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Analgesic Effect of Intrathecal Administration of Orexin on Neuropathic Pain in Rats

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Abstract. *Orexin-A, a hypothalamic peptide found in the neurons of the lateral hypothalamus, has been shown to modulate pain. We examined whether orexin could alleviate heat-evoked hyperalgesia in rats caused by chronic constriction injury (CCI) of the sciatic nerve. Orexin-A, orexin-B, the vehicle, or orexin-A-antiserum was intrathecally administered to CCI rats. Paw withdrawal latency (PWL) was measured from 30 to 300 minutes after injection, which was repeated for 2 days. Orexin-A administration normalized ΔPWL (PWL in the CCI side minus PWL in the control side) and inhibited heat-evoked hyperalgesia in CCI rats, while orexin-A antiserum inhibited the normalization of heat-evoked hyperalgesia caused by orexin-A two-fold. In contrast, orexin-B had no significant effect. These results suggest that orexin-A may be applicable for treatment of neuropathic pain.*

A hypothalamic peptide known as orexin (hypocretin) is found in the neurons of the lateral hypothalamus (1, 2) and has been reported to be involved with feeding behavior and energy homeostasis (3), sleep/wakefulness (4, 5), the sympathetic nervous system (6) and pain (7-9). Further, some reports have noted that the peptide may be an important anti-nociceptive and anti-hyperalgesic agent (7-9). Orexin fibers also have a role in sensory processing, while the presence of robust projections from the hypothalamus to lamina 1 of the spinal cord strongly implicates orexin in the modulation of nociceptive pathways (8, 10). In addition, results of behavioral tests using an inflammatory pain model suggested that orexin-A is a potent analgesic (7, 9).

Peripheral inflammation induced by intra-plantar injection of carrageenan and formalin are models of inflammatory pain in animals, while chronic constriction injury (CCI) of the sciatic nerve (11), one of the most widely used models for the study of neuropathic pain, has been reported to induce an inflammatory response in the ipsilateral hind paw (12, 13). The aim of the present study was to investigate the alleviation of heat-evoked hyperalgesia by orexin in a rat neuropathic pain model as well as the effect of orexin antiserum.

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Key Words: Orexin, neuropathic pain, CCI model, rat.

Materials and Methods

Fifty-one adult (approximately 7 weeks old) Sprague-Dawley rats, weighing between 200 and 300 g, were housed in individual cages with a 12-hour light/dark cycle (lights on at 8:00 am) and a constant room temperature of 23+/-2˚C, with access to food *ad libitum*. Each rat was anesthetized with sodium pentobarbital (50 mg/kg, *i.p.*, supplemented as necessary) and an experimental nerve injury was induced in the right hind limb. According to the method described in detail in a previous report (11), CCI was induced using 4 loosely constrictive ligatures of 4.0 chromic gut tied around the right sciatic nerve at mid-thigh level. In each animal, an identical contralateral dissection was also performed, omitting the ligatures (sham surgery as a control). The muscle and skin tissues were then closed in layers. Following surgery, the rats were housed individually in cages for 10 days. Orexin-A and orexin-B (Peptide Institute, Inc., Osaka, Japan) were dissolved in saline and given as a bolus in volumes of 1, 10, or 30mcg/10 μl, followed by 10 μl of saline to wash the peptide. Orexin-A-antiserum (Peptide Institute, Inc.) (1:500 or 1:5000 dilution) was given as a bolus in 10 μl, followed by 10 μl of saline to wash the antiserum. For the intrathecal injection, polyethylene tubing (Intramedic™ PE-10, Becton Dickinson and Co. Japan, Tokyo, Japan) was passed through a slit in the dura between segments T11-T12 and T12-T13

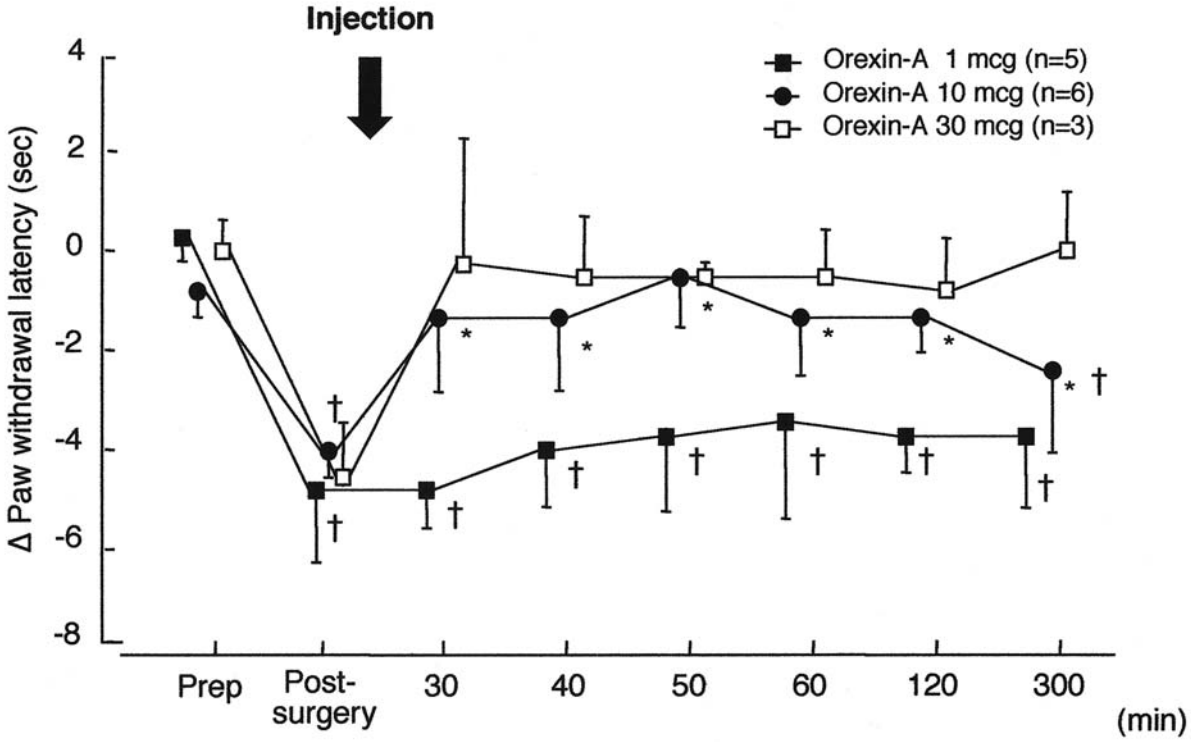

Figure 1. *Analgesic effect of orexin-A on heat-evoked hyperalgesia.*
ΔPWL results are displayed as mean±SD. In the orexin-A 30 mcg group (open square), data is presented without statistical analysis because the number was too small. On post-surgical day 10, ΔPWL was significantly decreased as compared with that at pre-surgery in the orexin-A 1 mcg (filled square) and 10 mcg (filled circle) groups. Thirty minutes after intrathecal administration of orexin-A, ΔPWL was nearly 0 in the orexin-A 10 mcg and 30 mcg groups. The effects continued until 300 minutes after administration.
†: $p<0.01$ vs. preparation (prep) within a group.
**: $p<0.01$ vs. after surgery within a group.*

and the position of the tubing was verified visually at the end of each experiment. The experimental protocols were approved by the institutional animal care committee of the Division of Clinical Medical Science, Graduate School of Biomedical Sciences, Hiroshima University, Japan.

The thermal nociceptive threshold was measured using a device similar to that described by Hargreaves *et al.* (14). Paw withdrawal latency (PWL) was measured on both sides with 10-minute intervals between successive trials. Of the 51 rats, 37 showed ipsilateral PWL that was at least 1 standard deviation (SD) shorter than the mean PWL in the controls. These were used as CCI model rats.

An additional group of rats were given an administration of 10 or 30 mcg of orexin-A and used as normal controls.

The CCI model rats were divided into 3 groups according to the administration of orexin-A, orexin-B, or the vehicle, which were each given as an intrathecal administration of 1, 10, or 30 mcg (orexin-A), 10 mcg (orexin-B), or 0 mcg (vehicle), with 10 μl saline from 10 to 12 days after the surgery. In the 3 orexin-A groups, PWL was measured at 30, 40, 50, 60, 120 and 300 minutes. In the orexin-A 10 mcg and orexin-B 10 mcg groups, PWL was also measured 1, 2 and 3 days following injection. Rats who received orexin-A at 10 mcg were measured for PWL at 30, 40 and 50 minutes, then 1 hour after administration they received an intrathecal administration of orexin-A-antiserum and on the first and second day following injection were measured for PWL at 30, 40, 50 and 120 minutes. Orexin-A antiserum (Peptide Institute, Inc.) (1:500 or 1:5000 dilution) was given as a bolus in 10 μl, followed by 10 μl of saline to wash the antiserum. These doses of orexin-A antiserum had been previously shown to significantly attenuate the orexin-A-induced pressor response.Differences in PWL (ΔPWL: PWL in CCI side minus PWL in control side) among groups were analyzed using a Kruskal-Wallis test, followed by a Mann-Whitney *U*-test. Sequential differences in ΔPWL within a group were analyzed using one-way ANOVA, followed by Fischer's predicted least significant difference test. $p<0.05$ was considered statistically significant.

Results

All CCI rats showed ipsilateral PWL that was at least 1 standard deviation (SD) shorter than the mean PWL in the

Table I. *Paw withdrawal latency (PWL).*

		Baseline	POD 7	PTT 30**	PTT 40**	PTT 50**	PTT 60**	PTT 120**	PTT 300**
Orexin-A 1 mcg	Sham-side	13.7±1.5	13.9±1.0	14.6±2.3	14.1±1.3	14.1±1.9	13.0±2.0	13.3±2.0	14.6±1.0
	CCI-side	13.8±1.3	9.0±1.4 *	9.8±1.8*	10.0±0.5*	10.5±1.2*	9.6±2.5*	9.6±1.8*	10.7±1.6*
Orexin-A 10 mcg	Sham-side	12.4±1.1	12.8±2.1	13.2±2.5	12.4±2.5	10.8±2.0	10.4±2.0	13.1±2.4	12.7±1.9
	CCI-side	11.8±0.9	8.8±1.8 *	12.0±2.6	10.9±2.9	10.4±2.3	9.7±2.2	11.8±2.2	10.4±2.2 *
Orexin-A 30 mcg	Sham-side	11.1±1.5	12.1±0.6	12.1±1.6	12.9±2.9	12.1±1.0	11.8±1.1	14.4±0.5	14.7±0.2
	CCI-side	11.2±1.0	7.2±0.8	11.7±1.1	12.4±1.7	11.5±1.1	11.2±0.3	13.6±0.9	14.6±1.0

Baseline: 1 week before surgery, POD: post-operative day, PTT: post treatment time
*$p<0.05$ compared with sham side, by Wilcoxon signed-ranks test
** Minutes
Orexin-A 30 mcg group was not subjected to statistical analysis due to the small number (n=3).
Data are shown as mean ± SD

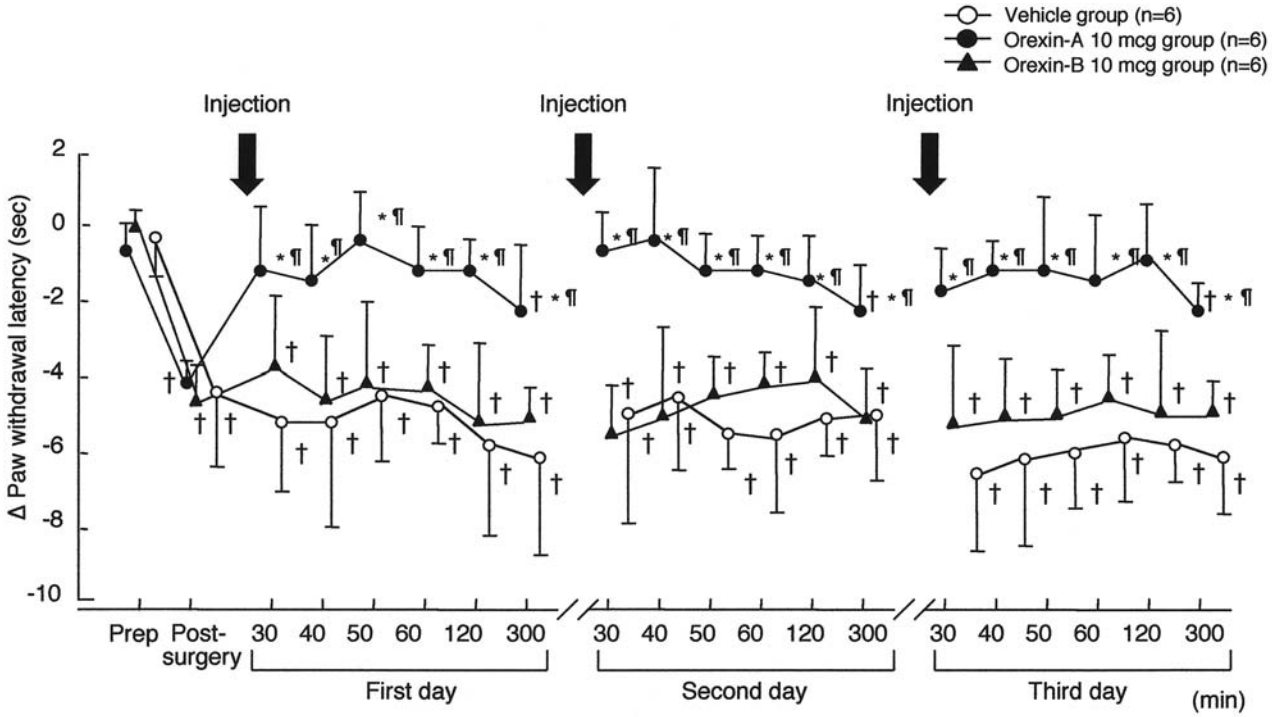

Figure 2. *Analgesic effect of orexin or the vehicle on heat-evoked hyperalgesia.*
In the orexin-A 10 mcg group (filled circle), ΔPWL was nearly 0 following intrathecal administration and then significantly increased. In the orexin-B 10 mcg (filled triangle) and vehicle (open circle) groups, ΔPWL following intrathecal injection did not significantly change.
†: $p<0.01$ vs. preparation (prep) within a group.
**: $p<0.01$ vs. after surgery within a group.*
: $p<0.05$ vs. vehicle group.

normal controls. Orexin-A at 1, 10 and 30 mcg did not change PWL in the normal rats (data was not shown). However, orexin-A at 10 and 30 mcg normalized ΔPWL in the CCI rats, while doses of intrathecal orexin-A greater than 10 mcg showed an analgesic effect (Figure 1, Table I). Orexin-A normalized ΔPWL by three-fold from 30 to 120 minutes (Figure 2), however, at 300 minutes after injection, it caused a reduction of ΔPWL.

There were no significant differences between the orexin-B and vehicle groups and orexin-B did not inhibit heat-evoked hyperalgesia (Figure 2).

The first injection of orexin-A normalized ΔPWL, while orexin-A antiserum, given 1 hour after orexin-A, inhibited that normalization (Figure 3). Both doses of orexin-A antiserum, 1:500 and 1:5000, inhibited the normalization of heat-evoked hyperalgesia by orexin-A two-fold.

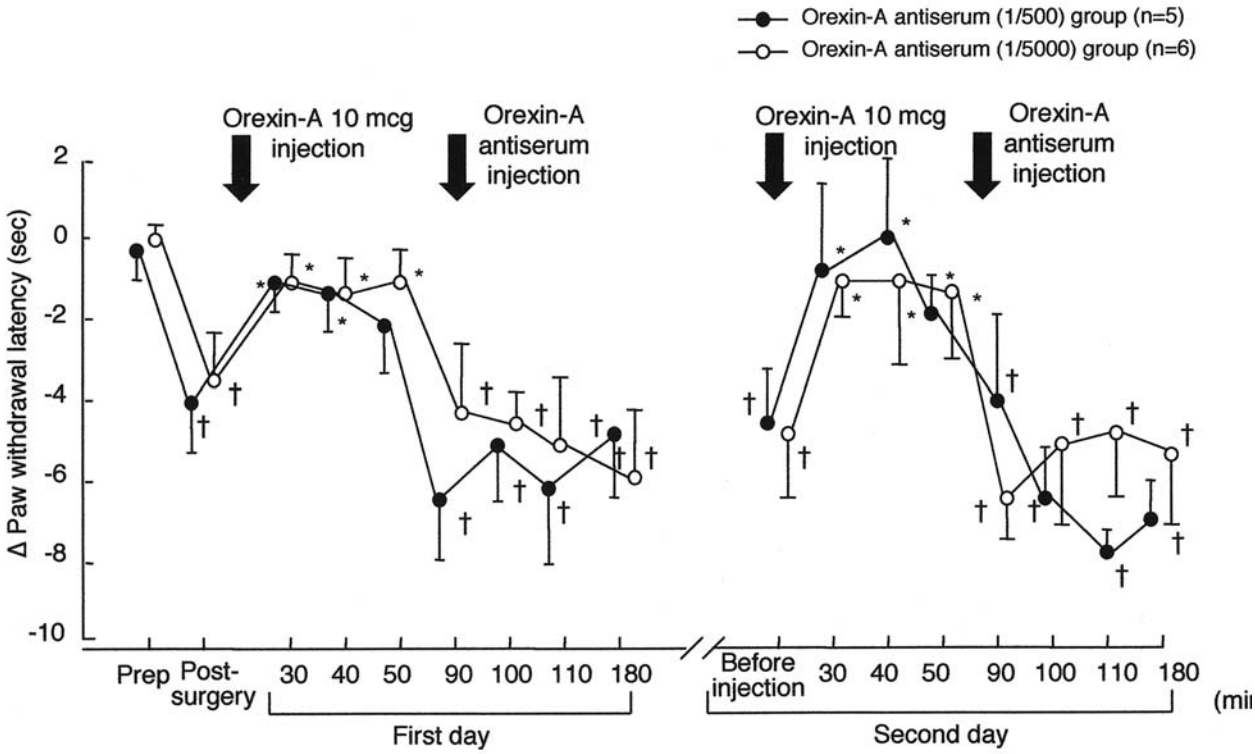

Figure 3. *Effects of orexin-A and orexin-A antiserum on heat-evoked hyperalgesia.*
†: p<0.01; vs. preparation within a group.
**: p<0.01; vs. after surgery within a group.*

Discussion

The present results are the first known to demonstrate the alleviation of heat-evoked hyperalgesia by orexin-A in CCI rats, whereas orexin-B had no effect. Orexin-A has been shown to have an anti-nociceptive effect in thermal and visceral nociceptive tests using mice and rats, as well as an anti-hyperalgesic effect in animals with carrageenan-induced thermal hyperalgesia when given *via* intravenous and intracerebral-ventricular routes (7). In another examination, intrathecal injection of orexin-A, but not orexin-B, decreased the sum of flinches in phase 1 and 2 in a formalin test and increased hot plate latency (9).

Peripheral inflammation induced by intra-plantar injection of carrageenan and formalin are models of inflammatory pain in animals. Further, experimental CCI of the sciatic nerve (11) is one of the most widely used models for the study of neuropathic pain and has been reported to induce an inflammatory response in the ipsilateral hind paw (12, 13). We previously reported the usefulness of etodolac, a cyclooxygenase 2 inhibitor, in the treatment of neuropathic pain using this model (15).

Orexin-B is also known to have excitatory effects on certain superficial dorsal horn neurons, some of which exert inhibitory influences on other cells in the region; this is consistent with the idea that orexin has a role in orchestrating reactions related to arousal, including nociceptive pain and temperature sense (8). However, in the present experiments, intrathecal orexin-A suppressed heat-evoked hyperalgesia in CCI rats, whereas orexin-B did not. Therefore, orexin-B probably has an insignificant role in the regulation of pain or nociceptive response in the central nervous system.

In the present study, when an intrathecal administration of orexin-A-antiserum was used to inhibit the action of orexin-A, slight but insignificant changes of mean arterial pressure and heart rate were seen, whereas orexin-A alone significantly induced a pressor response (16). The present results also showed that orexin-A antiserum attenuated orexin-A-mediated heat-evoked hyperalgesia. Although naloxone had no effect on orexin-A-mediated responses in mice with carrageenan-induced thermal hyperalgesia and rats with formalin-induced inflammatory pain, the orexin receptor antagonist SB-334867 inhibited those responses (7, 9). Further, pre-treatment with an NMDA antagonist, though

not a serotonin antagonist, to the trigeminal motor nucleus abolished the excitatory response of the masseter muscle to orexin-A application (17). As a result, we suggest that the anti-nociceptive effect of orexin-A may be related to the NMDA receptor in CCI rats, without activation of the opiate or serotonergic systems.

In conclusion, an intrathecal administration of orexin-A alleviated heat-evoked hyperalgesia in CCI rats, suggesting that it is applicable for the treatment of neuropathic pain, whereas orexin-B showed no such protective effect.

References

1 De Lecea L, Kilduff TS, Peyron C, Gaorexin-B, Foye PE, Danielson PE, Fukuhara C, Battenberg ELF, Gautvik VT, Bartlett II FS, Frankel WN, van den Pol AN, Bloom FE, Gautvik KM and Sutcliffe JG: The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. Proc Natl Acad Sci USA *95*: 322-7, 1998.

2 Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, Williams SC, Richardson JA, Kozlowski GP, Wilson S, Arch JR, Buckingham RE, Haynes AC, Carr SA, Annan RS, McNulty DE, Liu WS, Terrett JA, Elshourbagy NA, Bergsma DJ and Yanagisawa M: Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. Cell *92*: 573-85, 1998.

3 Toshinai K, Date Y, Murakami N, Shimada M, Mondal MS, Shimbara T, Guan JL, Wang QP, Funahashi H, Sakurai T, Shioda S, Matsukura S, Kangawa K and Nakazato M: Ghrelin-induced food intake is mediated *via* the orexin pathway. Endocrinology *144*: 1506-12, 2003.

4 Chemelli RM, Willie JT, Sinton CM, Elmquist JK, Scammell T, Lee C, Richardson JA, Williams SC, Xiong Y, Kisanuski Y, Fitch TE, Nakazato M, Hammer RE, Saper CB and Yanagisawa M: Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. Cell *98*: 437-51, 1999.

5 Overeem S, Scammell TE and Lammers GJ: Hypocretin/orexin and sleep: implications for the pathophysiology and diagnosis of narcolepsy. Curr Opin Neurol *15*: 739-45, 2002

6 Shirasaka T, Nakazato M, Matsukura S, Takasaki M and Kannan H: Sympathetic and cardiovascular actions of orexins in conscious rats. Am J Physiol Regul Integr Comp Physiol *277*: R1780-5, 1999.

7 Bingham S, Davey PT, Babbs AJ, Irving EA, Sammons MJ, Wyles M, Jeffrey P, Cutler L, Riba I, Johns A, Porter RA, Upton N, Hunter AJ and Parsons AA: Orexin-A, an hypothalamic peptide with analgesic properties. Pain *92*: 81-90, 2001.

8 Grudt TJ, van den Pol AN and Perl ER: Hypocretin-2 (orexin-B) modulation of superficial dorsal horn activity in rat. J Physiol *538*: 517-25, 2002.

9 Yamamoto T, Nozaki-Taguchi N and Chiba T: Analgesic effect of intrathecally administered orexin-A in the rat formalin test and in the rat hot plate test. Br J Pharmacol *137*: 170-6, 2002.

10 Van den Pol AN: Hypothalamic hypocretin (orexin): robust innervation of the spinal cord. J Neurosci *19*: 3171-82, 1999.

11 Bennett GJ and Xie YK: A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. Pain *33*: 87-107, 1988.

12 Daemen MARC, Kurvers HAJM, Kitslaar PJEHM, Slaaf DW, Bullens PHJ and Van den Wildenberg FAJM: Neurogenic inflammation in an animal model of neuropathic pain. Pain *20*: 41-5, 1998.

13 Okamoto K, Martin DP, Schmelzer JD, Mitsui Y and Low PA: Pro- and anti-inflammatory cytokine gene expression in rat sciatic nerve chronic constriction injury model of neuropathic pain. Exp Neurol *169*: 386-91, 2001.

14 Hargreaves K, Dubner R, Brown F, Flores C and Joris J: A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. Pain *32*: 77-88, 1988.

15 Suyama H, Gaus S, Moriwaki K, Kawamoto M and Yuge O: Effect of COX2 inhibitor (etodorac) on neuropathic pain in a rat model. Anesthesiology *93*: A-931, 2000. (www.asa-abstracts.com)

16 Antunes VR, Brailoe GC, Kwok EH, Scruggs P and Dun NJ: Orexins / hypocretins excite rat sympathetic preganglionic neurons *in vivo* and *in vitro*. Am J Physiol Regul Integr Comp Physiol *281*: R1801-7, 2001.

17 Peever JH, Lai YY and Siegel JM: Excitatory effects of hypocretin-1 (orexin-A) in the trigeminal motor nucleus are reversed by NMDA antagonism. J Neurophysiol *89*: 2591-600, 2003.

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