Published in final edited form as:

West J Nurs Res. 2016 March ; 38(3): 292–307. doi:10.1177/0193945915610083.

Lateral Hypothalamic Stimulation Reduces Hyperalgesia Through Spinally-Descending Orexin-A Neurons in Neuropathic Pain

Jacob Wardach¹, Monica Wagner¹, Younhee Jeong², and Janean E. Holden^{1,2,†}

¹The University of Michigan School of Nursing, 400 N. Ingalls Bldg, Ann Arbor, MI 48109-5482, Tel: 01-734-763 7667; Fax 01-734-936-5525

²College of Nursing Science, Kyung Hee University, 26 Kyunghee-daero, Dongdaemun-gu, Seoul 130-701, Korea, Tel: 82-2-961-2210; Fax: 82-2-961-9398

Abstract

No evidence to date shows that lateral hypothalamic (LH) stimulation produces orexin-A mediated antinociception in the spinal cord dorsal horn (SCDH) in a model of neuropathic pain. We conducted experiments to examine the effect of orexin-A-mediated LH stimulation in female rats with chronic constriction injury (CCI) on thermal hyperalgesia. Rats receiving carbachol into the LH demonstrated antinociception on both the left CCI and right non-ligated paws ($p < 0.05$). Rats were given carbachol in the LH followed by intrathecal injection of the orexin-1 receptor (OX1) antagonist SB-334867, which blocked LH-induced antinociception compared to control groups ($p < 0.05$) in the left paw, but not in the right paw. These findings support the hypothesis that LH stimulation produces antinociception in rats with thermal hyperalgesia from neuropathic pain via an orexin-A connection between the LH and the SCDH. Identification of this pathway may lead to studies using orexins to manage clinical pain.

Neuropathic pain is caused by a lesion or disease of the somatosensory nervous system. One out of ten people suffers from neuropathic pain (Cunha et al., 2015; Gilron, Baron, & Jensen, 2015), and the World Health Organization (WHO) considers it a major public health issue (2006). However, the management of neuropathic pain remains challenging because it doesn't respond consistently to the commonly used opioid and NSAIDs analgesics.

Although there has been some improvement in treatment options based on research on neuropathic pain, patients report that their pain is not managed effectively, and neuropathic pain negatively affects their daily life activities and quality of life (Berger, Dukes, & Oster, 2004; Cocito et al., 2006; Daousi, Benbow, Woodward, & MacFarlane, 2006; Werhagen, Hultling, & Molander, 2007). Consequently, it is necessary to pursue new options for neuropathic pain treatment. One approach to improve the management is to understand innate pain modulation mechanisms and apply them to develop appropriate interventions.

[†]Corresponding author holdenje@umich.edu.
wardach@umich.edu, mawag@umich.edu, yjeong2@khu.ac.kr

There is now substantial evidence that the lateral hypothalamus (LH) is part of a descending system that modulates nociception in the spinal cord dorsal horn and that stimulating the LH produces analgesia (antinociception), although the mechanisms for this effect have not been fully determined (Dafny et al., 1996; Holden et al., 2014). One way that the LH facilitates antinociception may be through spinally descending orexin neurons.

The orexins are neuropeptides with cell bodies located in the hypothalamus and particularly in the LH/perifornical region (Peyron et al., 1998; van den Pol, Gao, Obrietan, Kilduff, & Belousov, 1998). Orexin-A and orexin-B preferentially activate orexin-1 receptors (OX₁) and orexin-2 receptors (OX₂), respectively (Gotter, Webber, Coleman, Renger, & Winrow, 2012).

Anatomical and behavioral evidence support a role for LH orexin neurons in nociceptive processing in the dorsal horn. Previous work in male rodents has shown that orexin-A and orexin-B, and their respective receptors OX₁ and OX₂, are densely located in the superficial lamina of the spinal cord, an area known to be involved in nociception (Cluderay, Harrison, & Hervieu, 2002; Guan et al., 2004). In behavioral studies, differences have been observed for orexin-A and orexin-B. Orexin-B either increases nociception (Bartsch, Levy, Knight, & Goadsby, 2004), produces less potent antinociception than orexin-A (Cheng, Chou, Hwang, & Chiou, 2003), or produces no effect (Suyama et al., 2004). Orexin-A given intravenously or intrathecally, and selectively acting at OX₁ receptors, produces significant antinociception (Holland, Akerman, & Goadsby, 2005; Jeong & Holden, 2009b; Sakurai et al., 1998). Orexin-A-mediated antinociception is blocked by intrathecal injection of the OX₁ receptor antagonist, SB-334867 (Kajiyama et al., 2005). However, there are no studies to date that report that directly stimulating the LH in perifornical area produces antinociception mediated by OX₁ receptors in the dorsal horn.

The purpose of this study is to examine whether LH-induced antinociception is mediated in part by spinally descending orexin-A neurons. To test the hypothesis, we conducted three behavioral experiments in which we: (1) stimulated the LH with the cholinergic agonist carbamoyl chloride (carbachol); (2) gave the OX₁ receptor antagonist SB-334867 intrathecally in a dose that blocks the effects of orexin-A (Cheng et al., 2003; Jeong & Holden, 2009b); and (3) evaluated intrathecal microinjections for control. The chronic constriction injury (CCI) was used to model thermal hyperalgesia of neuropathic pain (Bennett & Xie, 1988). The paw withdrawal latency (PWL) was used to test responses to a thermal stimulus.

Methods

The Institutional Animal Care Committees at the University of Michigan approved the respective experimental protocols used in this study. The experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, revised 1996). All efforts were made to minimize animal suffering, reduce the numbers of animals used, and use alternatives to in vivo experiments.

Animals

Female Sprague-Dawley rats (250-350 g; Charles River, Portage, MI) were used in this study and were randomly assigned to either treatment or control groups.

To reduce the possibility of estrous cycle influence, no two rats were taken from the same cage on the same day because female cage mates are known to cycle together. All rats were maintained on a 12-hour day/night schedule with free access to food and water. To reduce the risk of mirror image effects on the non-ligated paw in the CCI protocol (Kim & Chung, 1997), we used separate control animals rather than having each animal serve as its own control and no comparisons between left and right PWLs were made. A total of 63 rats were used for behavioral study. Thirteen rats were excluded for improper cannula placement or surgical anesthesia attrition. Fifty rats were included in data analysis as reported, and each rat was used only once.

Procedures

CCI procedure—This procedure has been outlined in detail elsewhere (Holden et al., 2014). Briefly, under isoflurane anesthesia, the left thigh of the rat was infused with bupivacaine (0.5%; 0.10 ml) and the common sciatic nerve was exposed at the level of the mid-thigh. Proximal to the sciatic trifurcation, four ligatures (4.0 chromic gut) were tied loosely around the nerve about 1 mm apart. The length of the nerve affected was 4 to 5 mm long. The ligatures were tied to lightly constrict the diameter of the nerves, and standardized by observing for the initial twitching of the paw as the ligature was tightened. The muscle was sutured with 4.0 chromic gut and the incision closed with wound clips. Each rat received a subcutaneous injection of buprenorphine (0.3 mg/ml) at a dose of 0.05 mg/kg, was recovered, and returned to its cage. Fourteen days after the CCI procedure, rats were tested for thermal hyperalgesia.

Experiment 1: Carbachol or saline microinjection in the LH—Each rat was lightly anesthetized with intraperitoneal sodium pentobarbital 35 mg/kg, which removes conscious perception of pain but leaves withdrawal reflexes intact. The scalp was then infused with the local anesthetic lidocaine (1%; 0.15 ml). Immediately after anesthesia, the rats were immobilized in a stereotactic apparatus which restrains the head and allows manipulation of a microinjector on three planes. Using aseptic technique, a 2-cm incision was made, and the muscle and fascia retracted. A 23-gauge stainless steel guide cannula was lowered through a burr hole into the region of the left LH defined by the following stereotactic coordinates: AP −1.5 mm from bregma, lateral +1.6 mm, vertical +2.2 mm, incisor bar set at −2.5 mm. A 30-gauge stainless steel injection cannula was connected to a 10 µl syringe by a length of PE-10 polyethylene tubing filled with either saline or a solution of carbachol (500 nmol) in normal saline injected in a volume of 0.5 µl (Sigma Chemical Co., St. Louis, MO). All solutions were made fresh daily and filtered through a 0.2 µm syringe prior to use. The injection cannula was then inserted and extended approximately 3 mm beyond the end of the guide cannula. After a baseline response latency measurement was taken and recorded, either saline or carbachol was injected into the LH over a 1 min period using an electronic syringe pump. The injection cannula was left in place for an additional 60 sec following completion of the microinjection to reduce flow of drug up the guide cannula. Response latencies were

then measured at 5 min intervals until a return to baseline or for 60 min. In a separate group of rats the LH was pretreated with the cholinergic antagonist atropine sulfate (5 µg/0.5 µl NS) microinjected over 1 min, followed by carbachol (500 nmol) microinjection and paw withdrawal latency measurement.

Experiment 2: Carbachol microinjection in the LH followed by intrathecal microinjection of orexin-A antagonist—In addition to preparation for cerebral microinjection as described above, each rat had an intrathecal catheter constructed from PE-10 polyethylene tubing inserted through an incision in the cisterna magna and the tip positioned near the lumbar enlargement. Following carbachol microinjection into the LH as above, PWL was measured at 1, 5, and 10 min. Then, either SB-334867 (dissolved in DMSO; Sigma Chemical Co.), or DMSO or physiological saline was injected intrathecally in a volume of 30 µl using an electronic syringe pump at a rate of 30 µl/min. Paw response latencies were then measured at 1 min post injection and then every 5 min for additional 45 min for a total of 60 min. Because DMSO is known to have anesthetic properties, we used saline as a comparison for control.

Experiment 3: Intrathecal injection only—In the third experiment, rats were prepared for intrathecal cannulation only as previously described. After a baseline response latency, either SB-334867 (30 nmol), DMSO, or saline was microinjected into the lumbar space in a volume of 30 µl and response latencies were measured at 1 min and then every 5 min for 45 min.

Analgesiometric testing procedures—To determine the effect of LH stimulation on thermal nociception, the paw withdrawal test was used. The paw withdrawal response to noxious stimuli, especially to noxious radiant heat, is a valid and useful measurement of unilateral changes in nociceptive responses, and has been commonly used to test nociception in rodents (Holden et al., 2014; Yeomans & Proudfit, 1994). In this procedure, the hairy surface of the hindpaw was exposed to a focused beam of radiant heat using an infrared analgesimeter (37360, Ugo Basile, Italy). The skin of the hairy surface of the paw is relatively less affected by environmental conditions than the plantar surface with its thicker foot pads (Yeomans & Proudfit, 1994).

The radiant beam of infrared light was adjusted to a maximum intensity of 145 mW/cm² and the time interval between the onset of skin heating and the withdrawal response was measured electronically. The intensity of the analgesimeter is consistent at the level of ±1%. The longer the latency, the greater the antinociceptive effect. In the absence of a response, skin heating was terminated after 15 sec to prevent burning. Response latencies represent the average within group time to withdrawal for each time point. Baseline response latencies were approximately 5-8 sec. Temperature was measured with a rectal probe at baseline and at 50 min. Heart rate, blood pressure, and mean arterial pressure were measured pre-injection and following the final latency measurement using a tail cuff and Coda monitor (Kent Scientific; Torrington, CT, USA).

Histology—Following testing, animals were overdosed with sodium pentobarbital and decapitated. The brains were taken and drop fixed in a solution of 10% neutral-buffered

formalin. To determine the position of the microinjection sites relative to the LH, 40- μ m transverse brain sections were cut from blocks of tissue that contained the visible injection cannula tract using a cryostat microtome. The sections were rinsed in cold phosphate-buffered saline PBS, (10 mM), mounted on gel-coated slides, stained with 0.05% neutral red, dehydrated through a series of alcohols and xylenes, and cover slipped. The placement of the microinjection cannula was determined by locating the most ventral position of the cannula tip in serial sections by brightfield microscopy. Tracings of the appropriate sections were then made using the Neurolucida imaging system (Microbrightfield, Colchester, VT). The tracings were compared with drawings from the atlas of Paxinos and Watson to verify that the cannula was within the LH (Paxinos & Watson, 2009). The data were excluded if the cannula was not in the LH. Verification of approximate intrathecal cannula location was made following laminectomy. India ink was injected into the cannula and the tip was visualized in relation to the dorsal horn. Data from rats in which the cannula was positioned in the ventral horn were excluded.

Statistical Analysis

Treatment groups consisted of between 5 and 11 rats. Paw withdrawal response latencies are presented as the mean $\pm$ SEM. Statistical comparisons among treatment groups across multiple time points were made using two-way repeated measures ANOVA, and comparisons among means at specific time points were made using the Holm-Sidak test for multiple post-hoc comparisons. The analysis was generated using SigmaPlot software, version 11.0 of the Systat Software for Windows.

Results

Microinjector Placement

Figure 1 represents microinjector placement in rats that received carbachol microinjection in Experiment 1. Most of the injection sites were located within the area defined as the LH (Paxinos & Watson, 2009). One microinjection of carbachol outside of the LH in the ventral thalamus produced latency change for nociception. Because it is well known that areas around the LH produce antinociception when stimulated, data were analyzed only for microinjections within the LH in all experiments.

Experiment 1: Effect of Carbachol Microinjection in the LH on Nociception

Microinjection of carbachol in the LH at sites shown in Fig. 1 produced antinociception on left (ligated side) paw responses compared to baseline latencies. Comparisons between groups showed a significant effect of carbachol compared to normal saline (two way repeated measures $p < 0.001$; Fig. 2A). Post hoc comparisons demonstrated that rats receiving carbachol in the LH kept their left paw on the thermal stimulus significantly longer than control rats (9.09 ± 0.24 vs 5.21 ± 0.23 sec; carbachol vs saline; $p < 0.05$), an analgesic effect. Atropine pretreated rats withdrew their paw faster than carbachol treated rats (5.5 ± 0.26 sec; $p < 0.05$) and did not significantly differ from control rats, indicating that the cholinergic agonist, carbachol, was acting at cholinergic receptors on cells in the LH.

On the non-ligated right side, carbachol produced significantly longer PWL compared to other groups ($p = 0.002$; Fig. 2B). Carbachol treated rats had significantly longer withdrawal latencies than both saline control rats and rats pretreated with atropine (8.54 ± 0.68 vs 4.64 ± 0.63 and 6.00 ± 0.73 sec, carbachol, saline and atropine pretreated groups, respectively; $p < 0.05$), while atropine rats were not significantly different from the saline control group.

There were no significant differences among the three groups (carbachol, saline, or atropine/carbachol) for systolic or diastolic blood pressure or mean arterial pressure, nor were there any significant effects from giving carbachol in the LH for either heart rate or temperature (data not shown; $p > 0.05$).

Experiment 2: Carbachol-Induced Antinociception: Effect of Intrathecal Injection of the Orexin-A Antagonist SB-334867

Because DMSO, the vehicle for SB-334867, has known analgesic properties, both DMSO and normal saline withdrawal latencies were compared. There was no statistical difference between intrathecal injection of saline and DMSO groups in the left (8.99 ± 0.28 vs 8.39 ± 0.38 sec; $p > 0.05$) and right PWL (9.71 ± 0.38 vs 8.90 ± 0.49 sec; $p > 0.05$), so DMSO was used as vehicle control for subsequent comparisons. Following microinjection of carbachol in the LH at sites similar to those in Fig. 1, intrathecal injection of the orexin-1 receptor antagonist SB-334867 significantly altered left PWL ($p < 0.001$; Fig. 3A). Intrathecal injection of SB-334867 blocked carbachol-induced antinociception compared to DMSO control (5.37 ± 0.33 vs 8.39 ± 0.38 sec) after 1 min post intrathecal injection lasting through 45 min.

Figure 3B shows that intrathecal microinjection of SB-334867 following carbachol-induced LH antinociception produced no significant change in right PWL compared to rats given DMSO (8.01 ± 0.42 vs 8.90 ± 0.49 sec; $p > 0.05$).

Experiment 3: Intrathecal injection only

In the absence of LH stimulation, there were no significant differences among groups on either the left or the right paw latencies for any intrathecal injection (data not shown, $n = 5$ per group; $p > 0.05$). This finding indicates that the effect of SB-334867 was not due to volume of solution in the dorsal horn, that SB-334867 did not have any agonist effect, and that this orexin pathway was not tonically active.

Discussion

Findings from the present study provide evidence that LH stimulation activates spinally projecting orexin-A neurons acting at OX_1 receptors in the dorsal horn to produce antinociception in rats with thermal hyperalgesia from CCI. Our behavioral studies support and extend the work of Suyama et al. (2004) who showed that intrathecal application of orexin-A, but not orexin-B, decreases thermal hyperalgesia in CCI rats; the work of Yamamoto, Saito, Shono, Aoe, & Chiba (2003), who used partial sciatic nerve ligation, and found that intrathecal or intracerebroventricular administration of orexin-A attenuated mechanical allodynia, another sign of neuropathic pain; the work of Kajiyama et al. (2005) who found that intrathecal orexin-A, but not B, attenuated mechanical allodynia and thermal

hyperalgesia in a model of diabetic neuropathy; and the work of Jeong and Holden (2009b) who found that stimulating the posterior hypothalamus (PH) produced orexin-A mediated antinociception in CCI rats.

The mechanisms whereby orexin-A produces antinociception in the dorsal horn have not been fully identified. In dorsal horn neurons, both orexin-A and orexin-B are excitatory, producing post-synaptic depolarization by inhibiting K⁺ channels (Doroshenko & Renaud, 2009). Although orexins have been shown to act presynaptically in the hypothalamus to release GABA and glutamate (Acuna-Goycolea & van den Pol, 2009), these presynaptic mechanisms have not been demonstrated in the dorsal horn. It is possible that, in our experiments, orexin-A was acting at OX₁ receptors located on presynaptic terminals from brainstem neurons that inhibit transmission of nociceptive responses, such as GABA or glycine terminals, long known to descend from nucleus raphe magnus and periaqueductal gray (Willis & Westlund, 1997). Orexin-A could also be acting postsynaptically on inhibitory interneurons that reduce thermal hyperalgesia (Bardoni et al., 2013). While Leonard and Kukkonen (2014) reported on the work of Ishibashi, Gumenchuk, and Leonard (2012) showing that orexin-A can inhibit neuronal excitability in serotonergic dorsal raphe neurons through enhanced small-conductance Ca²⁺ activated K⁺ channels, this inhibitory action for orexin-A has not been shown elsewhere. Taken together, these findings underscore the need for further study in the mechanisms of orexins-induced decrease of thermal hyperalgesia.

In the present study, only the left sciatic nerve was ligated. Stimulating the left LH with carbachol produced antinociception on both the left and right paws as shown in Fig. 2. While the LH projection to the spinal cord is primarily ipsilateral (Hancock, 1976), the LH also sends projections to brainstem areas that in turn project to both the ipsilateral and contralateral dorsal horn, including the rostral ventromedial medulla that provides serotonin to the dorsal horn (Antal, Petko, Polgar, Heizmann, & Storm-Mathisen, 1996) and the pontine A7 catecholamine cell group that provides norepinephrine to the dorsal horn (Clark & Proudfoot, 1991). However, we found that intrathecal application of SB-334867 blocked orexin-A-induced antinociception in the left paw, but not the right non-ligated paw. This finding supports the idea that the orexin-A projection from the LH to the dorsal horn is primarily ipsilateral.

We chose not to do microinjector placement controls for this study, in which we deliberately stimulate areas around the LH to determine responses to carbachol. We have previously shown that microinjection of carbachol into areas adjacent to the LH, including the ventral thalamus (Holden et al., 2014; Jeong & Holden, 2009a), the nigrostriatal bundle (Holden, Van Poppel, & Thomas, 2002) and the internal capsule (Holden et al., 2002, 2014; Holden, Farah, & Jeong, 2005) produces withdrawal latencies similar to baseline measurements. It has also been well-established that areas adjacent to the LH, including the PH (Jeong & Holden, 2009b; Jeong, Moes, Wagner, & Holden, 2012), the dorsal hypothalamic nucleus (Biagioni et al., 2013) and certain places in the thalamus (Son, Kim, Kim, & Lee, 2014) all produce antinociception. To repeat placement controls to show what is already known does not correspond with our commitment to reduce the number of animals used in our studies whenever possible.

A limitation of the present study is the difficulty in determining the precise size of injection. As indicated previously (Holden et al., 2014; Jeong et al., 2012), many factors contribute to injection size, including the features of the solution, injector tip size, and neuroanatomical features of the injection site (Lum, Nguyen, & Felpel, 1984; Myers & Hoch, 1978; Nicholson, 1985; Sakai, Swartz, & Woody, 1979). Our smaller injection size and the relatively hydrophilic nature of carbachol limit the average spread of injection (Grossman & Stumpf, 1969; Martin, Knuepfer, & Westfall, 1991; Myers & Hoch, 1978). Additionally, we showed previously that carbachol microinjection into the PH in an area directly adjacent to the LH produces antinociception that was blocked by intrathecal injection of SB-334867 (Jeong & Holden, 2009b). The exact borders of the PH and LH are not clearly defined, and it is reasonable to assume that some of the solution from our microinjections crossed the border into the PH. Nevertheless, the compelling fact is that orexin-containing neurons are only in the hypothalamus, and particularly the LH and perifornical area. This fact then supports the evidence that our microinjections stimulated orexins-containing neurons because we excluded microinjections outside the LH and we blocked the antinociceptive effect of orexin-A effect with a specific orexin-A intrathecal antagonist.

We used female rats in this study. Sex differences have been an issue in clinical and experimental settings. Almost half of publications do not report the sex of rats and the rest use predominantly males (Fillingim, King, Ribeiro-Dasilva, Rahim-Williams, & Riley, 2009; Mogil & Chanda, 2005). Holden et al. (2014) demonstrated sex differences as well as pain type differences for LH stimulation; however, testing both males and females is beyond the scope of the present study, which was not focused on sex differences, but on the pathway itself. Lack of male/female comparisons is a limitation and further comparisons of sex differences are needed regarding orexins and the orexins pathway.

In summary, the results of the present study may be combined with the results from other studies to provide converging anatomical and functional evidence in support of the hypothesis that the LH mediates antinociception in part through a direct orexin-A connection with the spinal cord dorsal horn. The full role of the LH in descending modulation of nociception remains to be determined. Defining the role of orexins in pain is a fundamental process that can lead to further studies targeting the LH-dorsal horn orexins pathway. It is possible that therapeutics aimed at activating the LH could ultimately produce improved clinical pain management composed of both pharmacological and non-pharmacological treatments. These treatments include not only drugs that promote orexin-mediated analgesia, but also deep brain stimulation, which is currently being done for conditions such as Parkinson's disease, or relaxation and massage therapy that involves hypothalamic function. Definition of the pathway is the first necessary step to using it in clinical applications.

Acknowledgments

This work is supported by USPHS grant NR04778 from the National Institute of Nursing Research (NINR) at the National Institutes of Health (JEH).

References

- Acuna-Goycolea C, van den Pol AN. Neuroendocrine proopiomelanocortin neurons are excited by hypocretin/orexin. *Journal of Neuroscience*. 2009; 29(5):1503–1513. doi:10.1523/JNEUROSCI.5147-08.2009. [PubMed: 19193897]
- Antal M, Petko M, Polgar E, Heizmann C, Storm-Mathisen J. Direct evidence of an extensive GABAergic innervation of the spinal dorsal horn by fibres descending from the rostral ventromedial medulla. *Neuroscience*. 1996; 73(2):509–518. [PubMed: 8783266]
- Bardoni R, Takazawa T, Tong C, Choudhury P, Scherrer G, MacDermott AB. Pre and postsynaptic inhibitory control in the spinal cord dorsal horn. *Annals of the New York Academy of Sciences*. 2003; 1279(1):90–96.
- Bartsch T, Levy M, Knight Y, Goadsby P. Differential modulation of the nociceptive dural input to [hypocretin] orexin A and B receptor activation in the posterior hypothalamic area. *Pain*. 2004; 109(3):367–378. [PubMed: 15157698]
- Bennett GJ, Xie Y. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. *Pain*. 1988; 33(1):87–107. [PubMed: 2837713]
- Berger A, Dukes EM, Oster G. Clinical characteristics and economic costs of patients with painful neuropathic disorders. *Journal of Pain*. 2004; 5(3):143–149. doi:10.1016/j.jpain.2003.12.004. [PubMed: 15106126]
- Biagioni AF, de Freitas RL, da Silva JA, de Oliveira RC, de Oliveira R, Alves VM, Coimbra NC. Serotonergic neural links from the dorsal raphe nucleus modulate defensive behaviours organised by the dorsomedial hypothalamus and the elaboration of fear-induced antinociception via locus coeruleus pathways. *Neuropharmacology*. 2013; 67:379–394. [PubMed: 23201351]
- Cheng JK, Chou RC, Hwang LL, Chiou LC. Antiallodynic effects of intrathecal orexins in a rat model of postoperative pain. *Journal of Pharmacology and Experimental Therapeutics*. 2003; 307(3):1067–1071. doi:10.1124/jpet.103.056663.
- Clark FM, Proudfit HK. The projection of noradrenergic neurons in the A7 catecholamine cell group to the spinal cord in the rat demonstrated by anterograde tracing combined with immunocytochemistry. *Brain Research*. 1991; 547(2):279–288. [PubMed: 1884202]
- Cluderay J, Harrison D, Hervier G. Protein distribution of the orexin-2 receptor in the rat central nervous system. *Regulatory Peptides*. 2002; 104(1):131–144. [PubMed: 11830288]
- Cocito D, Paolasso I, Pazzaglia C, Tavella A, Poglio F, Ciaramitaro P, Padua L. Pain affects the quality of life of neuropathic patients. *Neurological Sciences*. 2006; 27(3):155–160. doi:10.1007/s10072-006-0660-5. [PubMed: 16897626]
- Currow DC, Clark K, Kamal A, Collier A, Agar MR, Lovell MR, Ritchie C. The population burden of chronic symptoms that substantially predate the diagnosis of a life-limiting illness. *Journal of Palliative Medicine*. 2015; 18(6):480–485. doi:10.1089/jpm.2014.0444. [PubMed: 25859908]
- Dafny M, Dong W, Prieto-Gomez C, Reyes-Vazquez C, Stanford J, Qiao J. Lateral hypothalamus: Site involved in pain modulation. *Neuroscience*. 1996; 70(2):449–460. [PubMed: 8848153]
- Daousi C, Benbow SJ, Woodward A, MacFarlane IA. The natural history of chronic painful peripheral neuropathy in a community diabetes population. *Diabetic Medicine*. 2006; 23(9):1021–1024. [PubMed: 16922710]
- Doroshenko P, Renaud L. Acid-sensitive TASK-like K conductances contribute to resting membrane potential and to orexin-induced membrane depolarization in rat thalamic paraventricular nucleus neurons. *Neuroscience*. 2009; 158(4):1560–1570. [PubMed: 19135504]
- Fillingim RB, King CD, Ribeiro-Dasilva MC, Rahim-Williams B, Riley JL III. Sex, gender, and pain: A review of recent clinical and experimental findings. *Journal of Pain*. 2009; 10(5):447–485. doi:10.1016/j.jpain.2008.12.001. [PubMed: 19411059]
- Gilron I, Baron R, Jensen T. Neuropathic pain: Principles of diagnosis and treatment. *Mayo Clinic Proceedings*. 2015; 90(4):532–545. [PubMed: 25841257]
- Gotter AL, Webber AL, Coleman PJ, Renger JJ, Winrow CJ. International union of basic and clinical pharmacology. LXXXVI. Orexin receptor function, nomenclature and pharmacology. *Pharmacological Reviews*. 2012; 64(3):389–420. doi:10.1124/pr.111.005546. [PubMed: 22759794]

- Grossman SP, Stumpf WE. Intracranial drug implants: An autoradiographic analysis of diffusion. *Science* (New York, N. Y.). 1969; 166(3911):1410–1412.
- Guan J, Wang Q, Hori T, Takenoya F, Kageyama H, Shioda S. Ultrastructure of orexin-1 receptor immunoreactivities in the spinal cord dorsal horn. *Peptides*. 2004; 25(8):1307–1311. [PubMed: 15350698]
- Hancock M. Cells of origin of hypothalamo-spinal projections in the rat. *Neuroscience Letters*. 1976; 3(4):179–184. [PubMed: 19604883]
- Holden JE, Farah EN, Jeong Y. Stimulation of the lateral hypothalamus produces antinociception mediated by 5-HT 1A, 5-HT 1B and 5HT 3 receptors in the rat spinal cord dorsal horn. *Neuroscience*. 2005; 135(4):1255–1268. [PubMed: 16165284]
- Holden JE, Van Poppel AY, Thomas S. Antinociception from lateral hypothalamic stimulation may be mediated by NK 1 receptors in the A7 catecholamine cell group in rat. *Brain Research*. 2002; 953(1):195–204. [PubMed: 12384253]
- Holden JE, Wang E, Moes JR, Wagner M, Maduko A, Jeong Y. Differences in carbachol dose, pain condition, and sex following lateral hypothalamic stimulation. *Neuroscience*. 2014; 270:266–235.
- Holland PR, Akerman S, Goadsby PJ. Orexin 1 receptor activation attenuates neurogenic dural vasodilation in an animal model of trigeminovascular nociception. *Journal of Pharmacology and Experimental Therapeutics*. 2005; 315(3):1380–1385. [PubMed: 16160082]
- Ishibashi, M., Gumenchuk, I., Leonard, CS. Orexin-A enhances a slow afterhyperpolarization (sAHP) and slows steady firing in serotonergic dorsal raphe (DR) neurons. Poster session presented at the meeting of the Society for Neuroscience; New Orleans, LA. Oct, 2012
- Jeong Y, Holden JE. Lateral hypothalamic-induced alpha-adrenoceptor modulation occurs in a model of inflammatory pain in rats. *Biological Research for Nursing*. 2009a; 10(4):331–339. doi: 10.1177/1099800408325053. [PubMed: 19114413]
- Jeong Y, Holden JE. The role of spinal orexin-1 receptors in posterior hypothalamic modulation of neuropathic pain. *Neuroscience*. 2009b; 159(4):1414–1421. [PubMed: 19409203]
- Jeong Y, Moes JR, Wagner M, Holden JE. The posterior hypothalamus exerts opposing effects on nociception via the A7 catecholamine cell group in rats. *Neuroscience*. 2012; 227:144–153. [PubMed: 23036619]
- Kajiyama S, Kawamoto M, Shiraishi S, Gaus S, Matsunaga A, Suyama H, Yuge O. Spinal orexin-1 receptors mediate anti-hyperalgesic effects of intrathecally-administered orexins in diabetic neuropathic pain model rats. *Brain Research*. 2005; 1044(1):76–86. [PubMed: 15862792]
- Kim KJ, Chung JM. Comparison of three rodent neuropathic pain models. *Experimental Brain Research*. 1997; 113(2):200–206. [PubMed: 9063706]
- Leonard C, Kukkonen J. Orexin/hypocretin receptor signaling: A functional perspective. *British Journal of Pharmacology*. 2014; 171(2):294–313. [PubMed: 23848055]
- Lum JT, Nguyen T, Felpel LP. Drug distribution in solid tissue of the brain following chronic local perfusion utilizing implanted osmotic minipumps. *Journal of Pharmacological Methods*. 1984; 12(2):141–147. [PubMed: 6536817]
- Martin JR, Knuepfer MM, Westfall TC. Hemodynamic effects of posterior hypothalamic injection of neuropeptide Y in awake rats. *American Journal of Physiology*. 1991; 261:H814–H824. 3 Pt. 2. [PubMed: 1887927]
- Mogil JS, Chanda ML. The case for the inclusion of female subjects in basic science studies of pain. *Pain*. 2005; 117(1-2):1–5. [PubMed: 16098670]
- Myers R, Hoch D. 14 C-dopamine microinjected into the brain-stem of the rat: Dispersion kinetics, site content and functional dose. *Brain Research Bulletin*. 1978; 3(6):601–609. [PubMed: 318183]
- Nicholson C. Diffusion from an injected volume of a substance in brain tissue with arbitrary volume fraction and tortuosity. *Brain Research*. 1985; 333(2):325–329. [PubMed: 3995298]
- Paxinos, G., Watson, C. The rat brain in stereotaxic coordinates. Academic Press; San Diego, CA: 2009.
- Peyron C, Tighe DK, van den Pol AN, de Lecea L, Heller HC, Sutcliffe JG, Kilduff TS. Neurons containing hypocretin (orexin) project to multiple neuronal systems. *Journal of Neuroscience*. 1998; 18(23):9996–10015. [PubMed: 9822755]

- Sakai M, Swartz BE, Woody C. Controlled micro release of pharmacological agents: Measurements of volume ejected in vitro through fine tipped glass microelectrodes by pressure. *Neuropharmacology*. 1979; 18(2):209–213. [PubMed: 431788]
- Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, Yanagisawa M. Orexins and orexin receptors: A family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell*. 1998; 92(4):573–585. [PubMed: 9491897]
- Son BC, Kim DR, Kim HS, Lee SW. Simultaneous trial of deep brain and motor cortex stimulation in chronic intractable neuropathic pain. *Stereotactic and Functional Neurosurgery*. 2014; 92(4):218–226. doi:10.1159/000362933. [PubMed: 25073491]
- Suyama H, Kawamoto M, Shiraishi S, Gaus S, Kajiyama S, Yuge O. Analgesic effect of intrathecal administration of orexin on neuropathic pain in rats. In *Vivo* (Athens, Greece). 2004; 18(2):119–123.
- van den Pol AN, Gao XB, Obrietan K, Kilduff TS, Belousov AB. Presynaptic and postsynaptic actions and modulation of neuroendocrine neurons by a new hypothalamic peptide, hypocretin/orexin. *Journal of Neuroscience*. 1998; 18(19):7962–7971. [PubMed: 9742163]
- Werhagen L, Hultling C, Molander C. The prevalence of neuropathic pain after non-traumatic spinal cord lesion. *Spinal Cord*. 2007; 45(9):609–615. [PubMed: 17160075]
- Willis W, Westlund K. Neuroanatomy of the pain system and of the pathways that modulate pain. *Journal of Clinical Neurophysiology*. 1997; 14(1):2–31. [PubMed: 9013357]
- World Health Organization. *Neurological disorders: Public health challenges*. Author; Geneva, Switzerland: 2006.
- Yamamoto T, Saito O, Shono K, Aoe T, Chiba T. Anti-mechanical allodynic effect of intrathecal and intracerebroventricular injection of orexin-A in the rat neuropathic pain model. *Neuroscience Letters*. 2003; 347(3):183–186. [PubMed: 12875916]
- Yeomans DC, Proudfit HK. Characterization of the foot withdrawal response to noxious radiant heat in the rat. *Pain*. 1994; 59(1):85–94. [PubMed: 7854807]

Microinjector Placement

Figure 1.

A schematic depiction of microinjection sites in the LH for PWL in rats receiving 500 nmol of carbachol in Experiment 1. Most of the injection sites were located within the border of the LH between AP -2.12 and -3.30 mm from bregma in the rostro-caudal extent. The symbols represent the PWL at 15 min after microinjection of carbachol as follows: (○) 11-15 sec; (□) 7-10 sec. An injection located in the ventral thalamus (■) elicited antinociception and was excluded from data analysis. Abbreviations: AMG, amygdala; DM, dorsomedial hypothalamic nucleus; f, fornix; ic, internal capsule; LH, lateral hypothalamus; PH, posterior hypothalamus; VM, ventromedial hypothalamic nucleus.

Carbachol Microinjection in the LH Reduced Antinociception in Both the Left and Right Paws

Figure 2.

Experiment 1. Microinjection of carbachol in the LH produced increases in PWL across 60 min of testing. Normal saline or carbachol (500 nmol) was microinjected into the LH at time -1. Time-course changes are shown in (A) for left PWL or (B) for right PWL produced by microinjection of saline (■; n = 8) or carbachol (○; n = 7). In a separate group of rats (◆; n = 6), pretreatment with atropine in the LH blocked PWLs following carbachol microinjection. Mean response latencies ± SEM, $p < 0.05$.

Blocking OX₁ Receptors Decreased Antinociception Only in the Left Paw

Figure 3.

Experiment 2. Antinociception from microinjection of carbachol (500 nmol) in the LH was blocked by intrathecal injection of the OX₁ receptor antagonist SB-334867. (A) Time course of the effects of either SB-334867 in DMSO (○, n = 8) or DMSO (▲, n = 6) injected intrathecally following carbachol microinjection in the LH on left PWL. (B) Time course of the effects of intrathecal injection of either SB-334867 in DMSO or DMSO following carbachol microinjection in the LH on right PWL. Mean response latencies ± SEM.