Phosphorylation of ERK in Trigeminal Spinal Nucleus Neurons Following Passive Jaw Movement in Rats with Chronic Temporomandibular Joint Inflammation

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Aims: *To elucidate the neuronal mechanisms underlying chronic pain of the temporomandibular joint (TMJ), expression of phosphorylated extracellular signal-regulated kinase (pERK) in the trigeminal spinal nucleus caudalis (Vc) was studied in rats with a chronically inflamed TMJ.* ***Methods:*** *Complete Freund's adjuvant (CFA) was injected in the left TMJ region of rats anesthetized with pentobarbital (50 mg/kg intraperitoneally). Face temperature of the TMJ region was measured periodically after CFA injection. Two weeks after CFA injection, passive jaw movement with 4-, 6-, and 15-mm distances was carried out in inflamed and naive rats for 5, 15, and 30 minutes. pERK expression was studied in the medulla and upper cervical cord after passive jaw movement.* ***Results:*** *Face temperature was significantly increased 2 days after CFA injection and returned to the preoperative level 7 days later. The pERK-like immunoreactive (LI) cells were observed in the dorsal portion of the rostral Vc in inflamed rats after passive jaw movement, and a small number of pERK-LI cells were observed in naive rats after passive jaw movement. No pERK-LI cells were observed in the TMJ of inflamed rats without jaw movement. The number of pERK-LI cells increased following increases in the jaw-movement distance and duration.* ***Conclusion:*** *These findings suggest that the dorsal portion of the rostral Vc may be involved in mediating chronic pain following TMJ inflammation and that the intracellular ERK cascade may be involved.* J OROFAC PAIN 2007; 21:225–231

Key words: chronic inflammation, extracellular signal-regulated kinase, jaw movement, temporomandibular joint, trigeminal spinal nucleus

Acute inflammation of the temporomandibular joint (TMJ) region causes sensitization of C-fibers in the TMJ capsule as well as neurons in the trigeminal spinal nucleus caudalis (Vc; also termed the medullary dorsal horn).[1–3] However, little is known about the neuronal mechanisms underlying chronic TMJ pain following peripheral inflammation.

The extracellular signal-regulated kinase (ERK) is 1 of the mitogen-activated protein kinases activated by calcium influx in dorsal root ganglion (DRG) and dorsal horn (DH) neurons.[4–7] It has been reported that C-fiber but not A-fiber stimulation induces phosphorylated ERK (pERK) expression in DH neurons.[5] It has been also reported that pERK expression peaks in the DRG and in DH neurons within 10 minutes after noxious stimulation.[4,7,8] Recently, Shimizu et al reported that pERK-LI cells are expressed in Vc within 10 minutes following capsaicin stimulation of the tooth pulp.[8] These data suggest that pERK is a potential marker of the excitation of DH neurons following noxious stimulation.

Thus, it was decided to analyze the change in pERK expression following jaw movement in rats with chronic TMJ inflammation in order to elucidate the neuronal mechanisms underlying chronic TMJ pain.

Materials and Methods

Lateral face temperature measurement (inflamed rats: n = 5, naive rats: n = 5), histologic analysis of the TMJ (2 days after injection of complete Freund's adjuvant [CFA]: n = 5; 14 days after CFA injection: n = 5), and pERK immunohistochemistry (inflamed rats: n = 45; naive rats: n = 45) were performed on male Sprague-Dawley rats (250 to 365 g).

The study was approved by the Animal Experimentation Committee at Nihon University School of Dentistry. The animals were treated according to the guidelines of the International Association for the Study of Pain.[9]

CFA Injection into the TMJ Region and Measurement of the Lateral Face Temperature

Rats were anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneally). The inflammatory agent CFA was suspended in an oil-saline (1:1) emulsion, and a volume of 0.05 mL was injected into the left TMJ region through the facial skin with a 27-gauge needle under an optical microscope. One day later, rats were anesthetized, and the face temperature on the ipsilateral and contralateral sides was measured by a computer-assisted infrared thermograph (Thermo tracer TH3100ME, NEC-SANEI).

Passive Jaw Movement

Fourteen days after CFA injection, rats were anesthetized with sodium pentobarbital (50 mg/kg intraperitoneally) and placed on a flat plate. The gingiva around the mandibular incisors was locally anesthetized with 2% lidocaine. The hook of an electrically controlled vibrator was then placed on the gingiva around the mandibular incisors. Passive jaw movement was applied at 1 Hz (distance: 4 mm, 6 mm, and 15 mm; duration: 5 minutes, 15 minutes, and 30 minutes, n = 5 in each group). Passive jaw movement was also applied to naive rats (n = 5 in each group).

pERK Immunohistochemistry

Rats were perfused through the aorta with 200 mL 0.9% saline followed by 500 mL 4% paraformaldehyde in 0.1 mol/L phosphate buffer (PBS, pH 7.4) 5 minutes after passive jaw movement. Sections 30 μm wide were cut from the brainstem, and every fourth section was collected in PBS. Since a detailed pERK immunohistochemical procedure was reported previously,[8] the procedure is only briefly described here. Free-floating tissue sections were incubated in rabbit anti-Phospho-p44/42 MAP kinase antibody (1:1000, Cell Signaling Technology) and biotinylated goat anti-rabbit IgG (1:600; Vector Labs). The pERK-like immunoreactive (LI) cells were visualized using 0.035% 3,3'-diaminobenzidine-tetra HCl (DAB, Sigma).

Under a light microscope, the pERK-LI cells were drawn using a camera lucida drawing tube. The number of pERK-LI cells was counted from every eighth section. The total number of pERK-LI cells from 3 of these sections was calculated, and the mean number of pERK-LI cells (per section) was obtained for each rat.

Statistical Analysis

Statistical analysis was performed using analysis of variance (ANOVA) followed by a Fisher protected least significant difference (LSD) test, a Newman-Keuls test, a Dunnett test, or a Scheffé test. A Student *t* test or Welch's *t* test was also used as appropriate. Differences were considered significant at $P < .05$. Results are presented as means ± standard error of the mean (SEM).

Results

Face Temperature and TMJ Inflammation

The face temperature was significantly increased on the ipsilateral side compared to the contralateral side 2 days after CFA injection into the left TMJ and gradually decreased thereafter (Figs 1a and 1b). High-temperature areas were widely distributed over the whole lateral face, sometimes extending to the neck region (Fig 1a). The accumulation of a large number of inflammation-related cells, such as neutrophils and macrophages, was observed 2 days after CFA injection (Fig 2a). Many inflammation-related cells were packed in the space between the condylar head and capsule. However, no inflammation-related cells were observed at 14 days after CFA injection (Fig 2b).

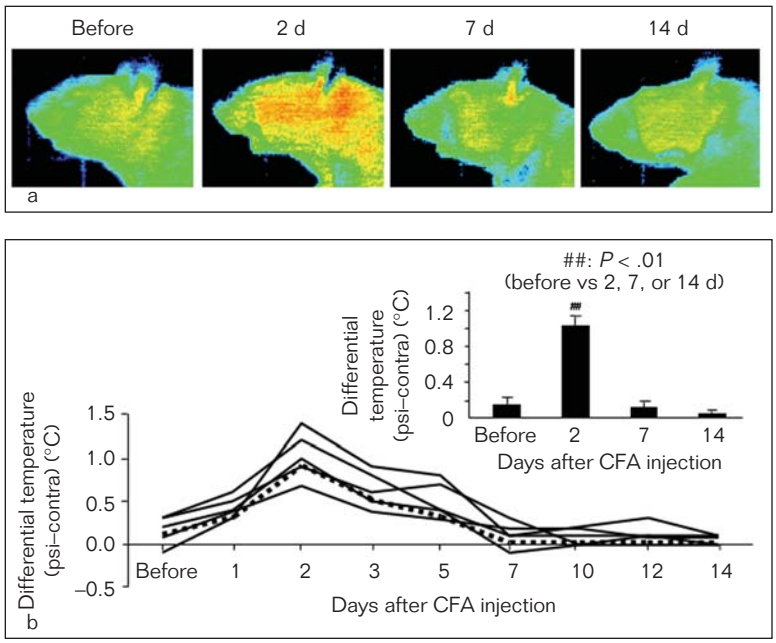


Fig 1 Change in face temperature following CFA injection into the left TMJ. *(a)* Typical example of the lateral face temperature following CFA injection. *(b)* Time-course of change in face temperature. The inset graph indicates the mean temperature difference between the ipsilateral and contralateral sides before and 2, 7, and 14 days after CFA injection. Dotted line in *b* indicates median face temperature.

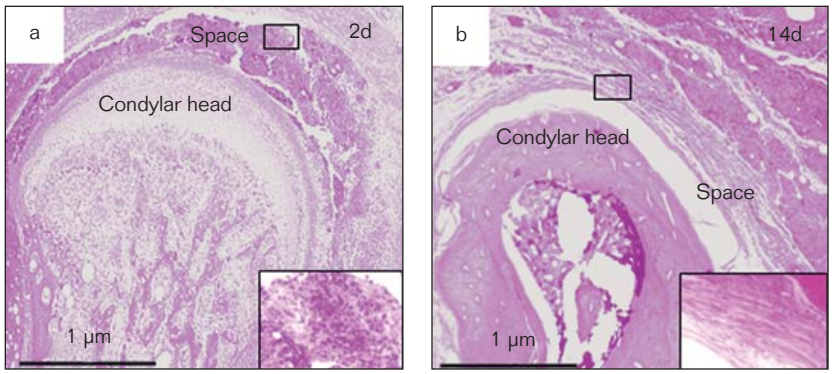


Fig 2 The TMJ capsule *(a)* 2 days and *(b)* 14 days after CFA injection into the left TMJ region. Insets are high-magnification photomicrographs of the areas indicated by the smaller squares.

Modulation of pERK Expression Following Change in Distance and Duration of Jaw Movement

A large number of pERK-LI cells were expressed in the superficial laminae of the dorsal portion of the ipsilateral Vc following 15 mm of passive jaw movement for 15 minutes at 14 days after TMJ CFA injection, as illustrated in Figs 3a to 3c. The pERK-LI cells were ipsilaterally dominant. pERK-LI cells were observed on the side contralateral to the CFA injection as well (Fig 3d). A small number of pERK-LI cells were expressed bilaterally in the Vc following 15 mm of passive jaw movement for 15 minutes in the rats without CFA (Figs 3e and 3f). However, no pERK-LI cells were observed in the TMJ-inflamed rats without jaw movement, as illustrated in Fig 3g.

The rostro-caudal distribution of pERK-LI cells for different jaw-movement distances and durations is illustrated in Figs 4a to 4c. A small number of pERK-LI cells were expressed following jaw movement in naive rats (Fig 4b). However, the number and the distribution area of pERK-LI cells were increased in the rostral Vc following jaw movements in the CFA-treated rats (Fig 4c). The increase in the number and distribution area of pERK-LI cells was predominantly seen on the side ipsilateral to the CFA injection site, but slight change was observed on the contralateral side.

The relationship between the mean number of pERK-LI cells and jaw movement duration and distance is illustrated in Figs 4d and 4e. More pERK-LI cells were expressed following 15 and 30 minutes of jaw movement with 15 mm of jaw movement than with 4 mm of jaw movement on

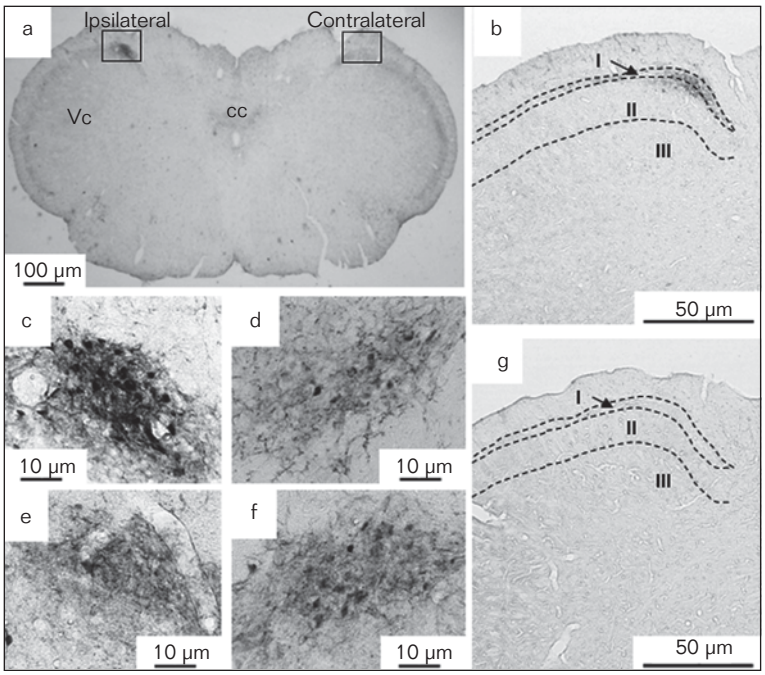


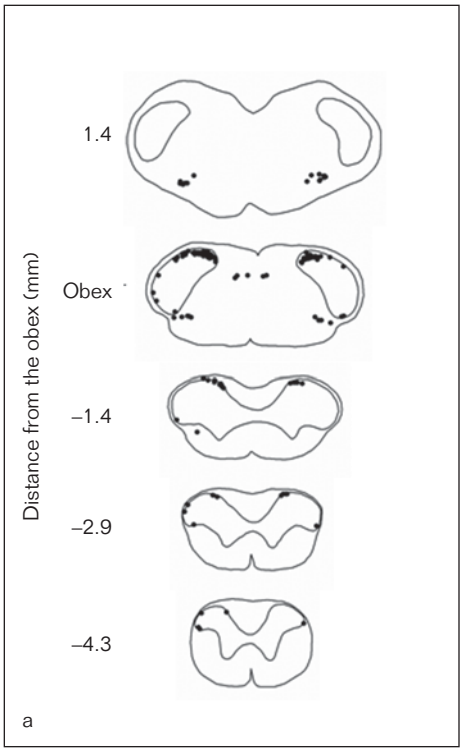
Fig 3 pERK-LI cells in Vc. *(a)* Vc at low magnification following 15 mm of jaw movement for 15 minutes at 14 days after CFA injection into the left TMJ. *(b and c)* The ipsilateral Vc at high magnification following 15 mm of jaw movement for 15 minutes at 14 days after CFA injection into the left TMJ. *(d)* The contralateral Vc at high magnification following 15 mm of jaw movement for 15 minutes *(e and f)* The ipsilateral and contralateral Vc, respectively, at high magnification in a naive rat following 15 mm of jaw movement for 15 minutes. *(g)* The ipsilateral Vc at high magnification in a CFA-injected rat without jaw movement. I = lamina I, II = lamina II, III = lamina III.

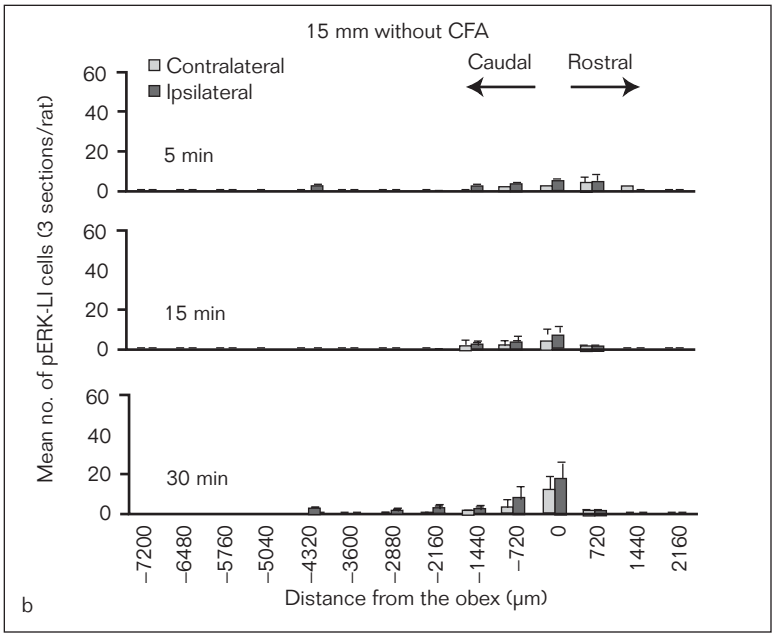
the side ipsilateral to CFA injection ($P < .01$). A stronger expression of pERK-LI cells was observed at the 15-mm distance compared to 4 mm for 30 minutes of jaw movement. The number of pERK-LI cells was also significantly larger in rats, with longer duration of jaw movement on the ipsilateral side to CFA injection after 15 mm of jaw movement (Fig 4d). However, no obvious increase in the number of pERK-LI cells was observed on the contralateral side (Fig 4e).

Discussion

An increase in the face temperature occurred after CFA injection into the TMJ region. It is well known that a change in skin temperature of an inflamed region is a good indicator of inflammation.[10,11] Face temperature was maximal 2 days after the CFA injection. Evans' blue extravasation was measured in previous studies to verify the extent of inflammation.[12,13] It has been reported that Evans' blue extravasation in the TMJ capsule is significantly increased 2 to 3 days after TMJ CFA injection. These findings suggest that acute inflammation became maximal 2 to 3 days after CFA injection. No increase in face temperature was noted 14 days after CFA injection, suggesting that the TMJ inflammation was in a chronic state at this period.

It has been reported that CFA injection into the TMJ region induces a variety of changes in Vc neuronal activity.[1] Three days after CFA injection, receptive field size was significantly expanded, and background activity and heat-evoked responses were significantly increased. These changes in neuronal activity are thought to result from central sensitization of the Vc nociceptive neurons. The Vc nociceptive neurons modulated by CFA injection into the TMJ region were distributed in the middle portion of the Vc. On the other hand, jaw movement caused a strong expression of pERK-LI cells in the dorsal portion of rostral Vc following CFA injection into the TMJ region in the present study. It is likely that many rostral Vc neurons are recruited during jaw movement in rats with chronic TMJ inflammation. In a single-unit recording study, the limited location of the cells responding to TMJ CFA-induced inflammation (the middle part of Vc) was probably due to the limited sampling in those experiments, and it is likely that recordings in the dorsomedial part of the rostral Vc would also have revealed evidence of hyperexcitability.[1] In the present study, it was observed that the ERK phosphorylation was enhanced following an increase in the duration and distance of jaw movement in the rats with chronic TMJ inflammation. A slight increase in pERK-LI cells was also observed in the noninflamed rats after





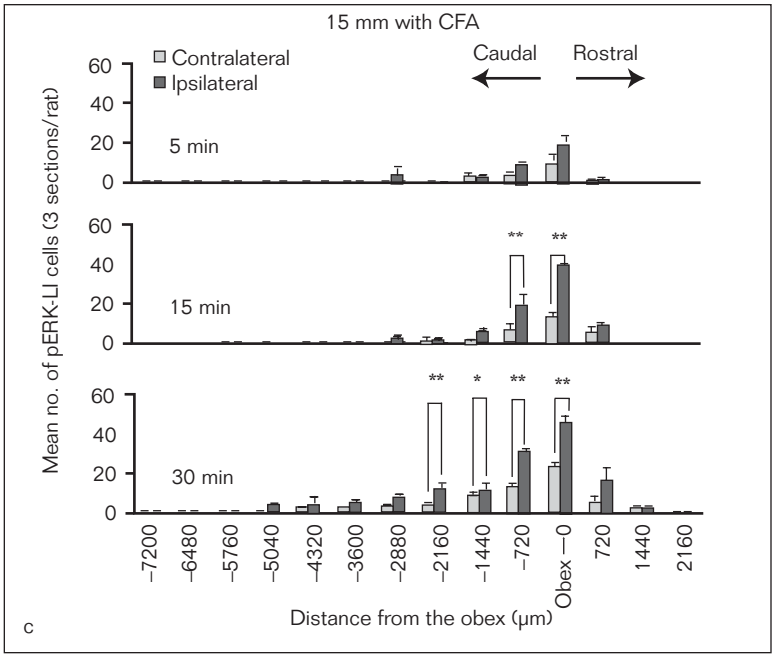
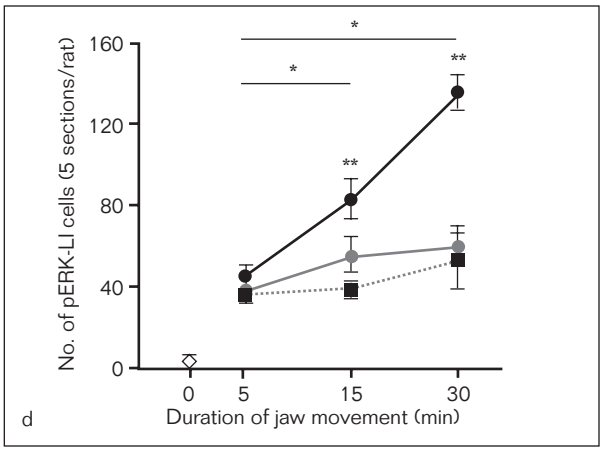
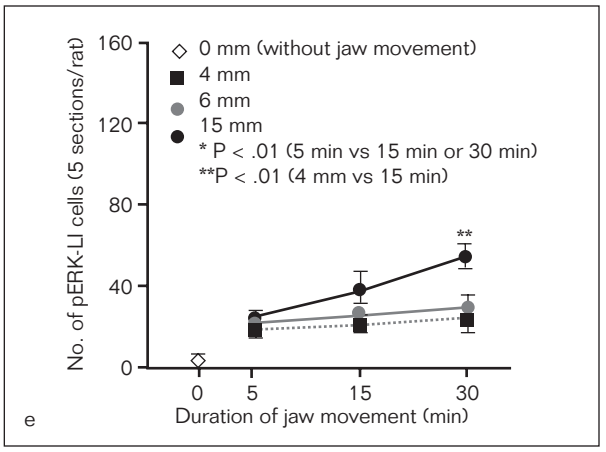


Fig 4 The rostro-caudal distribution of pERK-LI cells in the Vc and upper cervical spinal cord following jaw movement in the rats with TMJ CFA treatment. *(a)* Camera lucida drawings of sections from Vc and upper cervical spinal cord in the CFA-injected rat with jaw movement (distance: 15 mm, duration: 30 minutes). *(b and c)* Rostro-caudal distribution of pERK-LI cells in the rats with CFA injection following passive jaw movement. *(d and e)* Change in the mean number of pERK-LI cells in *(d)* the ipsilateral and *(e)* the contralateral Vc and upper cervical cord following an increase in the jaw movement distance and duration. $^{*}P < .05$; $^{**}P < .01$.





jaw movement. It is very important that pERK was expressed only after jaw movement; this indicates that ERK was not phosphorylated under chronic TMJ inflammation unless mechanical stimulation was also present. It has been reported that Fos-positive neurons are expressed in the Vc and upper cervical cord after CFA injection into the TMJ region of rats with and without jaw movement.[13–16] This suggests that the Fos-LI cells are expressed as the result of an increase in spontaneous and evoked activities following TMJ inflammation. On the other hand, ERK phosphorylation occurs as the result of evoked activity but not spontaneous activity. This suggests that the intracellular ERK cascade is involved in the dynamic responses of nociceptive neurons in Vc.

It has been reported that many Fos-LI cells are expressed in the Vi/Vc, Vc, and the upper cervical cord following CFA injection into the TMJ region.[13–16] A large number of pERK-LI cells were found in the present study in the dorsal portion of the rostral Vc and overlapped with the areas where Fos protein-LI cells have been reported to be expressed after TMJ inflammation. However, significant expression of pERK-LI cells in the upper cervical spinal cord in the CFA-treated rats following passive jaw movement was not observed. It has been reported that Fos protein is expressed in spinal dorsal horn neurons 0.5 to 1.0 hours following noxious stimulation of the skin, whereas pERK is expressed within 10 minutes. The time-course difference in expression between pERK and Fos protein may reflect the distribution difference of positive neurons in the Vi/Vc zone and upper cervical spinal cord. Furthermore, there are a number of intracellular transduction pathways that produce Fos protein. The ERK phosphorylation cascade is thought to be 1 of the pathways for Fos production. It is likely that the different intracellular transduction cascades for Fos production and ERK phosphorylation are involved in the distribution differences between pERK and Fos-positive neurons.

It is probable that the pERK expression in the rostral Vc neurons is an earlier event in TMJ nociception compared to that of Fos expression. Together with previous Fos studies, the present ERK study strongly suggests that the Vi/Vc neurons, rostral Vc neurons, and the upper cervical cord neurons are involved in chronic TMJ pain.

Conclusions

A large number of phosphorylated extracellular signal-regulated kinase-like immunoreactive cells were observed in lamina I of the dorsal portion of the rostral Vc of TMJ-inflamed rats after passive jaw movement. The number of pERK-LI cells gradually increased following increases in jaw movement, distance, and duration. These findings suggest that neurons in the dorsal portion of the rostral Vc may be involved in chronic TMJ pain following TMJ inflammation through an intracellular MAP kinase signal transduction cascade that involves ERK phosphorylation.

Acknowledgments

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