Opposing effects of cervical spinal cold block on spinal itch and pain transmission

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Abstract

Inactivation of descending pathways enhanced responses of spinal dorsal horn neurons to noxious stimuli, but little is known regarding tonic descending modulation of spinal itch transmission. To study effects of cervical spinal cold block on responses of dorsal horn neurons to itch-evoking and pain-evoking stimuli, single-unit recordings were made from superficial dorsal horn wide dynamic range and nociceptive-specific-type neurons in pentobarbital-anesthetized mice. Intradermal histamine excited 17 units. Cold block starting 1 minute after intradermal injection of histamine caused a marked decrease in firing. The histamine-evoked response during and following cold block was significantly lower compared with control histamine-evoked responses in the absence of cold block. A similar but weaker depressant effect of cold block was observed for dorsal horn unit responses to chloroquine. Twenty-six units responded to mustard oil allyl isothiocyanate (AITC), with a further significant increase in firing during the 1-minute period of cold block beginning 1 minute after AITC application. Activity during cold block was significantly greater compared with the same time period of control responses to AITC in the absence of cold block. Ten units’ responses to noxious heat were significantly enhanced during cold block, while 6 units’ responses were reduced and 18 unaffected. Cold block had no effect on mechanically evoked responses. These results indicate that spinal chemonociceptive transmission is under tonic descending inhibitory modulation, while spinal pruriceptive transmission is under an opposing, tonic descending facilitatory modulation.

Keywords

Itch; Pain; Tonic descending modulation; Spinal dorsal horn; Cold block

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Conflict of interest statement

The authors declare that they have no financial conflict of interest with regard to the content of this report.
Introduction

The spinal transmission of nociceptive information is well known to be under tonic and phasic descending modulatory influences\[^1\text{-}^4\]. Serotonergic, GABAergic and other neurons in the rostral ventromedial medulla (RVM) have descending projections to the spinal cord dorsal horn where they exert presynaptic and postsynaptic inhibitory effects on nociceptive afferent input. It is thought that spinal nociceptive transmission is inhibited by activity of RVM OFF and facilitated by activity of RVM ON cells that are inhibited and excited, respectively, just before the occurrence of a nociceptive reflex\[^5,^6\]. Early studies reported that the effect of reversible spinalization to eliminate descending modulation resulted in an enhancement (disinhibition) of responses of spinal dorsal horn neurons to noxious thermal stimulation, usually with little or no effect on responses to non-noxious mechanical stimuli\[^7\text{-}^"^12\]. For thoracic neurons with viscerosomatic nociceptive input, reversible spinalization had mixed effects with neurons in the dorsal horn exhibiting enhanced responses while more ventrally situated neurons exhibited reduced responses\[^13,^14\]. These studies suggest a predominant tonic descending inhibitory effect on nociceptive transmission through the spinal dorsal horn.

Our knowledge of descending modulation of the spinal transmission of itch-related signals is much more limited. Itch appears to be transmitted by neurons in the superficial dorsal horn of the spinal cord that respond to pruritic as well as algogenic stimulation of the skin (for recent reviews\[^15\text{-}^"^20\]). Recent studies suggest that spinal itch transmission may be under descending noradrenergic\[^21\] and serotonergic\[^22,^23\] modulation. Scratching the skin inhibits the pruritogen-evoked responses of dorsal horn neurons via both segmental and supraspinal mechanisms\[^24\]. There is otherwise little information as to how spinal itch-signaling neurons are modulated by descending pathways. For this reason, we have presently investigated whether spinal pruriceptive neurons are under tonic descending modulation. We hypothesized that cold block of the upper cervical spinal to reduce or eliminate descending influences from the brain would alter the responses of dorsal horn neurons to pruritogenic stimulation of the skin. An abstract of this work has appeared\[^25\].

Methods

The study was approved by the University of California, Davis, Institutional Animal Care and Use Committee. Methods are similar to those described previously\[^26\] and are schematically depicted in Figure 1. A total of 61 Male C57Bl/6 mice were anesthetized with sodium pentobarbital (60 mg/kg ip). Laminectomies were made over the lumbar and upper cervical spinal cords which were bathed in warm saline. Single-unit recordings were made from neurons in the superficial lumbar dorsal horn. In some studies wide dynamic range (WDR)-type neurons were identified by their response to mechanical stimulation of the ipsilateral hindpaw receptive field. WDR units responded at higher frequency to pinch than light brush stimuli. WDR neurons were tested for the effect of cervical cold block on brush and von Frey-evoked responses. Many WDR neurons responded to noxious thermal stimulation of the receptive field using a computer-controlled Peltier thermode that raised the skin temperature from 34 to 52°C over 10 seconds. In some experiments, we specifically searched for chemosensitive units (eg, Akiyama et al\[^26\]), by selecting units that exhibited...
spontaneous firing following a small (0.1–0.5 μL) intradermal (id) injection of histamine or chloroquine in the ipsilateral hindpaw. This search strategy increased the likelihood that the unit responded to a subsequent injection of histamine or chloroquine. After recording responses to id injection of histamine (50 μg in 1 μL) or chloroquine (100 μg in 1 μL), the mechanosensitive receptive field was characterized. Units responsive to pinch but not low-threshold stimuli were categorized as nociceptive-specific. Most units that responded to noxious heat and/or histamine or chloroquine also responded to topical application of mustard oil (80%, 2 μL).

Cold block was accomplished by delivering chilled (0°C) Ringers to the exposed upper cervical spinal cord. This method was previously shown to reduce the intraspinal temperature to 21°C, and was verified to be equivalent to complete cervical transection[24]. After a 1-minute period of cold block, the cervical cord was rapidly rewarmed to body temperature by replacing the chilled saline with warmed saline.

Effects of cold block were tested as follows. For mechanical stimuli, light brushing or an innocuous von Frey monofilament was applied to the mechanosensitive receptive field. Neuronal activity was recorded for the 10 second epochs preceding and following the initiation of each stimulus. Stimuli were repeated once per min. After a stable baseline response was established, the cervical cord was cooled for a period of 1 minute. Forty seconds after initiating the cold block, the next-subsequent mechanical stimulus was applied, followed by rapid rewarmin to body temperature. The effect of the cold block was assessed as the response magnitude during cold block divided by the response magnitude before cold block (within-animal comparison). For thermal stimuli, neuronal activity was recorded for 30 second epochs before and after the onset of heating. Heat stimuli were delivered every 3 minutes. After establishing a stable response level, cold block was initiated. The next-subsequent heat stimulus was delivered 30 seconds after starting the cold block. The effect of the cold block was assessed as the response magnitude to heat during cold block divided by the response magnitude before cold block (within-animal comparison). Responses to mechanical and thermal stimuli with and without cold block were compared by paired t test with P < 0.05 considered to be significant. The effect of cold block on chemically evoked responses was tested as follows. One minute after id injection of histamine or chloroquine, or application of allyl isothiocyanate (AITC), the cold block was initiated for 1 minute. Activity was recorded continuously before and after cessation of the cold block. The effect of cold block was assessed as the response 1–2 and 1–3 minutes after application of the chemical (ie, during and after cold block), divided by the response during the same time intervals in the absence of cold block, recorded in different animals (between-animal comparison). We also compared the difference between the number of action potentials during the peak response (pre-cold block, 0–1 min after chemical application) and post-cold block (1–2 min after chemical application). The idea was that any increase or decrease in firing due to cold block would differ from control units whose firing during the same interval was not influenced by cold block. Responses and response differences pre-cold versus post-cold block were compared using unpaired t tests with P < 0.05 considered to be significant.
At the completion of recording an electrolytic lesion was made at the recording site. The spinal cord was postfixed in 10% paraformaldehyde, cut in 40 μm sections and examined under the light microscope to identify lesion sites.

Results

A total of 64 units was recorded in 61 mice. Forty-six percent were wide dynamic range-type and 54% were nociceptive-specific. The mean recording depth was 153.5 ± 10 (SE) μm below the cord surface. Lesion sites that were histologically recovered were distributed in the superficial dorsal horn (Fig. 2).

An example of a unit’s responses to heat and histamine is shown in Figure 3. This superficial dorsal horn unit gave reproducible responses to repeated noxious heat stimuli before cervical cold block. During cold block the response to heat was enhanced, followed by a return to the pre-cold block level (Fig. 3, left). The same unit responded to id injection of histamine (Fig. 3, right black peristimulus-time histogram [PSTH]). One minute post-injection, the spinal cold block was applied, resulting in an apparent reduction of the histamine-evoked response which was brief. For comparison, the response of another unit (recorded in a separate animal in the absence of cold block) is shown by the gray PSTH. It gave a robust and prolonged response lasting ~20 minutes.

Cold block inhibits histamine-evoked responses

Seventeen cells responsive to id histamine were tested with cold block, and 57 control units responded to histamine in the absence of cold block (data from Akiyama and colleagues\textsuperscript{26–28}). Figure 4A shows the averaged PSTH of the 57 control units, and Figure 4B shows the averaged response of 17 units to histamine with subsequent cold block. The response 1 minute posthistamine was significant for both groups (to 613% and 456% of baseline, respectively, \( P < 0.001 \), paired t test). In Figure 4C, the averaged responses with (gray PSTH) and without cold block (black PSTH) are superimposed to show a reduction in histamine-evoked firing during and following the cold block. Neuronal firing during and after cessation of the cold block was significantly lower (\( P < 0.001 \) for time period 1–3 minutes posthistamine, unpaired \( t \) test) compared with firing during the corresponding time periods in the units not subjected to cold block. The difference in histamine-evoked firing pre- vs. post-cold block was also significantly lower compared with the difference in firing during the same time intervals in the absence of cold block (\( P < 0.05 \), unpaired \( t \) test). These data indicate that responses to histamine are reduced during cold block.

Cold block inhibits chloroquine-evoked responses

Twenty-six units responded to id chloroquine followed by cold block, and 25 control units received id chloroquine without cold block (data from Akiyama et al\textsuperscript{28}). Figure 5A shows the averaged response of the control units, and Figure 5B shows the averaged response followed by cold block 1 minute postchloroquine. There was a significant increase in firing during the first minute postchloroquine for both groups of neurons (to 408% and 629%, respectively; \( P < 0.001 \) for both, paired \( t \) test). There was a significant reduction in firing during the 1-minute period of cold block compared with the same time period in units not
receiving cold block ($P < 0.005$, unpaired $t$ test). However, the effect of cold block was smaller for chloroquine-evoked compared with histamine-evoked firing. The difference in chloroquine-evoked firing pre- vs. post-cold block was also significantly lower compared with the controls ($P < 0.05$, unpaired $t$ test). These data indicate that responses to chloroquine are reduced during cold block.

**Cold block enhances AITC-evoked responses**

Twenty-six units were tested with AITC and cold block. All of the AITC-sensitive units tested additionally responded to chloroquine ($n = 15$) or histamine ($n = 6$) or both ($n = 1$). Averaged responses of dorsal horn units to cutaneous application of AITC in control units not receiving cold block (data from Akiyama and colleagues\cite{28,29}), and in units receiving subsequent cold block, are shown in Figures 6A and B, respectively. The PSTHs are superimposed in Figure 6C to show increased firing during cold block (black PSTH). Neuronal firing during cold block was significantly greater ($P < 0.05$, unpaired $t$ test) compared with the control units not receiving cold block. The difference in AITC-evoked firing pre vs. post-cold block was also significantly higher compared with control responses with no cold block ($P < 0.05$, unpaired $t$ test). These results indicate that cold block enhanced AITC-evoked firing.

**Mixed effects of cold block on noxious heat-evoked responses**

Of 34 noxious heat-sensitive units, responses of 10 were enhanced (by $> 25\%$) during cervical cold block, responses of 6 were reduced (by $> 25\%$) and responses of the remaining 18 units were unaffected. Figures 7A and B show the averaged heat-evoked responses of the units whose responses were enhanced and reduced, respectively, during cold block. The enhancement and reduction in mean responses during cold block were both significant ($P < 0.01; P < 0.05$, respectively; paired $t$ tests).

**No effect of cold block on mechanically evoked responses**

Lightly brushing the receptive field evoked responses in 9 WDR units tested. Figure 8A shows that cold block had no significant effect on brush-evoked responses or preceding baseline activity. Four units tested exhibited responses to low-threshold von Fry stimulation of the receptive field. Again, there was no effect of cold block (Fig. 8B).

**Discussion**

A main outcome is that neuronal activity elicited by id injection of the pruritogens histamine and chloroquine was reduced during cervical spinal cold block, indicating a reduction in tonic descending facilitatory modulation (disfacilitation). In contrast, cold block enhanced neuronal activity elicited by the algogen AITC, and in many cases noxious heat as well, implying disruption of tonic descending inhibitory modulation (disinhibition). Cold block did not affect neuronal responses to low-threshold mechanical stimuli. The results suggest differential tonic descending modulation of various somatosensory submodalities, and in particular support the existence of opposing tonic effects on spinal transmission of itch-related versus pain-related activity.
Pain-modulatory descending pathways originate from neurons in the RVM and other brainstem regions\([2–4]\). Subsets of RVM neurons consist of ON and OFF cells that are thought to exert descending facilitatory and inhibitory effects, respectively, on spinal nociceptive transmission\([5,6]\). Many RVM-spinal projection neurons contain serotonin (5-hydroxytryptamine = 5-HT)\([30,31]\), and intrathecal delivery of 5-HT inhibits spinal nocifensive reflexes and neuronal firing\([3,32]\). However, the role of 5-HT in acute descending pain modulation by ON or OFF cells is uncertain. Of intracellularly labeled RVM ON and OFF cells, none were immunohistochemically labeled for 5-HT\([33]\). Moreover, 13 intracellularly stained RVM neurons that were immunopositive for 5-HT exhibited a steady firing rate and could not be classified as ON or OFF\([34,35]\). It was suggested that such serotonergic neurons may play a role in tonic, but not phasic, descending pain modulation. A role for serotonergic RVM neurons in descending facilitation of itch is supported by 2 recent studies. Liu et al\([22]\) reported that intra-RVM neurotoxic ablation of serotonergic neurons significantly reduced scratching elicited by compound 48/80 in mice. Zhao et al\([23]\) reported that depleting supraspinal 5-HT reduced chloroquine-evoked scratching behavior, while increasing central levels of 5-HT enhanced scratching via crosstalk between 5-HT1A and gastrin releasing peptide receptors coexpressed in spinal itch-signaling neurons. The present results support the existence of a tonic descending itch-facilitatory pathway. Cervical cold block, which reduced or eliminated tonic descending influences, resulted in a marked suppression of histamine-evoked neuronal activity and a weaker but significant attenuation of chloroquine-evoked neuronal firing. Interestingly, the cold block suppressed histamine-evoked activity for a prolonged period; we speculate that interruption of tonic descending facilitation by cold block prevented the histamine-evoked buildup of firing in dorsal horn neurons by some unknown mechanism. Our findings are consistent with the above-noted studies suggesting that 5-HT has a role in tonic descending facilitation of spinal itch-transmitting neurons. It would be interesting to investigate if antagonizing 5-HT1A receptors at the spinal level might block the tonic descending facilitation observed presently.

In contrast, allergic itch-related biting behavior was enhanced by neurotoxic ablation of catecholaminergic but not serotonergic neurons, and was increased by the α-adrenoceptor antagonist phenolamine, supporting a tonic descending noradrenergic inhibitory modulation of spinal itch signaling\([21]\). Conceivably, separate, tonically active inhibitory and facilitatory descending pathways modulate spinal itch transmission. Our data indicate that disabling all descending pathways has a net disfacilitatory effect.

In addition to 5-HT, GABA, and glycine are also expressed in RVM-spinal projection neurons\([14,36–38]\). About 80% of neurons projecting from RVM onto intraspinal afferent terminals were GABAergic, with the majority also expressing the proenkephalin gene Penk1\([38]\). A recent study has identified a descending, GABAergic pathway from RVM that inhibits mechanical pain\([39]\). GABAergic fibers from RVM inhibit spinal interneurons expressing Penk1, resulting in reduced presynaptic inhibition of mechanonociceptive (but not thermonociceptive) input\([39]\). Descending inhibition of nociceptive spinal reflexes elicited by electrical stimulation in RVM was blocked by systemic or intrathecal administration of the μ-opioid antagonist naloxone\([40,41]\) suggesting that spinal release of
enkephalin may inhibit segmental nociceptive transmission. It is currently not known if descending GABAergic pathways and enkephalinergic spinal interneurons influence spinal itch transmission.

Scratching behavior and spinal pruriceptive neurons are suppressed by agonists of GABA-A and GABA-B,[24,42,43], glycine,[24] and κ-opioid receptors.[26,44] Descending pathways from RVM may interact with spinal inhibitory interneurons that release GABA, glycine or dynorphin to modulate the segmental transmission of itch signals, but exact mechanisms are currently unknown.

The role of μ-opioid receptors in the transmission of itch is complex. Spinal administration of morphine enhanced scratching in primates[45] and responses of pruriceptive spinal neurons in rats,[46], possibly via an action at the MOR1D receptor subtype that dimerizes with the gastrin releasing peptide receptor to activate spinal itch-signaling neurons.[47]. Intracisternal[48,49] or intramedullary[50–52] administration of morphine elicited robust scratching behavior in monkeys and rats. In contrast, intracisternal, but not spinal, administration of the μ-opioid antagonist naloxone inhibited scratching.[53]. These findings suggest that spinal itch transmission is under tonic descending modulation from medullary neurons that are activated or suppressed by μ-opioid agonists and antagonists, respectively. The exact pathways and mechanisms of this tonic opioidergic modulation are currently unknown.

**Tonic descending modulation of spinal pain transmission**

In contrast to disfacilitation of itch, our data indicate that cold block disinhibited neuronal responses to the algogen AITC and frequently also disinhibited responses to noxious heat (Fig. 7A), implying that spinal pain transmission is under tonic descending inhibition. The disinhibitory effect of cold block on spinal neuronal responses to noxious heat is consistent with earlier reports[7–12]. Our observation that cold block did not affect spinal neuronal responses to mechanostimulation is also consistent with the earlier literature. However, in a minority of cases neuronal responses to noxious heat were suppressed (disfacilitated) during cold block (Fig. 7B). Disfacilitation of responses of visceroreceptive spinal neurons during spinal cold block has previously been reported[13,54].

All of the presently recorded neurons that responded to AITC also responded to histamine and/or chloroquine, yet cold block had opposing effects on algogen-evoked versus pruritogen-evoked responses. This implies that the tonic descending modulatory effects are exerted presynaptically to differentially modulate pruriceptive and nociceptive inputs to the spinal neurons.

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**References**

[1]. Fields HL, Basbaum AI. Brainstem control of spinal pain-transmission neurons. Annu Rev Physiol 1978;40:217–48. [PubMed: 205165]
[2]. Heinricher MM, Tavares I, Leith JL, et al. Descending control of nociception: Specificity, recruitment and plasticity. Brain Res Rev 2009;60:214–5. [PubMed: 19146877]

[3]. Millan MJ. Descending control of pain. Prog Neurobiol 2002;66: 355–474. [PubMed: 12034378]

[4]. Ossipov MH, Dussor GO, Porreca F. Central modulation of pain. J Clin Invest 2010;120:3779–87. [PubMed: 21041960]

[5]. Fields HL. Pain modulation: expectation, opioid analgesia and virtual pain. Prog Brain Res 2000;122:245–53. [PubMed: 10737063]

[6]. Fields HL, Heinricher MM, Mason P. Neurotransmitters in nociceptive modulatory circuits. Annu Rev Neurosci 1991;14:219–45. [PubMed: 1674413]

[7]. Dickhaus H, Pauser G, Zimmermann M. Tonic descending inhibition affects intensity coding of nociceptive responses of spinal dorsal horn neurones in the cat. Pain 1985;23:145–58. [PubMed: 4069718]

[8]. Duggan AW, Morton CR. Tonic descending inhibition and spinal nociceptive transmission. Prog Brain Res 1988;77:193–211. [PubMed: 3064167]

[9]. Hall JG. Supraspinal inhibition of spinal neurones responding to nociceptive stimulation. Neurosci Lett 1979;14:165–9. [PubMed: 530496]

[10]. Handwerker HO, Iggo A, Zimmermann M. Segmental and supraspinal actions on dorsal horn neurons responding to noxious and non-noxious skin stimuli. Pain 1975;1:147–65. [PubMed: 1235979]

[11]. Li HS, Monhemius R, Simpson BA, et al. Supraspinal inhibition of nociceptive dorsal horn neurones in the anaesthetized rat: tonic or dynamic? J Physiol 1998;506 (pt 2):459–69. [PubMed: 9490872]

[12]. Necke R, Hellon RF. Noxious thermal input from the rat tail: modulation by descending inhibitory influences. Pain 1978;4:231–42. [PubMed: 634623]

[13]. Cervero F Supraspinal connections of neurones in the thoracic spinal cord of the cat: ascending projections and effects of descending impulses. Brain Res 1983;275:251–61. [PubMed: 6626982]

[14]. Reichling DB, Basbaum AI. Contribution of brainstem GABAergic circuitry to descending antinociceptive controls: I. GABA-immunoreactive projection neurons in the periaqueductal gray and nucleus raphe magnus. J Comp Neurol 1990;302:370–7. [PubMed: 2289975]

[15]. Akiyama T, Carstens E. Neural processing of itch. Neuroscience 2013;250:697–714. [PubMed: 23891755]

[16]. Bautista DM, Wilson SR, Hoon MA. Why we scratch an itch: the molecules, cells and circuits of itch. Nat Neurosci 2014;17:175–82. [PubMed: 24473265]

[17]. Green D, Dong X. The cell biology of acute itch. J Cell Biol 2016;213:155–61. [PubMed: 27114499]

[18]. Kremer AE, Feramisco J, Reeh PW, et al. Receptors, cells and circuits involved in pruritus of systemic disorders. Biochim Biophys Acta 2014;1842:869–92. [PubMed: 24568861]

[19]. LaMotte RH, Dong X, Ringkamp M. Sensory neurons and circuits mediating itch. Nat Rev Neurosci 2014;15:19–31. [PubMed: 24356071]

[20]. Potenzieri C, Undem BJ. Basic mechanisms of itch. Clin Exp Allergy 2012;42:8–19. [PubMed: 21645138]

[21]. Gotoh Y, Omori Y, Andoh T, et al. Tonic inhibition of allergic itch signaling by the descending noradrenergic system in mice. J Pharmacol Sci 2011;115:417–20.E. [PubMed: 21372505]

[22]. Liu C, Liu TT, He ZG, et al. Inhibition of itch-related responses by selectively ablated serotonergic signals at the rostral ventromedial medulla in mice. Int J Clin Exp Pathol 2014;7:8917–21. [PubMed: 25674265]

[23]. Zhao ZQ, Liu XY, Jeffry J, et al. Descending control of itch transmission by the serotonergic system via 5-HT1A-facilitated GRP-GRPR signaling. Neuron 2014;84:821–34. [PubMed: 25453842]

[24]. Akiyama T, Iodi Carstens M, Carstens E. Transmitters and pathways mediating inhibition of spinal itch-signaling neurons by scratching and other counterstimuli. PLoS One 2011;6:e22665. [PubMed: 21818363]
[25]. Carstens E, Iodi Carstens M, Akiyama T, et al. Opposing effects of cervical spinal cold block on spinal itch and pain transmission. Acta Dermato-venerol 2017;97:1026–6.

[26]. Akiyama T, Nagamine M, Davoodi A, et al. Intradermal endothelin-1 excites bombesin-responsive superficial dorsal horn neurons in the mouse. J Neurophysiol 2015;114:2528–34. [PubMed: 26311187]

[27]. Akiyama T, Carstens MI, Carstens E. Excitation of mouse superficial dorsal horn neurons by histamine and/or PAR-2 agonist: potential role in itch. J Neurophysiol 2009;102:2176–83. [PubMed: 19625538]

[28]. Akiyama T, Tominaga M, Takamori K, et al. Role of spinal bombesin-responsive neurons in nonhistaminergic itch. J Neurophysiol 2014;112:2283–9. [PubMed: 25122701]

[29]. Akiyama T, Carstens MI, Piecha D, et al. Nalfurafine suppresses pruritogen- and touch-evoked scratching behavior in models of acute and chronic itch in mice. Acta Derm Venereol 2015;95:147–50. [PubMed: 24890341]

[30]. Bowker RM, Westlund KN, Sullivan MC, et al. Descending serotonergic, peptidergic and cholinergic pathways from the raphe nuclei: a multiple transmitter complex. Brain Res 1983;288:33–48. [PubMed: 6198030]

[31]. Jones SL, Light AR. Serotonergic medullary raphespinal projection to the lumbar spinal cord in the rat: a retrograde immunohistochemical study. J Comp Neurol 1992;322:599–610. [PubMed: 1383285]

[32]. Yaksh TL, Wilson PR. Spinal serotonin terminal system mediates anti-nociception. J Pharmacol Exp Ther 1979;208:446–53. [PubMed: 581884]

[33]. Potrebic SB, Fields HL, Mason P. Serotonin immunoreactivity is contained in one physiological cell class in the rat rostral ventromedial medulla. J Neurosci 1994;14 (pt 2):1655–65. [PubMed: 7510333]

[34]. Leung CG, Mason P. Physiological survey of medullary raphe and magnocellular reticular neurons in the anesthetized rat. J Neurophysiol 1998;80:1630–46. [PubMed: 9772227]

[35]. Mason P Physiological identification of pontomedullary serotonergic neurons in the rat. J Neurophysiol 1997;77:1087–98. [PubMed: 9084584]

[36]. Antal M, Petkó M, Polgár E, et al. Direct evidence of an extensive GABAergic innervation of the spinal dorsal horn by fibres descending from the rostral ventromedial medulla. Neuroscience 1996;73:509–18. [PubMed: 873266]

[37]. Hossaini M, Goos JA, Kohli SK, et al. Distribution of glycine/GABA neurons in the ventromedial medulla with descending spinal projections and evidence for an ascending glycine/GABA projection. PLoS One 2012;7:e35293. [PubMed: 22558137]

[38]. Zhang Y, Zhao S, Rodriguez E, et al. Identifying local and descending inputs for primary sensory neurons. J Clin Invest 2015;125:3782–94. [PubMed: 26426077]

[39]. François A, Low SA, Sypek EI, et al. A brainstem-spinal cord inhibitory circuit for mechanical pain modulation by GABA and enkephalins. Neuron 2017;93:822–39. e6. [PubMed: 28162807]

[40]. Zorman G, Hentall ID, Adams JE, et al. Lumbar intrathecal naloxone blocks analgesia produced by microstimulation in the rat medulla. Brain Res 1982;236:77–84. [PubMed: 6276023]

[41]. Zorman G, Belcher G, Adams JE, et al. Lumbar intrathecal naloxone blocks analgesia produced by microstimulation of the ventromedial medulla in the rat. Brain Res 1982;236:77–84. [PubMed: 6279238]

[42]. Cevikbas F, Braz JM, Wang X, et al. Synergistic antipruritic effects of gamma aminobutyric acid A and B agonists in a mouse model of atopic dermatitis. J Allergy Clin Immunol 2017;140:454–64. e2. [PubMed: 2823084]

[43]. Foster E, Wildner H, Tudeau L, et al. Targeted ablation, silencing, and activation establish glycineric dorsal horn neurons as key components of a spinal gate for pain and itch. Neuroreport 2015;85:1289–304. [PubMed: 25789756]

[44]. Kardon AP, Polgár E, Hachişuka J, et al. Dynorphin acts as a neuromodulator to inhibit itch in the dorsal horn of the spinal cord. Neuron 2014;82:573–86. [PubMed: 24726382]

[45]. Ko MC, Song MS, Edwards T, et al. The role of central μ opioid receptors in opioid-induced itch in primates. J Pharmacol Exp Ther 2004;310:169–76. [PubMed: 15044556]
[46]. Moser HR, Giesler GJ Jr. Itch and analgesia resulting from intrathecal application of morphine: contrasting effects on different populations of trigeminothalamic tract neurons. J Neurosci 2013;33:6093–101. [PubMed: 23554490]

[47]. Liu XY, Liu ZC, Sun YG, et al. Unidirectional cross-activation of GRPR by MOR1D uncouples itch and analgesia induced by opioids. Cell 2011;147:447–58. [PubMed: 22000021]

[48]. Andoh T, Yageta Y, Konno M, et al. Evidence for separate involvement of different mu-opioid receptor subtypes in itch and analgesia induced by supraspinal action of opioids. J Pharmacol Sci 2008;106:667–70. [PubMed: 18403901]

[49]. Moser HR, Giesler GJ Jr. Itch elicited by intradermal injection of serotonin, intracisternal injection of morphine, and their synergistic interactions in rats. Neuroscience 2014;274:119–27. [PubMed: 24875173]

[50]. Thomas DA, Anton F, Kenshalo DR Jr, et al. Noradrenergic and opioid systems interact to alter the detection of noxious thermal stimuli and facial scratching in monkeys. Pain 1993;55:63–70. [PubMed: 7904058]

[51]. Thomas DA, Hammond DL. Microinjection of morphine into the rat medullary dorsal horn produces a dose-dependent increase in facial scratching. Brain Res 1995;695:267–70. [PubMed: 8556343]

[52]. Thomas DA, Williams GM, Iwata K, et al. The medullary dorsal horn. A site of action of morphine in producing facial scratching in monkeys. Anesthesiology 1993;79:548–54. [PubMed: 8363081]

[53]. Kuraishi Y, Yageta Y, Konno M, et al. Intracisternal, but not intrathecal, injection of naloxone inhibits cutaneous itch-related response in mice. Biol Pharm Bull 2008;31:2143–5. [PubMed: 18981588]

[54]. Tattersall JE, Cervero F, Lumb BM. Effects of reversible spinalization on the visceral input to viscerosomatic neurons in the lower thoracic spinal cord of the cat. J Neurophysiol 1986;56:785–96. [PubMed: 3783220]
Figure 1.
Experimental set-up (see text for further explanation).
Figure 2.
Recording sites in superficial dorsal horn. Dots represent histologically recovered lesion sites compiled on a representative section through the lumbar spinal cord.
Figure 3.
Example of effects of cervical cold block on a lumbar spinal unit’s responses to noxious heat and intradermal (id) histamine. The left-hand PSTH (bin width: 1 s) shows the unit’s responses to repeated noxious heat stimuli (52°C, 10 s at arrows). The upper right figurine of the ipsilateral hindpaw shows the receptive field (black), and the figurine below it shows the recording site in the superficial dorsal horn. The right-hand PSTH in black shows the same unit’s response to id injection of histamine in the receptive field. The bar with dashed lines show the 1-minute duration of the cervical cold block. The gray PSTH superimposed shows the response of a unit recorded in a different animal to id histamine in the absence of cold block. PSTH indicates peristimulus-time histogram.
Figure 4.
Cold block reduces neuronal responses to intradermal (id) histamine. A, Averaged PSTH (error bars: SEM) of 57 superficial dorsal horn units to id histamine (data from Akiyama and colleagues\cite{26–28}). B, Averaged PSTH of 17 units to id histamine. Cervical cold block was applied for 1 minute, starting 1 minute after histamine (bar with dashed lines). C, Averaged PSTHs from A (black) and B (gray) have been enlarged and are superimposed to show difference in time course of histamine-evoked responses (error bars omitted for clarity). *Spike count 1–3 minutes posthistamine was significantly different for units with vs. without cold block (P < 0.001, unpaired t test). PSTH indicates peristimulus-time histogram.
Figure 5.
Cold block inhibits spinal neuronal response to intradermal chloroquine. Averaged response of 25 units to intradermal chloroquine (CQ). Format as in Figure 4A (data from Akiyama et al\cite{28}). B, Averaged response of 26 units to chloroquine, with cold block (format as in Fig. 4B). C, PSTHs from A (black) and B (gray) have been enlarged and are superimposed. *P < 0.01, unpaired t test. PSTH indicates peristimulus-time histogram.
Figure 6. Cold block enhances dorsal horn neuronal responses to AITC. Format as in Figures 4 and 5. A, Averaged PSTH of control unit responses to AITC in absence of cold block (data from Akiyama and colleagues[28,29]). B, Averaged AITC-evoked response with cold block. C, PSTHs from A (gray) and B (black) have been enlarged and are superimposed. *P < 0.05, unpaired t test. AITC indicates allyl isothiocyanate; PSTH, peristimulus-time histogram.
Figure 7.
Cold block has mixed effects on responses of dorsal horn units to noxious heat. Units were grouped according to whether their response to noxious heat was enhanced (A) or reduced (B) by > 25% during cold block. A, Graph plots mean (± SEM) neuronal activity before (open bars) and during application of heat (black bars). Gray bar: enhanced response during cold block. B, As in (A), showing reduced response during cold block. *P < 0.05, paired t test.
Figure 8.
No effect of cold block on mechanically evoked responses of dorsal horn neurons. A, Mean responses (error bars: SEM) before (open bars) and following repeated application of brush stimulation of the receptive field (black bars). B, As in (A) for responses to probing receptive field with low-threshold von Frey filament. N.s.: cold block did not significantly affect evoked responses ($P > 0.05$, paired t test).