Original article

Modulatory effects of repeated psychophysical stress on masseter muscle nociception in the nucleus raphe magnus of rats

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Abstract: Psychophysical stress can cause neural changes that increase nociception in the orofacial region, particularly the masseter muscle (MM). The nucleus raphe magnus (NRM), which is located in the brain stem, serves the crucial role of regulating nociception through descending modulatory pain control. However, it remains unclear if neural activities in the NRM are affected under psychophysiological stress conditions. This study conducted experiments to assess (1) whether neural activity, indicated by Fos protein expression in an NRM that has experienced MM injury, is affected by the stress of repeated forced swim tests (FST); and (2) whether the selective serotonin reuptake inhibitor fluoxetine administered daily after an FST could affect the number of Fos-positive neurons in the NRM. Results revealed that the stress from repeated FSTs significantly increased the number of Fos-positive neurons in an NRM that had been affected by MM injury. Fluoxetine inhibited increases in the number of Fos-positive neurons in the NRM that occurred as a result of FSTs, but this was not observed in sham rats. These findings indicate that the stress from FSTs could increase nociceptive neural activity in an NRM that has experienced MM injury. This could be due, in part, to changes in serotonergic mechanisms.

Keywords: masseter muscle, nucleus raphe magnus, pain, repeated forced swim stress, rostral ventromedial medulla, serotonin

Introduction

Exposure to repeated psychophysical stress has modulatory effects on orofacial nociception [1]. Multiple brain structures mediate the experience of pain in a complex manner. Nociceptive transmission can be affected by psychophysical stress conditions, which can influence neural functioning with regard to pain circuits in the brain [2,3]. Prior studies have found that repeated forced swim tests (FSTs), which can induce psychophysiological stress conditions, enhanced nociceptive neural excitabilities in the trigeminal subnucleus caudalis (Vc) region [4-6], with the Vc region being well documented as a critical area for mediating nociception in deep orofacial tissues, such as masseter muscle (MM) [7,8] and temporomandibular joints [4].

The nucleus raphe magnus (NRM), the area of the rostral ventromedial medulla (RVM) in the caudal brainstem, is a key relay in the pathway of descending pain control to the Vc region [9,10]. It is, therefore, possible that neural changes in the NRM under pathological conditions could cause increases in nociception in the trigeminal nerve [7,8]. Ample studies have revealed that the dysfunction of the NRM could affect nociceptive responses in spinal pain models, but it remains unclear if psychophysical stress conditions could modulate nociceptive neural activities in the NRM related to trigeminal noxious inputs in the Vc region. Notably, neural functions in the NRM in trigeminal pain models appear to be different to those in spinal pain models [11,12].

Serotonin (5HT) is a key factor in descending pain control pathways as well as mediating psychological stress and nociceptive responses [2,3,6,13,14]. Serotonergic neurons display nociceptive responses indicated by Fos protein expression in an NRM subjected to noxious stimuli in the peripheral regions [15,16]. Chronic restraint stress modulates tryptophan hydroxylase biosynthesis of serotonin production in the NRM [17], and antidepressant agents, such as selective serotonin reuptake inhibitors (SSRIs), reduce tryptophan hydroxylase levels in the NRM [18]. Recent studies revealed that the daily administration of the SSRI fluoxetine reduced nociceptive neural activities at the Vc region after FSTs [8]. These findings suggested that dysfunction of the serotonergic mechanism in the brain could affect neural activity in the Vc region by adversely influencing descending pain control pathways including NRM functions [13]. However, it remains unknown how SSRIs affect MM nociception in the NRM, especially under FST conditions.

In this study, Fos immunohistochemical procedures were conducted to quantify the MM nociception in the NRM under repeated FSTs. Despite several limitations, Fos protein is often employed as a marker of excitability to assess neural functioning in the central nervous system for psychophysiological stress conditions and pain processing [19,20]. The aims of this study were to clarify if repeated psychophysical stress had modulatory effects on Fos expression in the NRM, and if administering daily doses of an SSRI could affect Fos expression in the NRM after noxious stimulation of the MM.

Materials and Methods

Animals

Experiments were conducted in accordance with the International Association for the Study of Pain [21], reviewed by the Institutional Animal Care and Use Committee, and approved by the President of Niigata University (#SA00351). All experiments performed using animals in this study were in accordance with the ethical standards of the institution or practice where the studies were conducted. Efforts were made to minimize the number of animals used for the experiments as well as their suffering. This report includes no studies with human participants. Sprague Dawley rats (Male, 250-280 g, Charles River, Yokohama, Japan) were employed. Rats were housed in plastic cages (two rats per cage) and had access to food and water freely for at least 5 days before stress conditioning was conducted. Cages were maintained at a temperature of 25 ± 2°C and were light-controlled protected units (12:12 h of light-dark cycles with light beginning at 8:00 a.m.).

Repeated forced swim stress tests (FST)

For repeated FSTs, each rat was placed in a plastic cylinder (diameter 30 cm, height 50 cm) containing 20 cm water (25-27°C) for 10 min/day between 09:00 a.m. and 11:00 a.m. for 3 days (Days-3, -2, and -1) [22,23] (Fig. 1). Fresh water was used for each session. Sham rats served as the controls and were placed in an empty swim chamber on the same schedule. Rats were dried in a warm environment after each FST session. Noxious stimulation to the MM region was conducted with formalin on Day-0 at 24 h after the last FST.

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MM injury

MM stimulation was induced with 0% formalin (i.e. saline, 0.05 mL), 1% formalin, and 5% formalin (0.05 mL) injected into the central portion of the left MM under general anesthesia with three types of mixed anesthetic agents. A combination anesthetic drug was prepared with 0.3 mg/kg of medetomidine, 4.0 mg/kg of midazolam, and 5.0 mg/kg of butorphanol. MM injection of formalin was conducted on Day-0 in the FST-conditioned group (0% formalin [n = 5]; 1% formalin [n = 5]; and 5% formalin [n = 5]) and the sham-conditioned group (0% formalin [n = 5]; 1% formalin [n = 5]; 5% formalin [n = 5]). Additional rats were included as the controls. These rats received no stimulus to the MM region after FSTs (FST-N.S. group; n = 5), nor did the sham rat group who experienced FSTs (Sham-N.S. group, n = 5). Until the rats were euthanized, the plane of anesthesia of rats was kept at the point that the rats showed no withdrawal reflex evoked by noxious pinch stimulation to the hindpaw. All rats remained alive for 2 h after MM stimulation.

Fluoxetine effects on Fos responses in the RVM after FST

Rats were divided into six groups, which included the vehicle group (i.e. saline, 1 mL/kg) and the group that received fluoxetine diluted in saline (0.1 mg/kg, 1 mg/kg) under FST or sham conditions. Five rats were employed for each treatment. Drugs were given intraperitoneally 30 min after each FST and sham treatment on Day-3 to Day-1. Formalin (5% in saline, 0.05 mL) was injected to induce Fos expression in the RVM.

Tissue preparation and Fos immunohistochemistry

Two h after MM injury, rats were deeply anesthetized with three types of mixed anesthetic agents. They were perfused through the heart with 150 mL saline (4°C), followed by 400 mL of 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS) (PFA, pH 7.4; temperature, 4°C). The brainstem was removed and postfixed with PFA overnight. The next day, the brainstems were placed in sucrose (30% in 0.1 M PBS) for 2-3 days at 4°C. Brainstem transverse frozen sections (50 µm thick) were serially cut using a freezing microtome. Sections were collected in five wells containing 0.01 M PBS. The sections were processed for Fos immunohistochemistry from the brainstem (~10 mm to −11.5 mm caudal to the bregma). After being washed several times, sections were incubated in 5% normal goat serum (NGS) for 120 min in affinity-purified mouse c-Fos monoclonal antibody (1:2,000, Abcam, Cambridge, MA, 4°C, 40 h) or in rabbit serotonin polyclonal antibody (1:5,000; Immunostar, Hudson WI, USA), followed by biotinylated anti-rabbit IgG (1:200; Vector, Burlingame, CA, USA) for 2 h at RT. Fos immunostaining was abolished by omitting the primary antibody. The experiment for double labeling of Fos- and 5HT-immunoreactivity was conducted in the NRM to determine the neurochemical properties in Fos expressing cells. MM stimulation with 5% formalin was conducted. The procedures for Fos-immunostaining are described above. For 5HT immunostaining, after the completion of Fos-immunostaining, sections were incubated with rabbit serotonin polyclonal antibody (1:5,000; Immunostar, Hudson, USA), followed by biotinylated anti-rabbit IgG (1:200; Vector, Burlingame, CA, USA) for 2 h at RT. 5HT immunoreactivity was visualized by DAB alone activated by 0.01% peroxidase.

Data analysis

The experimental conditions, which were consistent with the initial report [24], Fos expressing cells were predominantly induced in the caudal area of the RVM region. Using a light microscope, the number of Fos-positive cells was quantified in the NRM, nucleus reticularis gigantocellularis pars alpha (GiA), ventral nucleus reticularis gigantocellularis (Gi), and nucleus lateralis paragigantocellularis (LPGi) at the level between −10 mm and −11.5 mm to the bregma (Fig. 2A, B). Under bright-field illumination, Fos-positive cells were counted if they contained a black, regularly shaped nucleus that was surrounded by a brown-stained perinuclear cytoplasmic region which had dendritic processes. On the other hand, 5HT positive cells were counted if they contained a well-defined nucleus that was surrounded by a brown-stained perinuclear cytoplasmic region. The number below the coronal section indicates the rostro-caudal distance from the bregma (mm). Gi, nucleus reticularis gigantocellularis; GiA, nucleus reticularis gigantocellularis pars alpha; LPGi, lateral nucleus reticularis paragigantocellularis; NRM, nucleus raphe magnus. The number “−” indicates the facial nucleus.
statistically analyzed by using analysis of variance followed by Bonferroni tests. The percentage of double labeling cells was estimated by the number of both Fos- and 5HT-expressing cells in the total number of Fos positive cells with or without 5HT expression in the NRM. A probability level less than 0.05 was significant. Sections from each animal were observed at a magnification of 100× to quantify the number of Fos-positive cells. The examiner was blinded to the treatment groups.

Results

The effects of FSTs on Fos responses in the RVM

The FST group with no stimulation (i.e. FST-N.S. group) or saline (i.e. 0% formalin) administered into the MM showed a small change in the number of Fos expressing cells in each area of the RVM, compared with the change in the sham rats (Fig. 3, *P* > 0.1). These findings indicated that injection procedures alone had no effect on neural activity in the RVM region. The effect of FSTs on the number of Fos-positive cells in each area after formalin injection was then tested. In general, Fos-positive cells were distributed in the caudal portion of the RVM in sham rats and FST rats (Fig. 2A, B). Compared with the saline (0% formalin) injection, an injection of 5% formalin to the MM region significantly increased the number of Fos-positive cells in the NRM (*P* < 0.001, Fig. 3A) and GiA (*P* < 0.001, Fig. 3B) in the sham rats and FST rats. Furthermore, compared with the sham rats, FST rats experienced increases in the number of Fos-positive cells in the NRM region evoked by 5% formalin (*P* < 0.01, Fig. 3A), indicating that FST stress could facilitate MM nociceptive responses in the NRM. In the Gi and LPGi, 1% and 5% formalin did not significantly enhance Fos responses in comparison with saline injection under FST and sham conditions (Fig. 3C, D). The percentages of Fos and 5HT double-labeled cells (Fig. 4) in the total number of Fos-positive cells, regardless of double labeling/section, were significantly greater in sham rats (27.5 ± 2.1%, *P* < 0.01) than in FST rats (17.6 ± 1.8%). These results indicated that the reduction of the percentages of double labeling cells/total number of Fos-positive cells after FSTs was due to increases in the number of Fos expressing cells with decreases in 5HT expressing cells.

Effects of fluoxetine on Fos expression in the NRM after FST

The effects of fluoxetine on Fos expression in the NRM after injecting 5% formalin to the MM region were determined (Fig. 5A). This location was chosen due to the findings that MM stimulation with formalin had a greater influence on the number of Fos-positive cells in the NRM compared with other areas in the RVM (Fig. 3). In FST rats, but not sham rats, fluoxetine significantly decreased the number of Fos-positive cells in the NRM compared with the vehicle-treated rats (0.1 mg/kg, *P* < 0.05; 1 mg/kg, *P* < 0.01, Fig. 5B). The number of Fos-positive cells was significantly increased in GiA in sham and FST rats (Fig. 3A), but fluoxetine did not reduce Fos responses significantly (*P* > 0.1).
This study had several novel findings. Firstly, repeated FSTs increased nociceptive neural activity, as indicated by Fos expression in the NRM, after formalin injection into the MM. These findings indicate that psychophysical stress conditions can affect the functioning of the NRM, which might lead to the dysfunction of descending pain controls. Secondly, repeated administration of an SSRI just after each FST may have prevented the FST-induced enhancement of Fos responses in the NRM by MM stimulation with formalin. These findings indicate that FSTs increased nociceptive responses in the NRM, which might be mediated, in part, by changes in serotonergic mechanisms. Several areas in the RVM, such as the NRM, GiA and LPGi regions, regulate nociception in trigeminal and spinal pain models [12,24]. Imaging studies that employ glucose utilization show increases in neural excitability in the NRM and GiA in rats with peripheral nerve injury and inflammation [26,27]. Furthermore, a recent report indicates the distinct roles of the NRM versus the LPGi in the regulation of neural activities in the spinal dorsal horn [29]. The results reveal that formalin injection into the MM increases Fos expression, especially in the NRM, but not in the Gi and LPGi regions for either group of rats. Furthermore, mapping studies using Fos immunoreactivity indicate that the contribution of each area in the RVM to nociceptive responses could be dependent on various pain conditions. For example, Fos expression evoked by noxious stimulation to the hindpaw was greater in the NRM than in other areas under persistent monoarthritis of the temporomandibular joint (TMJ) [24], whereas those by forepaw or visceral stimulation could cause greater Fos expression in the GiA than in other areas [16]. The results demonstrate that nociceptive neural activity is much greater in the NRM than in other areas of the RVM, which indicates that the NRM is more sensitive to MM injury compared with other areas in the RVM under repeated FST conditions. Therefore, this study focused on determining the effects of repeated and acute FSTs on neural activity, primarily in the NRM.

The NRM has descending pathways, which can regulate nociceptive neural activities in the Vc regions [10]. Changes intrinsic to the NRM are documented in various chronic pain conditions such as TMJ inflammation [24], headaches [29], visceral pain [30], and peripheral nerve injury [31,32]. Mounting evidence reveals that repeated psychological stress could enhance nociceptive responses related to the spinal and trigeminal nerve inputs [33,34]. As nociceptive neural properties in the NRM based on trigeminal nerve inputs were not similar to that seen after spinal nerve inputs [11,12], it is important to assess the effects of FSTs on functional changes in the NRM using trigeminal pain models rather than in spinal pain models. A previous report showed that the facilitatory effects of FST on nociception in MM regions occurred because of increases in Fos expression in the Vc region [8]. The results showed significant increases in Fos expression, primarily in the NRM evoked by MM injury after repeated FSTs, whereas the facilitatory effects of FSTs alone on Fos expression seem to be less than in other areas in the RVM. These findings suggest that enhanced neural activity in the NRM could have roles in increasing MM nociception with changes in neural activity in the Vc region. Serotonergic mechanisms are critically involved in the mediation of stress responses such as psychological distress [35,36] and stress-induced hyperalgesia [2,23]. Previous reports have indicated that stress conditioning influences serotonergic functioning such as the 5HT concentration in the central nervous system [37,38]. In contrast, modulating serotonergic mechanisms by antidepressants, such as SSRIs, could relieve stress-induced, depression-like behaviors and enhance nociception [8,23,39,40]. These findings suggest that serotonergic mechanisms have critical roles in mediating psychological distress and nociceptive processing. Many reports implicate 5HT in regulating pain responses and demonstrate that bulb-spinal/trigeminal serotonergic pathways exert modulatory influences on orofacial nociceptive signaling within the Vc and spinal cord [2,23]. However, evidence also indicates that serotonergic inputs to the NRM can affect neural activity in the NRM [41]. Several 5HT receptor subtypes can regulate nociception in the NRM [42] and the level of a certain subtype of 5HT receptor in the NRM is altered under pathological conditions [43]. These findings suggest that the involvement of serotonergic mechanisms in regulating neural activities in the NRM could be sensitive to several pathological conditions and could modulate descending outputs to the Vc region, which could affect orofacial nociception. Repeated SSRI administration after each FST was demonstrated to potentially enhance Fos expression evoked by MM injury in the NRM in FST rats but not in sham rats. These findings suggest that serotonergic functioning in the brain could be affected by repeated FSTs. At this point, it is unclear how SSRIs affect neural activity in the NRM. However, this effect could be related to the direct actions of SSRIs in the neural activities in the NRM or indirect effects of SSRIs on neural activity in remote areas of the brain in which descending mechanisms can regulate neural activity in the NRM. Further investigations would be required to determine the action sites of SSRIs which can inhibit Fos induction in the NRM after stress conditioning.

Previous neural recording experiments indicate that 5HT neurons in the NRM could be functionally classified as neutral cells, but not as ON-cells and OFF-cells [44,45]. In the present data, FSTs increase the number of Fos-positive cells in the NRM. However, the percentage of double labeling cells in the total number of Fos-positive cells with or without 5HT expression were decreased under FST conditions. These findings indicate that most Fos-positive cells, which were increased after FST sessions, were unlikely serotonergic neurons in the NRM, and that most Fos-positive cells, which were sensitive to FSTs, seemed to be ON- or OFF-cells. Furthermore, it is possible that FSTs could induce phenotype changes in serotonergic neurons from neutral cells to ON- or OFF-cells, as was seen in a persistent inflammatory pain model [46]. However, further functional experiments are required to determine this relationship.

In conclusion, FSTs increased the number of Fos-positive cells in an NRM affected by MM injury, possibly via changes in serotonergic functioning. These findings suggest that psychophysical stress conditions could influence neural activities in the NRM, which plays modulatory roles in deep craniofacial nociception.

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Conflict of Interest
The authors declare that they have no conflict of interest.

References
1. Slade GD, Ditschenko J, Bhagat K, Sigurdsson A, Fillingim RB, Belfer I et al. (2007) Influence of psychological factors on risk of temporomandibular disorders. J Dent Res 86, 1120-1125.
2. Imbe H, Iwai-Liao Y, Senba E (2006) Stress-induced hyperalgesia: animal models and putative mechanisms. Front Biosci 11:2179-2182.
3. Jennings EM, Okine BN, Roche M, Finn DP (2014) Stress-induced hyperalgesia. Prog in Neurobiol 121, 1-18.
4. Okamoto K, Tashiro A, Chang Z, Thompson R, Belfer I et al. (2012) Temporomandibular joint-evoked responses by spinomedullary neurons and masseter muscle are enhanced after repeated psychophysical stress. Eur J Neurosci 36, 2025-2034.
5. Okamoto K, Thompson R, Katagiri A, Belfer I et al. (2013) Testosterone status and psychophysical stress modulate temporomandibular joint input to medullary dorsal horn neurons in a lamina-specific manner in female rats. Pain 154, 1057-1064.
6. Okamoto K, Katagiri A, Raham M, Thompson R, Belfer I et al. (2015) Inhibition of temporomandibular joint input to medullary dorsal horn neurons by 5HT3 receptor antagonist in female rats. Neurosci 299, 35-44.
7. Kurose M, Imbe H, Nakatani Y, Hasegawa M, Fujii N, Takagi R et al. (2017) Bilateral increases in ERK activation at the spinomedullary junction region by acute masseter muscle injury during temporomandibular joint inflammation in the rats. Exp Brain Res 235, 913-921.
8. Nakatani Y, Kurose M, Shimizu S, Hasegawa M, Ikeda N, Yamamura K et al. (2018) Inhibitory effects of fluoxetine, an antidepressant drug, on masseter muscle nociception at the trigeminal subnucleus caudalis and upper cervical spinal cord regions in a rat model of psychophysical stress. Exp Brain Res 236, 2209-2221.
9. Li JL, Kaneo T, Nomura S, Li YQ, Mizuno N (1997) Association of serotonin-like immunoreactive axons with nociceptive projection neurons in the caudal spinal trigeminal nucleus of the rat. J Comp Neurol 384, 127-141.
10. Aicher SA, Hertsmes SM, Whittier KL, Hegarty DM (2012) Descending projections from the rostral ventromedial medulla (RVM) to trigeminal and spinal dorsal horns are morphologically and neurochemically distinct. J Chem Neuroanat 43, 103-111.
11. Eilrich J, Ulucan C, Schnell C (2000) 'Are 'neural cells' in the rostral ventro-medial hypothalamus subtypes of on- or off- cells? Neurosci Res 38, 419-423.
12. Khasabov SG, Malecha P, Noack J, Tabakov J, Okamoto K, Belfer I et al. (2015) Activation of rostral ventromedial medulla neurons by noxious stimulation of cutaneous and deep periauricular tissues. Neurosci Res 954, 94-99.
13. Millan MJ (2002) Descending control of pain. Prog Neurobiol 66, 355-474.
14. Okamoto K, Katagiri A, Raham M, Thompson R, Belfer I et al. (2015) Inhibition of temporomandibular joint input to medullary dorsal horn neurons by 5HT3 receptor antagonist in Female rats. Neurosci 299, 35-44.
15. Suzuki R, Morcuende S, Webber M, Hunt SP, Dickenson AH (2002) Superficial NK1-expressing neurons control spinal excitation through activation of descending pathways. Nat Neurosci 5, 1319-1326.
16. Chen T, Dong YX, Li YQ (2003) Fos expression in serotonergic neurons in the rat brainstem following noxious stimuli: an immunohistochemical double-labeling study. J Anat 203, 579-588.
17. Imbe H, Murakami S, Okamoto K, Iwai-Liao Y, Senba E (2004) Effects of acute and chronic restraint stress on nociceptive responses induced by formalin injected in rat’s TMJ. Pharmacol Biochem Behav 82, 338-344.
18. Huang J, Zhang M, Chen Y (2003) Fluoxetine hydrochloride (Prozac). Nat Rev Drug Discov 4, 764-774.
19. Gameiro GH, Andrade Ada S, De Castro M, Pereira LF, Tamberi CH, Veiga MC (2005) The effects of restraint stress on nociceptive responses induced by formalin injected in rat’s TMJ. Pharmacol Biochem Behav 82, 338-344.
20. Connor TJ, Kelly JP, Leonard BE (1997) Forced swim test-induced neurochemical endocrine and immune changes in the rat. Pharmacol Biochem Behav 58, 961-967.
21. Wong DT, Perry KW, Bymaster FP. (2005) Case history: the discovery of fluoxetine hydrochloride (Prozac). Nat Rev Drug Discov 4, 764-774.
22. Porsolt RD, Le Pichon M, Jalfre M (1977) Depression: a new animal model sensitive to antidepressant action. Nature 266, 730-732.
23. Cryan JF, Markou A, Lucki I (1997) Assessing antidepressant activity in rodents: recent developments and future needs. Trends Pharmacol Sci 23, 238-245.
24. Oh SH, Imbe H, Iwai-Liao Y (1997) TMJ inflammation increases Fos expression in the nucleus raphe magnus and gigantocellularis/rostral ventromedial medulla of lightly anesthetized rats. Neurosci Lett 226, 136-138.
25. Xie H, Ma F, Zhang YQ, Gao X, Wu GC (2002) Expression of 5-HT(2A) receptor mRNA on three classes of physiologically characterized putative pain modulating neurons in the rostral ventromedial medulla following noxious stimuli: an immunohistochemical double-labelling study. J Anat 203, 579-588.
26. MacGillivray L, Lagrou LM, Reynolds KB, Rosebuh P, Mazurek MF (2010) Role of serotonin transporter inhibition in the regulation of tryptophan hydroxylase in brainstem raphe nuclei: time course and regional specificity. Neurosci 171, 407-420.
27. Harris JA (1998) Using c-fos as a neural marker of pain. Brain Res Bull 45, 1-8.
28. Okamoto K, Imbe H, Kimura A, Donishi T, TamaI Y, Senba E (2007) Activation of central 5HT2A receptors reduces the craniofacial nociception of rats. Neurosci 147, 1090-1102.
29. Zimmermann M (1983) Ethical guidelines for investigations of experimental pain in conscious animals. Pain 16, 109-110.
30. Porsolt RD, Le Pichon M, Jalfre M (1977) Depression: a new animal model sensitive to antidepressant treatments. Nature 266, 730-732.
31. Cryan JF, Markou A, Lucki I (1997) Assessing antidepressant activity in rodents: recent developments and future needs. Trends Pharmacol Sci 23, 238-245.
32. Oh SH, Imbe H, Iwai-Liao Y (1997) TMJ inflammation increases Fos expression in the nucleus raphe magnus and gigantocellularis/rostral ventromedial medulla of lightly anesthetized rats. Neurosci Lett 226, 136-138.
33. de Oliveira R, de Oliveira RC, Falconi-Sobrinho LL, da Silva Soares R, Jr., Coimbra NC (2007) 5-Hydroxytryptamine2A/2C receptors of nucleus raphe magnus and gigantocellularis/rostral ventromedial medulla following noxious stimuli: an immunohistochemical double-labelling study. J Anat 203, 145-160.
34. Potrebic SB, Fields HL, Mason P (1994) Serotonin immunoreactivity is contained in one type of medullary pain facilitating neuron in the rat. J Neurophysiol 77, 1087-1098.
35. Adell A, Casanovas JM, Artigas F (1997) Comparative study in the rat of the actions of serotonin transporter inhibition in the regulation of tryptophan hydroxylase in brainstem subtypes of on-and off- cells? Neurosci Res 38, 419-423.