to rats 7 days or 14 days after nerve injury and tactile allodynia was measured for 6 hr.

Intrathecal drug administration

Surgery to place an indwelling catheter was conducted about 5–7 days before spinal nerve ligation. Under isoflurane (2.5%) anesthesia, rats were implanted with catheters for intrathecal injection according to a method described previously [52]. A polyethylene tube was inserted through the atlanto-occipital membrane to the lumbar enlargement (close to the L4-L5 segments) and externalized through the skin. Rats were injected intrathecally with drugs using a 25-μl Hamilton syringe with 28-gauge needle.

5,7-DHT administration

Rats were pretreated with desipramine hydrochloride (20 mg/kg, dissolved in 5% DMSO in saline, i.p., Sigma-Aldrich, Saint Louis, MO) to prevent uptake of the 5,7-DHT into noradrenergic neurons. After 45 min, rats received intrathecal injection of either saline or 5,7-DHT (100 μg, dissolved in 1% ascorbic acid in saline, Sigma-Aldrich) in a volume of 20 μl followed by 10 μl saline flush. This dose of 5,7-DHT has been reported to be sufficient to deplete endogenous spinal 5-HT [45,47]. Spinal nerve ligation was conducted 2 days after 5,7-DHT administration.

Immunohistochemistry

Seven days after spinal nerve ligation, vehicle or 5,7-DHT treated rats were deeply anesthetized with pentobarbital (100 mg/kg, i.p.) and perfused transcardially with 100 ml of phosphate-buffered saline (PBS, composition in mM: NaCl 137, KCl 2.7, KH_2PO_4 1.5, NaH_2PO_4 8.1; pH 7.4), followed by 250 ml of ice-cold 4% paraformaldehyde. The fifth lumbar (L5) segments of the spinal cord sections was removed and postfixed at 4°C for 5 hr and then transferred to 30% sucrose/PBS for 24 hr. Transverse L5 spinal cord sections (30 μm) were incubated for 2 hr at room temperature in a blocking solution (3% normal goat serum) and then incubated for 48 hr at 4°C with rat anti-serotonin monoclonal antibody (1:100, Millipore Corporation, Billerica, MA), mouse anti-OX42 antibody (1:1000, Chemicon, Temecula, CA) or rabbit anti-$P2X_4$ receptor antibody (1:1000, Alomone Labs, Jerusalem, Israel). Following incubation, tissue sections were washed and incubated for 3 hr at room temperature in the secondary antibody solution (goat anti-rat IgG-conjugated Alexa Fluor 546, goat anti-mouse IgG-conjugated Alexa Fluor 546 or goat anti-rabbit IgG-conjugated Alexa Fluor 488, 1:1000, Molecular Probes, Eugene, OR). The spinal cord sections were analysed using an LSM confocal imaging system (Carl Zeiss Japan, Tokyo, Japan).

Drugs

For *in vitro* experiments, adenosine 5'-triphosphate disodium salt (ATP), amitriptyline hydrochloride, citalopram hydrochloride, clomipramine, desipramine hydrochloride, doxepin hydrochloride, fluvoxamine maleate, imipramine hydrochloride, maprotiline, mianserin hydrochloride, milnacipran hydrochloride, nortriptyline hydrochloride, zonisamide sodium salt and TNP-ATP were purchased from Sigma-Aldrich and dissolved in BSS. Gabapentin (Toronto research chemicals Inc., North York, Ontario, Canada) and fluoxetine HCl (Biomol, Philadelphia, PA) were dissolved in BSS. Carbamazepine (Sigma-Aldrich) and paroxetine hydrochloride (Toronto Research Chemicals Inc.) were dissolved in 0.1% dimethyl sulfoxide (DMSO) in BSS. For *in vivo* experiments, paroxetine, fluvoxamine and citalopram were dissolved in 5% DMSO

in PBS. WAY100635 (Sigma-Aldrich), ketanserin (Sigma-Aldrich) and ondansetron (Sigma-Aldrich) were dissolved in 5% DMSO in saline for co-administration with paroxetine.

Statistical analysis

Differences between groups were analyzed using an unpaired t-test, a Friedman test with a Dunn's multiple comparison post-hoc test, a Kruskal-Wallis test with a Dunn's multiple comparison post hoc-test or a Mann-Whitney U-test. A p value < 0.05 was considered to be statistically significant.

Abbreviations

TCAs: tri-cyclic antidepressants; SSRIs: selective serotonin reuptake inhibitors; 5-HT: 5-hydroxytryptamine; NE: norepinephrine; 5,7-DHT: 5,7-dihydroxytryptamine; BSS: balanced salt solution; PBS: phosphate-buffered saline; ATP: adenosine 5'-triphosphate disodium salt; DMSO: dimethyl sulfoxide.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

KN, TI, MT and KI were responsible for experimental design. KN and TY participated in calcium imaging. HT participated in electrophysiology. KN and TI participated in animal surgery and behavioural experiments. KN participated in immunohistochemical experiments. KN, TI, MT and KI participated in manuscript writing. All authors read and approved the final manuscript.

Additional material

Additional File 1

Effect of citalopram on ATP-evoked $[Ca^{2+}]_i$ response via human $P2X_4$ receptors. Effect of pretreatment of cells with citalopram (10 μM, 10 min) on the ATP-evoked $[Ca^{2+}]_i$ response via human $P2X_4$ receptors. Citalopram has no effect on the ATP-evoked $[Ca^{2+}]_i$ response via human $P2X_4$ receptors. Data are means ± SEM of 164–181 cells.

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Additional File 2

*Effect of paroxetine on BzATP-evoked $[Ca^{2+}]_i$ response via rat $P2X_7$ receptors. Paroxetine (10 μM, 10 min) significantly inhibited the BzATP (100 μM, 20 sec) induced $[Ca^{2+}]_i$ response in rat $P2X_7$-expressed 1321N1 cells (***p < 0.001 by unpaired t-test). Data are means ± SEM of 95–113 cells.*

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慢性痛の痛覚情報認知機構

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要 旨

痛みは末梢神経から中枢神経である脊髄，脳へと神経伝達されて脳内で認知する．慢性痛の状態においてはこれらの神経系に機能的変化が引き起こされ，通常とは異なる経路や働きで脳に伝達される．末梢神経系では組織の慢性的な炎症などの結果としての有痛性瘢痕などが関与し，脊髄では脊髄視床路細胞の感作や可塑的変化が引き起こされ，これらの結果として脳内では情動系や前頭前野などの活動亢進がみられることとなる．このような神経系の機能変化はしばしば難治性であり，その病態を理解して慢性痛の治療に当たることが重要と考えられる．

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キーワード：慢性痛，神経可塑性，脳機能イメージング

はじめに

痛みとは，“組織の実質的あるいは潜在的な傷害に結びつくか，このような傷害を表す言葉を使って述べられる不快な感覚，情動体験である”と定義されている[1]．一方で慢性痛は，“急性疾患の通常の経過あるいは創傷の治癒に要する妥当な時間を超えて持続する痛み”と定義されている．このことについて過去の研究をみていくと，急性痛と慢性痛を鑑別する際に，しばしば傷害部の治癒が完全に得られていなければ急性痛の繰り返し，完全に治癒していれば慢性痛であるということがしばしば言及されている．一方で，実際の臨床の現場においては，完全な傷害部の治癒ということを前提として急性，慢性と鑑別することは実際上不可能なことがほとんどであり，両者は多くの場合混在しているものと考えられる．

そこで本稿では，脳内の痛覚情報認知機構に多大な影響を及ぼすと考えられる前述のような痛みの慢性化状態において引き起こされている末梢組織（末梢神経系）や脊髄レベルの神経の機能的変化に言及した上で，その結果としてわれわれが経験する痛みが脳内でどのように反映されているかについて述べたい．

〈Special Article〉 Pain and brain function
Cortical and peripheral neural networks in chronic pain condition
Takahiro Ushida, et al
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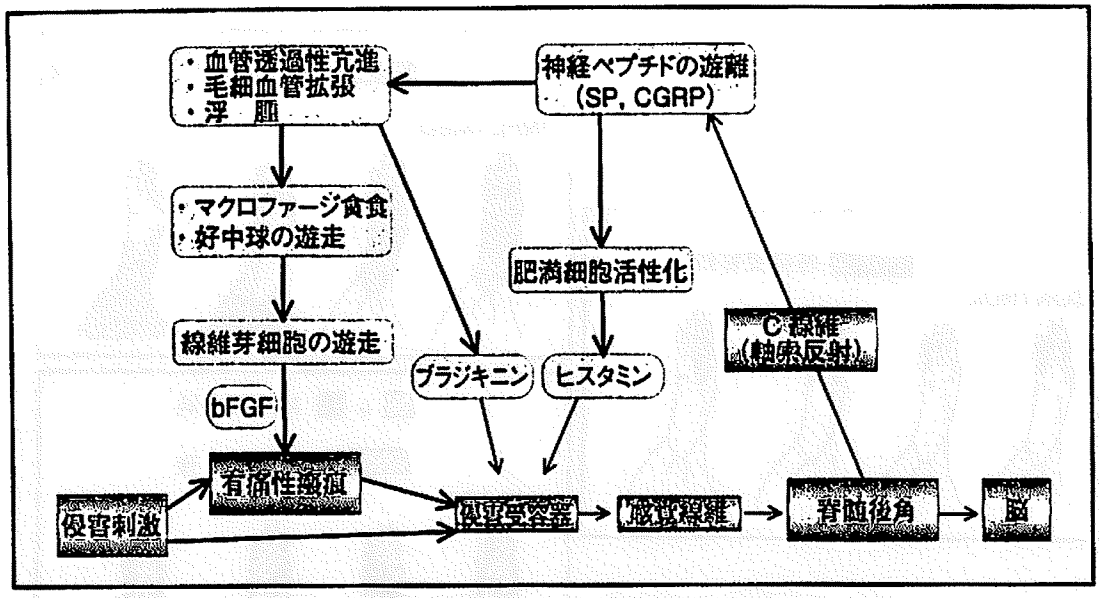

図1　神経原性炎症による痛みの発生と瘢痕形成

1．脳内痛覚情報認知に影響を及ぼす末梢・脊髄の神経の機能的変化

1）末梢組織における慢性的な痛みの機序

痛みの慢性化の最初のきっかけは外傷などに伴う急性痛であることが多く，慢性痛となっても末梢組織に引き起こされた病態は少なからず最終的に脳で経験する痛みに関与している．通常，末梢組織が傷害されると，サイトカインや神経ペプチド（サブスタンスP（SP）やカルシトニン遺伝子関連ペプチド（CGRP）など）などの活性化により傷害部は腫脹し，組織は炎症状態に陥り，時には肉芽の形成が引き起こされる．その後，炎症状態からの回復に伴って傷害組織は線維芽細胞などの活性化により線維化し，瘢痕化してくる．瘢痕組織が痛みの発生・維持に関わっていることは，瘢痕組織内における痛みに関与する神経ペプチドやサイトカイン，あるいは痛みを伝達する知覚神経線維の発現に関する以下の報告からも推測される．Henderson ら[2]は，傷と瘢痕において神経が再支配する際にSPやCGRPが増加していることを指摘し，それが感覚症状に関与するのではないかということを示している．また，Parkhouse ら[3,4]は，SPおよびCGRP陽性線維がhypertrophicな有痛性瘢痕組織の上皮に見つかっており，SPレベルを減少させることが瘢痕組織の増殖を抑えるであろうと述べている．また，活性度の高いケロイドでヒスタミン，セロトニンが高値を示す傾向があること[5,6]や瘢痕由来の線維芽細胞中にTGF-βなどのサイトカインの産生が増加していることが知られており[7]，末梢神経系の変容が痛覚情報伝達に様々な影響を与えていることが示唆される（図1）．代表的な難治性慢性腰痛症であるfailed back syndrome（FBS）は，脊柱管内の神経圧迫病変を取り除いたにもかかわらず慢性的に腰下肢痛が遷延する病態である．これらの痛みの原因としては，i）脊柱管内の病態（脊柱管内神経圧迫病変の残存，神経根癒着，癒着性くも膜炎），ii）関節障害などによる脊柱不安定要因とそれに併発する関節痛，iii）脊柱管外要素の関与，があるが，いずれの発症機序にも少なからず前述のような瘢痕組織内に存在する末梢神経系の機能異常が関与していると考えられる．したがって，

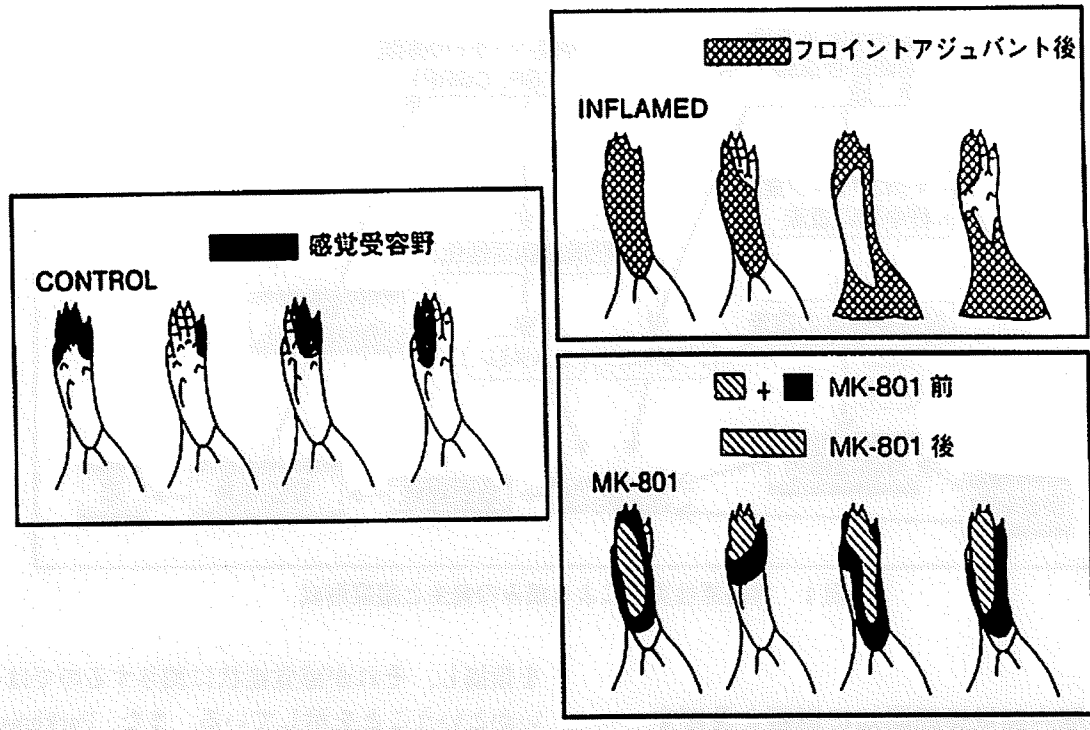

図2　単一脊髄後角細胞の感覚受容野の大きさの変化（文献8より引用改変）

今後は有痛性瘢痕などの組織（末梢神経系に起因する痛み）をどのようにして作らないようにするか，できてしまった場合にどのような工夫をして対処していくかについては大きな課題になると考えられる．

2）脊髄機能変化と痛みの慢性化

脊髄においては痛み伝達を担っている脊髄視床路細胞が持続的な侵害インパルスの持続により感作や可塑的変化を引き起こすことが知られている．脊髄視床路細胞に電極を刺入するなどして，その性格的変化を電気生理学的に調査してみると，正常状態では反応しなかったような弱い非侵害刺激にも応答を示すようになるほか，当初は反応しなかった感覚領域にも反応を示すようになる[8,9]（受容野の拡大）（図2）．これらの変化はおおよそ分単位で起こり，その変化の原因の一つとしてグルタミン酸受容体の一つであるNMDA受容体の活性化が大きな役割を担っていることが，これまでの研究で明らかにされてきている[10]．Grubbら[11]は，このような脊髄の感作が関節痛においても引き起こされていることを示している．臨床研究において，川田ら[12]は，股関節全置換術を行われた患者の術前の痛みの分布を調査した結果，ほとんどの症例において，股関節に限局しない大腿から下腿にまで広く分布する痛みに苛まれて手術に至っており，これらの患者に脊髄の機能的変化が引き起こされていることを示唆している（図3）．

また，神経障害性疼痛でしばしばみられるアロディニアは，非侵害刺激が痛みを引き起こす病態である．そのメカニズムとしてWoolfら[13]が提唱した脊髄内でのneural sproutingによる説や，Tsudaら[14]による脊髄や一次求心性線維に発現するATP受容体などの変化が関与している説があるが，いずれにしても触覚のような通常痛みを引き起こさない刺激が痛みを引き起こす病態であり，脊髄高位における神経伝達の機能変化が，脳で経験する痛みやその慢性化に大きな影響を及ぼしていることになる．

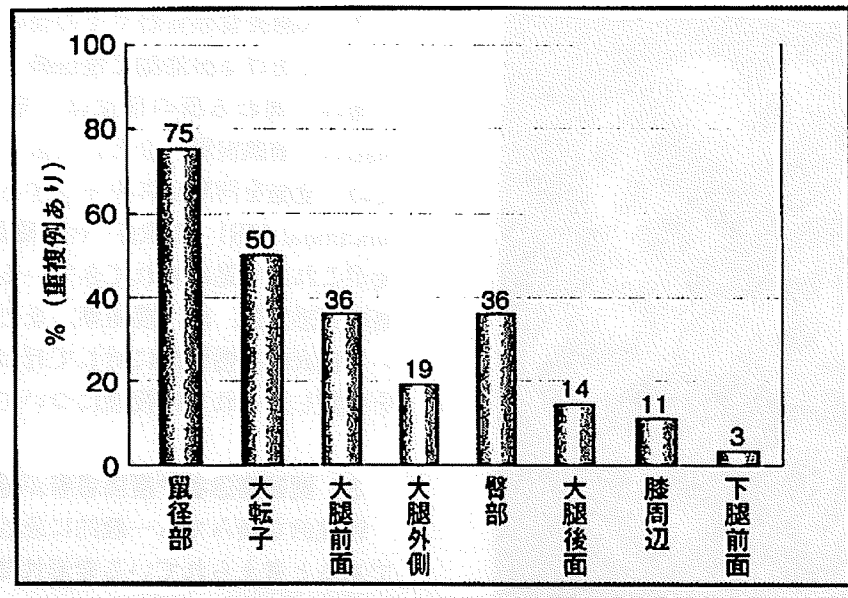

図3　股関節全置換術施行患者の術前の痛みの分布

2．慢性痛とそれに関与する脳活動部位

健常者では，主として脊髄視床路を上行してきた痛覚のシグナルは視床を介して第1次・第2次体性感覚野（SⅠ，SⅡ），島皮質，前帯状回，前頭前野内側部などのpain matrixと呼ばれる痛みに関連する大脳皮質部位に投射されることとなる．しかし，慢性痛においては，ここまで述べてきたように，神経系の感作や可塑的変化の結果，必ずしも同様の経路で痛覚としてのシグナルは伝達されて脳に伝導されてこないことに加えて，脳内では記憶や情動，自律神経系の影響など様々な要素が絡み合って痛み経験をしていくことから，脳活動にも大きな影響が生じることとなる．そこで今回は，これまで慢性疼痛疾患に対してこれまで行われてきた脳機能イメージング法の結果を中心に検証し，脳内痛覚認知機構について言及する．

1）視床の脳活動と慢性痛

視床は感覚の中枢と呼ばれ，痛覚の伝達においては先に述べた脊髄視床路が終末する部分でもあることから，古くから痛み研究のターゲットとして注目されている部分であり，動物実験などによって部位別の詳細な分析が行われてきている．視床には多くの核が存在するが，痛みの伝達系においては，外側脊髄視床路（＝新脊髄視床路）が終末している腹側基底核群と，前脊髄視床路（旧脊髄視床路）が終末している髄板内核群（主として外側中心核と束旁核）が重要な役割を果たしていることが知られている．前者は大脳皮質に主に投射する中継点であり，皮膚，内臓，筋，関節からの（識別性の）感覚に関与している．一方，後者は大脳辺縁系に投射し，痛みの情動等に関与するとされている．慢性痛における視床の役割については早期から注目されており，脳機能イメージング法として早くから用いられてきたsingle photon emission tomography（SPECT）やpositron emission tomography（PET）を用いた研究が行われてきている．

急性痛において視床の活動が引き起こされることが知られている一方で，慢性痛においては，疼痛状態において主に刺激伝達が引き起こされる対側の視床では活動がむしろ低下していると

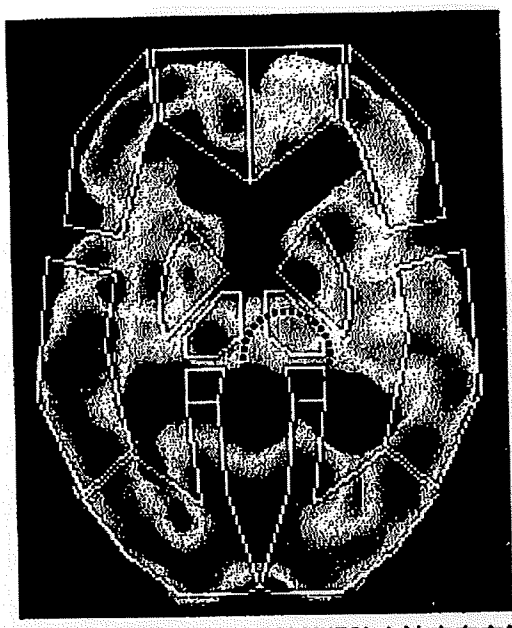

図4　右上肢に生じた慢性神経障害性疼痛症例（病歴3年）における視床血流の低下

いうことが散見されている[15,16]（図4）．このような対側視床の活動低下は，病期が長くなるにしたがって低下していることもわかってきており，中枢神経系の可塑的変化が起こっていることを示すものと考えられる[17]．このような変化の原因についての詳細は不明であるが，i）持続的な慢性的な痛みが抑制系を活性化し，視床の機能を抑制している，あるいはii）皮質間感覚野が慢性的な視床からの入力によって持続的に易興奮性の状態に陥っており，わずかな視床からの痛み信号の入力に対しても痛みを認識する状態になっている，iii）視床におけるシナプス伝達が非常に効率化されたため血流亢進を要さない，iv）神経の興奮性とは無関係に慢性の病的な頭蓋内血流動態自体の変化が引き起こされたことで，神経活動性と血流量の平衡関係が破綻した状態が引き起こされている，等が考えられるが，慢性的な痛みでも対側視床の活動亢進がみられる報告も散見され，今後更なる研究が必要と思われる．

2）大脳皮質感覚野やその他の脳部位における脳活動と慢性痛

痛みに関わる脳の部位は，先に述べたpain matrix（前頭前野，など）に広く広がっているため，全脳を同時にスキャンすることができるfunctional MRI（fMRI）や脳磁図を使った研究がこれまで進められてきている．以下では大脳皮質感覚野，島，前頭葉，などの各部位について代表的な慢性痛に対して行われた研究結果を検証し，それらの役割について触れる．

3）関節痛患者や腰痛患者が経験する脳活動

慢性的な痛みでも一般的に急性痛の繰り返しであると考えられている変形性膝関節症などの関節痛患者においては歩行時痛や局所的な圧痛を認める一方で自発痛を認めることは少ない．これらの患者の痛みは限局していることから，タスクとして圧痛部位を特殊なデバイスで刺激する方法が試みられている．その結果，変形性膝関節症の患者群では，圧痛点を圧迫刺激すると痛みの出現と視床を含む前述のpain matrixに有意な神経活動が観察されることがfMRI研究で明らかにされてきている[18]．

一方で慢性腰痛患者においては，自発痛の強さをパラメータとして用いることで自発痛（腰痛）と関連する脳の部位を調査する研究が行われている[18]．この結果では，前頭前野や前帯状回といった記憶や情動に関与すると考えられている部分に脳活動が観察されている（図5）．

このように，膝関節痛患者に痛刺激を加えた際の脳活動部位と慢性腰痛の自発痛に呼応して活動した脳部位は異なるパターンを生じた．この背景にはターゲットとしているものが動作時痛，自発痛と異なっていることも関与していることが考えられる．また，これらのことを考える際には，痛みが持つ特性，例えば，実際に患者が歩行している時に関節に生ずる痛みと今回の圧痛点の圧迫で生ずる痛みのメカニズムが脳内で異なっている可能性が高いことや，慢性腰

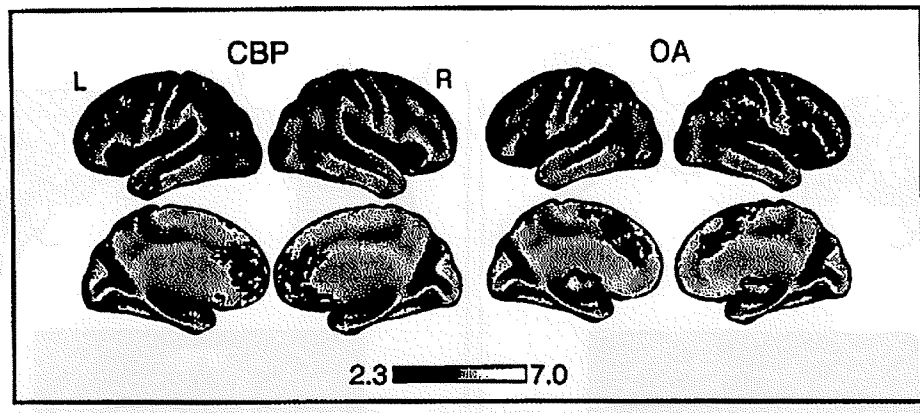

図5　腰痛患者（CBP）が自発痛を経験した際に活動した脳部位と変形性膝関節症（OA）の疼痛部位に刺激を加えた際に出現した脳活動部位（文献18より引用改変）
CBPが前頭前野や前帯状回を中心とした部位の活動を示したのに対して，OAでは視床も含めたpain matrixに広く活動を認めた

痛では，事故，仕事，収入などの社会的背景の影響がより強く反映されると考えられることについても考慮していかないといけない．

4）神経障害性疼痛患者に痛み刺激を加えた際の脳活動

アロディニアの痛みにおける脳活動を調べる目的で，Ikemotoら[19]は，患者の疼痛部位を通常では痛みを起こさない程度の機械刺激（非侵害刺激）を加えた時の脳活動をfMRIを用いて捉え，これを健常者に機械的侵害刺激および機械的非侵害刺激を加えた時の場合と比較する研究を行った．その結果，健常者に機械的侵害刺激を加えると，視床，S1，S2，帯状回，小脳における活動性の亢進が検出された．一方で患者群においては，VAS（visual analogue scale）において，健常者群よりも強い痛みが観察されたにもかかわらず，末梢からの痛みの主な中枢である視床の活動性は検出されず，S1，S2，帯状回（主として前帯状回）および運動野，補足運動野の活動が出現することを報告している．Peyronら[20]は，アロディニアを持つ患者の健側および患側に，通常では痛みを感じない程度の機械的刺激を与えた際の脳活動についての研究を行った結果，Ikemotoらと類似の結論に至っている．

またUshidaら[21]は，神経障害性疼痛患者における痛みの情動的側面を調べるため，視覚刺激のみを用いて実験を行った．手にアロディニアを有する神経障害性疼痛患者に対して手掌が筆で触られているバーチャル動画を見せたところ，前帯状回と内側前頭前野の活動が健常者群に比べて亢進していることがわかっている．前帯状回は健常者が「痛そうな画像」を見たときに賦活することが既に示されており[22]，したがって，手掌が筆で触られている動画は神経障害性疼痛患者に「痛そうな」情動を無意識のうちに惹き起こした可能性がある（**図6**）．また，内側前頭前野における痛みの認知的側面と情動的側面との統合に何らかの支障をきたしている可能性が示唆される．

5）慢性疼痛患者における機能的・解剖学的変化

慢性痛は，その特徴から情動的な要素が患者のquality of lifeへ大きく影響を及ぼし，脳活動パターンの変化を引き起こしているが，同時に解剖学的変化所見として，慢性疼痛病態下で