Antinociceptive Effects of Mirtazapine, Pregabalin, and Gabapentin After Chronic Constriction Injury of the Infraorbital Nerve in Rats

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Aims: To clarify the antiallodynic effects of the α2-adrenergic receptor antagonist mirtazapine compared with those of gabapentin and pregabalin in a rat model of orofacial neuropathic pain. **Methods:** Mirtazapine (10, 30, and 100 μg), gabapentin (10, 30, and 100 μg), and pregabalin (3, 10, and 30 μg) were administered intrathecally to eight male Sprague-Dawley rats with orofacial neuropathic pain induced by chronic constriction injury of the infraorbital nerve that had been carried out 2 weeks previously. Stimulation using von Frey filaments (1.0 to 15.0 g) applied to skin innervated by the injured infraorbital nerve enabled the measurement of mechanical thresholds 0 to 180 minutes after drug injection. Time-course data for the dose-response effects were analyzed using two-way analysis of variance and the post-hoc Tukey-Kramer multiple-comparison test. **Results:** Intrathecal administration of not only gabapentin and pregabalin but also mirtazapine reversed the lowered mechanical nociceptive thresholds produced by the nerve injury. The ED_{50} (95% confidence interval) was (in μg) 49.00 (39.71–58.29) for mirtazapine, 54.84 (46.12–63.56) for gabapentin, and 13.47 (11.24–15.69) for pregabalin. **Conclusion:** Intraspinal administration of either mirtazapine, gabapentin, or pregabalin reverses the lowered facial mechanical thresholds produced in a rat model of trigeminal neuropathic pain. *J Oral Facial Pain Headache 2014;28:61–67. doi: 10.11607/jop.1105*

Key words: *allodynia, gabapentin, mirtazapine, orofacial pain, pregabalin*

Neuropathic pain in the orofacial region can occur after direct injury to nerves or deafferentation after tooth extraction, dental implantation, facial trauma, or maxillofacial surgery. Previous reports have shown that some of the mechanisms involved in trigeminal neuropathic pain are different from other types of neuropathic pain.[1,2] For example, studies have shown that an injury to spinal nerves induces sympathetic sprouting in dorsal root ganglia, whereas injury to the trigeminal nerve does not induce ectopic sprouting of sympathetic fibers in the trigeminal ganglion.[1,3–6] Indeed, the incidence of sympathetically evoked pain syndromes is significantly lower after injuries to the face or jaws than to the limbs.[7] Several pharmacological strategies have proven efficacy against neuropathic pain; however, many neuropathic pain patients eventually become intolerant to these medications.[8] More generally, meticulous studies on the mechanisms of trigeminal neuropathic pain are needed to optimize treatment protocols being used in the clinic.

Various classes of antidepressants, such as tricyclic antidepressants (TCAs), serotonin and noradrenaline reuptake inhibitors (SNRIs), and selective serotonin reuptake inhibitors (SSRIs), in addition to the anticonvulsants gabapentin and pregabalin, have been indicated as first-line treatments for the management of orofacial neuropathic pain.[9–11] Their analgesic efficacy is independent of any antidepressant effects and is probably mediated by their actions on descending modulatory inhibitory controls. Antidepressants inhibit the reuptake at neuronal terminals of monoamines, including noradrenaline (NA) and serotonin (5-HT),[12] by inhibiting presynaptic transporters and by blocking postsynaptic

receptors. Most antidepressants have multiple sites of action and can produce antinociceptive effects at supraspinal,[13] spinal,[14] and peripheral sites.[15] A recent report has shown that trigeminal central sensitization can also be influenced by the NA system via effects on nociceptive neurons in the medullary dorsal horn.[16] It is therefore difficult to determine the mechanism of antinociceptive effects of systemically administered antidepressants.

Mirtazapine, a new dual-action antidepressant that enhances noradrenergic and serotonergic transmission,[17,18] has unique pharmacodynamics. Mirtazapine has little reuptake inhibition of NA or 5-HT. It enhances noradrenergic transmission instead by blocking neuronal α2-adrenergic autoreceptors, which are located on the noradrenergic cell bodies and presynaptic nerve terminals, thereby indirectly increasing NA release. Release of NA in turn enhances serotonergic neurotransmission mediated by α1-adrenoceptors on serotonergic cell bodies and presynaptic nerve terminals.[19,20] Mirtazapine also blocks 5-HT2A, 5-HT2C, and 5-HT3 receptors to inhibit various serotonergic side effects. However, α2-adrenoceptors (but not α1-adrenoceptors) are implicated in the antinociceptive effects of NA for neuropathic pain at the spinal level.[21] Among 5-HT receptor subtypes, the 5-HT2 receptor is the only receptor subtype which when activated can inhibit neuropathic pain mechanisms in the spinal cord.[22] A previous report showed that 5-HT has an important role in modulating spinal nociceptive transmission via 5-HT2C receptors.[23] Therefore, by antagonism of α2-adrenergic and 5-HT2A and 5-HT2C receptors, mirtazapine would be expected to block the analgesic effect mediated by descending modulatory inhibitory controls.

The anticonvulsants gabapentin and pregabalin are considered first-line agents for the management of neuropathic pain.[9–11] Their analgesic effects are related mainly to a reduction in central sensitization and nociceptive transmission through their action on the α2δ subunit of calcium channels. The antinociceptive effect of gabapentin may be produced by actions at spinal or supraspinal levels, or by a peripheral inhibitory action on ectopic afferent discharge.[24–26] Studies have shown that systemic administration of gabapentin reduced nociceptive behaviors in a mouse model of trigeminal neuropathic pain.[27] Intrathecal administration of gabapentin also produces antinociception in the orofacial formalin test in rats.[28] Studies have also shown that systemic pregabalin attenuates sensorimotor responses in an inflammatory tooth pain model[29] and suppresses both nociceptive behavior and trigeminal central sensitization in a rat neuropathic pain model involving infraorbital nerve (ION) transection.[30] However, it is not known if intrathecal administration of either of these drugs inhibits hypersensitivity in a trigeminal neuropathic pain model.

The aim of this study was to clarify the antiallodynic effects of mirtazapine compared with those of gabapentin and pregabalin in a rat model of orofacial neuropathic pain. This model involves damage to the ION, which has been shown to produce nociceptive behavior and trigeminal central sensitization.[30–32]

Materials and Methods

Surgical and experimental protocols were reviewed and approved by the Animal Care and Use Committee of Osaka University (Osaka, Japan) and were carried out according to guidelines set by the National Institutes of Health (Bethesda, MD, USA). Animals were treated according to the Guidelines of the International Association for the Study of Pain.[33]

Experimental Animals

Each group comprised eight male Sprague-Dawley rats (170 to 230 g at the time of surgery) that received only one drug at one dose (or vehicle). Rats had free access to chow and water, and were housed four to a cage at 22 ± 2°C with a 12-hour light–dark cycle (illumination from 0800 to 2000). Before surgery, rats were allowed ≥ 1 week to acclimatize to their housing. Male rats were selected for this study because trigeminal pain can vary across the menstrual cycle,[34] and sex-specific modulation of nociception may occur in the trigeminal region.[35]

Surgical Procedures and Behavioral Testing

Rats received a unilateral chronic constriction injury (CCI) to the right ION.[23,36] The ION was dissected free in the orbital cavity. Two nylon (5-0) ligatures were tied around the ION.

Rats were allowed to recover from the surgery for ≥ 7 days. After 7 days, a series of von Frey filaments (bending forces of 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, and 15.0 g), applied to the ipsilateral site innervated by the injured ION, was used to determine mechanical hypersensitivity to mechanical stimulation. Only rats with hyper-responsiveness to mechanical stimulation were used.

All behavioral analyses were conducted following the same protocol as in the authors' previous study.[23] The response threshold was defined as the lowest-force filament application that prompted at least three positive responses in five trials. If an animal made fewer than three pain responses to all the tested filaments, 15 g was taken to be the threshold. Motor function was evaluated by the righting reflex, stepping reflex, posture, and ambulation. The investigator was blinded to drug treatments being administered.

To test if spinal mechanisms may be important in orofacial neuropathic pain, intrathecal rather than intracisternal drug administration was used.[37] An intrathecal catheter was thus implanted for the injection of drugs into the upper cervical spine 7 days after nerve injury. A polyethylene tube (PE10) was advanced 10 mm caudally through a small hole in the atlanto-occipital membrane and dura. The catheter was then anchored surgically to the surrounding musculature to maintain its position. Rats were allowed to recover for 7 days before drug testing.

Experimental Protocol and Drugs

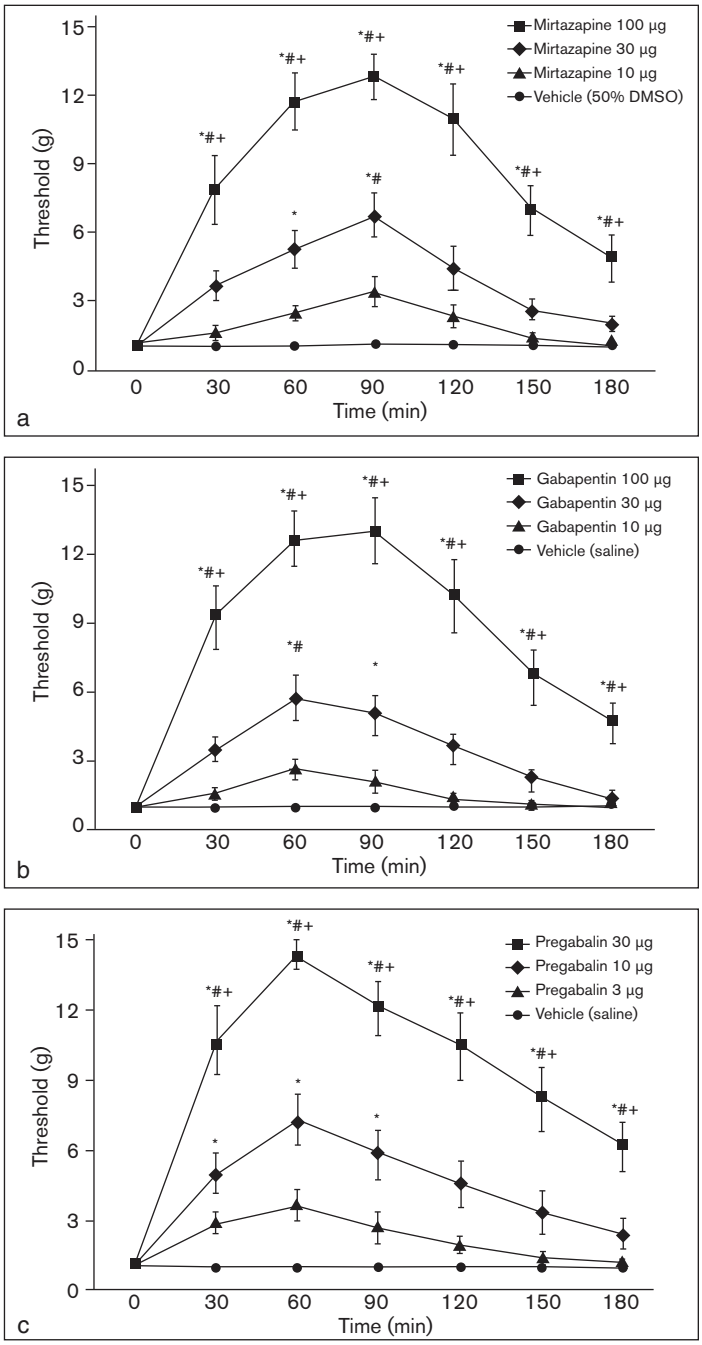
The time courses of the antiallodynic effects and the dose-response effects of intrathecally administered mirtazapine (10, 30, and 100 µg), gabapentin (10, 30, and 100 µg), and pregabalin (3, 10, and 30 µg) were examined. The drug doses were selected on the basis of previous work.[28,38] Gabapentin and pregabalin were dissolved in distilled water. Mirtazapine was dissolved in 50% dimethyl sulfoxide. Saline was used as a vehicle control for the studies with pregabalin and gabapentin, and 50% dimethyl sulfoxide was used as a vehicle control for the study with mirtazapine. Mirtazapine was provided by Meiji Seika (Tokyo, Japan) and gabapentin and pregabalin were purchased from Tocris Bioscience (Bristol, UK). Drugs were delivered in a 10-µL volume solution followed by 10 µL of saline to flush the catheter. Throughout the study, after determining the baseline value, withdrawal thresholds were measured at 30, 60, 90, 120, 150, and 180 minutes after drug injection (14 days after ION-CCI).

Data and Statistical Analyses

Data are expressed as the mean ± SEM. Time-course data are presented as the withdrawal threshold. The percentage of the maximal possible effect (%MPE) was calculated using the following formula:

$$\%\,\text{MPE} = (\text{post-drug threshold} - \text{pre-drug threshold}) / (15\ \text{g} - \text{pre-drug threshold}) \times 100$$

The time-course data for the dose-response effects were analyzed using two-way analysis of variance, and statistical differences were calculated post-hoc by using the Tukey-Kramer multiple-comparison test. The two factors assessed were time and drug dose. $P < .05$ was considered significant.



Figs 1a to 1c Time course of antiallodynic effects of intrathecally administered *(a)* mirtazapine, *(b)* gabapentin, and *(c)* pregabalin in rats with ION-CCI. Mechanical thresholds are expressed as mean ± SEM for eight rats in each group. *(a,b)* #$P < .05$ compared with the 10-µg-treated group. +$P < .05$ compared with the 30-µg-treated group. *$P < .05$ compared with the vehicle-treated group. *(c)* #$P < .05$ compared with the 3-µg-treated group. +$P < .05$ compared with the 10-µg-treated group. *$P < .05$ compared with the vehicle-treated group.

Results

Intrathecal administration of mirtazapine, gabapentin, and pregabalin produced dose-dependent antiallodynic effects. Figures 1a to 1c show the time course of the antiallodynic effects of each drug.

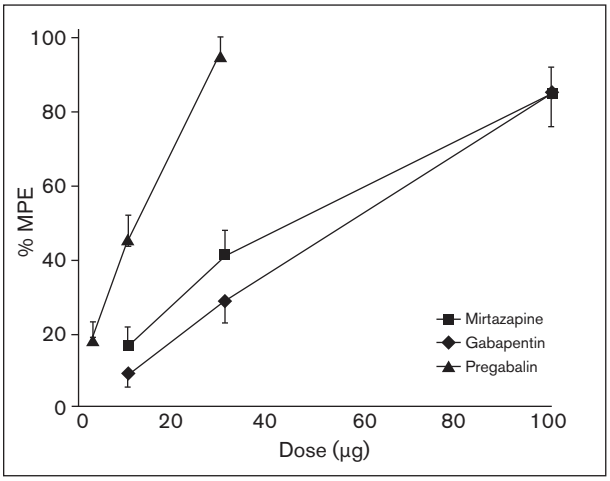


Fig 2 Dose-response curves showing the peak effect of intrathecally administered mirtazapine, gabapentin, and pregabalin in rats with ION-CCI. Data are expressed as the mean ± SEM of a percentage of the maximal possible effect (% MPE) for eight rats.

Table 1 ED_{50}, Peak Effect, and Duration of Action for the Three Drugs Tested

Drug	ED_{50} (95% CI), μg	Peak (min)	Duration of action (min)
Mirtazapine	49.00 (39.71–58.29)	90	≥ 180
Gabapentin	54.84 (46.12–63.56)	60	≥ 180
Pregabalin	13.47 (11.24–15.69	60	≥ 180

CI: confidence interval.

From 30 to 180 minutes post–drug injection (14 days after IO-CCI), mechanical thresholds after treatment with 100 μg of mirtazapine were significantly higher than those after treatment with 10 or 30 μg of mirtazapine or vehicle. Mechanical thresholds were significantly higher 60 to 90 minutes after treatment with 30 μg of mirtazapine than they were 60 to 90 minutes after treatment with vehicle. Moreover, mechanical thresholds were higher 90 minutes after treatment with 30 μg of mirtazapine than they were 90 minutes after treatment with 10 μg of mirtazapine.

Similarly, from 30 to 180 minutes after injection, mechanical thresholds after treatment with 100 μg of gabapentin were significantly higher than those after treatment with 10 or 30 μg of gabapentin or vehicle. Mechanical thresholds were significantly higher 60 to 90 minutes after treatment with 30 μg of gabapentin than they were 60 to 90 minutes after treatment with vehicle. Thresholds were likewise higher 60 minutes after treatment with 30 μg of gabapentin than they were 60 minutes after treatment with 10 μg of gabapentin.

From 30 to 180 minutes after injection, mechanical thresholds after treatment with 30 μg of pregabalin were significantly higher than those after treatment with 10 or 3 μg of pregabalin or vehicle. Mechanical thresholds were significantly higher 30 to 120 minutes after treatment with 10 μg of pregabalin than they were 30 to 120 minutes after treatment with vehicle. In addition, thresholds were higher 30 minutes after treatment with 10 μg of pregabalin than they were 30 minutes after treatment with 3 μg of pregabalin.

The peak effect of intrathecal administration of gabapentin or pregabalin occurred 60 minutes after injection, and the peak effect of mirtazapine occurred at 90 minutes (Table 1).

Figure 2 shows the dose-response relationship of the peak effect of each of the three drugs. The ED_{50} (95% confidence interval) was 49.00 μg (39.71–58.29 μg) for mirtazapine, 54.84 μg (46.12–63.56 μg) for gabapentin, and 13.47 μg (11.24–15.69 μg) for pregabalin. Motor function, as assessed by the righting reflex, stepping reflex, posture, and ambulation, was normal after intrathecal injection of all doses of these drugs (data not shown).

Discussion

To the best of the authors' knowledge, this is the first time that intrathecal administration of mirtazapine has been shown to attenuate nociceptive behavior in a rat model of trigeminal neuropathic pain. In this ION-CCI model in rats, intrathecal administration of mirtazapine produced the same strong antinociceptive effects as gabapentin and pregabalin. The brainstem–spinal descending NA and 5-HT systems suppress nociceptive signals from primary afferent neurons to the spinal and medullary dorsal horns.[16] Accordingly, intrathecal administration of adrenoceptor agonists and 5-HT receptor agonists suppresses allodynia in a rat model of neuropathic pain.[21,22] Mirtazapine has selective α2-adrenolytic properties, and blockage of somatodendritic and terminal α2-adrenoceptors facilitates noradrenergic transmission.[39] In turn, noradrenergic neurons originating from the locus coeruleus (LC) control the firing rate of serotonergic neurons in the raphe system and exert an α1-adrenoceptor–mediated tonic control upon 5-HT transmission.[40] A previous study showed that the LC, which is located in the brainstem and activated upon nociceptive stimulation, was altered in an animal model of chronic neuropathic pain. Importantly, antidepressants with antineuropathic effects restore LC-evoked activity in parallel with their behavioral analgesic effects.[41] In this study, there was sufficient time for the drugs to spread to the LC; therefore, the effects reported here may have been due to the effects of these drugs not only on the spinal cord but also on the brainstem.

Studies have demonstrated that 90 minutes after subcutaneous administration of 2 mg/kg mirtazapine in rats, hippocampal noradrenergic activity is

increased by 80%.[39] Similarly, subcutaneous administration of 2 mg/kg of mirtazapine in rats increased hippocampal levels of 5-HT by 80% when measured 45 minutes after injection.[39] In the present study, the peak antinociceptive effect of mirtazapine was also at 90 minutes, in accordance with findings described in an intraperitoneal injection study of neuropathic pain in the lower limbs.[42] Therefore, these data suggest that the time course of mirtazapine effects on noradrenergic activity may be more similar to that of intrathecal mirtazapine on the mechanical threshold than is the time course of mirtazapine effects on 5-HT. There is also evidence that mirtazapine might have a stronger action on the descending noradrenergic system than on the serotonergic system.[43] Indeed, mirtazapine has selective α2-adrenolytic properties and α1-adrenoceptors play an important role in the spinal antinociceptive effects of mirtazapine. In addition, studies have shown that spinal nerve injury induces sympathetic sprouting in dorsal root ganglia, but injury to the trigeminal nerve does not induce ectopic sprouting of sympathetic fibers in the trigeminal ganglion.[1,3–6] Posttraumatic interactions between sympathetic and sensory nerves play a role in the development of sensory hypersensitization. Thus, it is conceivable that the antinociceptive effects of mirtazapine on trigeminal neuropathic pain might be stronger than its effects on spinal neuropathic pain.

Pregabalin and gabapentin bind specifically to the α2δ subunits of voltage-dependent calcium channels in the central nervous system.[44] These drugs reduce the depolarization-induced calcium influx at nerve terminals, resulting in a reduction in the presynaptic release of excitatory neurotransmitters, including glutamate, substance P, and calcitonin gene-related peptide.[45,46] Systemic (intravenous, oral, intraperitoneal, and subcutaneous) and spinal (intrathecal) administration of gabapentin appear to yield similar effects on nociceptive behaviors in models of traumatic injury to spinal nerves.[47] One study has shown that systemic administration of gabapentin reduced nociceptive behaviors in a mouse model of ION-CCI,[27] and other recent studies have shown that systemic pregabalin attenuates sensorimotor responses and glutamate release in an inflammatory tooth pain model[28] and suppresses both nociceptive behavior and trigeminal central sensitization in a rat neuropathic pain model produced by ION injury.[37] The present study similarly demonstrated that spinal administration of pregabalin and gabapentin inhibited nociceptive behavior in a rat neuropathic pain model produced by ION-CCI. TCAs may be more efficacious than gabapentin or pregabalin; however, the drug interaction and side-effect profiles of gabapentin and pregabalin appear to be more favorable.[48] Pregabalin and gabapentin are also available for the treatment of trigeminal neuropathic pain.

Psychological disorders such as depression often coexist with and perpetuate chronic pain syndromes.[49,50] Antidepressants may be used in such cases, but TCAs and/or SSRIs can cause adverse side effects such as anorexia, defects in cardiac conduction, constipation, dry mouth, insomnia, nausea, orthostatic hypertension, sexual dysfunction, and urinary retention.[51] Mirtazapine exhibits very little binding to central and peripheral α1-adrenoceptors, muscarinic receptors, and dopaminergic receptors,[17] and thus does not cause the side effects related to these receptors. Interestingly, 5-HT neurotransmission through 5-HT2 receptors is one of the proposed causes of insomnia associated with SSRIs, and perhaps due to its effects as a 5-HT2 and 5-HT3 antagonist, mirtazapine can conversely improve the neurophysiological processes promoting sleep.[52] SSRIs may also initially worsen symptoms of anxiety that eventually resolve with chronic dosing; in contrast, mirtazapine is initially anxiolytic because of its blockade of 5-HT2 receptors.[53] Moreover, switching to mirtazapine from SSRIs has been shown to resolve drug-induced sexual dysfunction in 58% of individuals and improve sexual function in an additional 11% of subjects.[54] Mirtazapine has also been reported to reduce the nausea associated with the SSRI-induced serotonin syndrome,[55] and this is likely due to mirtazapine's affinity for 5-HT3 receptors, which is similar to that of ondansetron (a strong antiemetic agent). In one study, 5-HT–related side effects with mirtazapine were no different from placebo and were significantly less compared with SSRIs.[56] Mirtazapine has been shown to have a low overall dropout rate for a central-acting compound and a good/acceptable adverse-effect profile as reflected by the low rate of unexpected adverse events compared with other antidepressants such as TCAs, SNRIs, or SSRIs.[57,58] Thus, mirtazapine could become a more commonly used antidepressant in patients with chronic pain and depression.[51]

The mainstays of pharmacotherapy for neuropathic pain are currently antiepileptic drugs and TCAs.[9–11] Treatment of chronic neuropathies often requires long-term prescription medications that can have significant side effects. If individual drugs are only partially successful, combination approaches are sometimes employed. In combination studies with mirtazapine and other serotonergic and adrenergic agents, even if a low dose of mirtazapine that is ineffective on its own was used, the dose-response curve of 5-HT or clonidine (an adrenergic agonist) was in fact altered[43]; thus, mirtazapine may produce synergistic effects if combined with other drugs. While the present study has documented the antinociceptive

effect of mirtazapine by intrathecal administration in the rat trigeminal neuropathic pain model, it is yet to be determined whether this effect will translate to the clinical setting. However, there is certainly no reason to expect mirtazapine to exacerbate pain symptoms due to 5-HT2A and 5-HT2C blockade, and the present study provides good evidence that mirtazapine may show clinical antinociceptive efficacy.

Conclusions

The data presented here indicate that not only intrathecally administrated gabapentin and pregabalin but also mirtazapine attenuate nociceptive behavior manifested in a rat trigeminal neuropathic pain model. Mirtazapine produced strong antinociceptive effects to the same extent as gabapentin. The present study may contribute to better treatment of individuals with chronic orofacial pain, since clinicians treating orofacial neuropathic pain could use mirtazapine and expect appreciable antinociceptive effects and few adverse effects compared with TCAs and SSRIs. Further studies will be required to confirm the effects of mirtazapine, gabapentin, and pregabalin in the brain and to investigate drug interactions between serotonergic and adrenergic drugs and mirtazapine.

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