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Sensory Processing in the Deep Spinal Dorsal Horn of Neurokinin-1 Receptor Knockout Mice

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Background: **The neurokinin-1 receptor and its primary ligand, substance P, are widely recognized as contributing to the spinal processing of nociceptive stimuli, yet the specific function of the neurokinin-1 receptor remains unclear.**

Methods: **To better clarify these functions, the authors examined the neurophysiologic responses of L4–L5 neurons in the deep dorsal horn to acute mechanical, thermal, and electrical stimuli in knockout and wild-type mice. In addition, the capacity of knockout and wild-type mice to show wind-up to repeated C-fiber stimuli and to show sensitization after cutaneous mustard oil was assessed.**

Results: **A total of 68 nociceptive neurons (35 in knockout, 33 in wild type) in laminae III–V were studied. No differences in the acute responses of neurons in knockout and wild-type mice to graded mechanical, thermal, or electrical stimuli or in the acute responses to mustard oil were observed. However, wind-up to repeated electrical stimulation at C-fiber intensity was significantly attenuated in the knockout mice compared with wild type controls. In addition, mustard oil–induced mechanical hypersensitivity was significantly reduced in the knockout mice.**

Conclusions: **These results indicate that neurokinin-1 receptors do not play a significant role in the responses of nociceptive neurons in the deep spinal dorsal horn to acute noxious mechanical, thermal, electrical, or chemical stimuli. On the other hand, neurokinin-1 receptors are critical for the central hyperexcitability that is observed in these neurons with repeated C-fiber inputs and to the central sensitization induced by topical mustard oil application.**

THE neurokinin-1 (NK1) receptor and its chief ligand, substance P (SP), are important components in the spinal processing of nociceptive information. SP is contained in small dorsal root ganglion cells and primary afferent fibers likely to be nociceptors.[1] Noxious peripheral stimuli evoke release of SP into the dorsal horn,[2] and intrathecal injection of SP induces nocifensive behaviors.[3] SP specifically excites nociceptive spinal cells *in vivo*,[4] and NK1 antagonists are antihyperalgesic in models of postsurgical,[5] nerve injury,[6] chronic inflammatory,[7] capsaicin-,[8] and formalin-induced pain.[9] Nevertheless, infusion of SP into the dorsal horn had little effect on the spontaneous or evoked activity of primate spinothalamic neurons[10] and did not increase the behavioral responses to cutaneous formalin injection.[11] Furthermore, NK1 receptor antagonists have little effect on behavioral[12] or physiologic responses to acute nociceptive stimuli in spinal neurons.[8]

Wind-up, the phenomenon in which the physiologic responses of spinal neurons in several species increase with repeated stimulation of C fibers,[13] is also observed perceptually with repeated noxious stimuli in humans.[14] Wind-up, like central sensitization and hyperalgesia, is induced by C-fiber inputs and attenuated by *N*-methyl-D-aspartate (NMDA)[15] and NK1 receptor antagonists.[16] However, wind-up is short-lasting, whereas central sensitization and hyperalgesia persists a long time. Thus, wind-up likely models the initiating mechanisms of spinal sensitization[17] related to prolonged depolarization of spinal neurons by nociceptor drive.[18] Meanwhile, longer-lasting models of central sensitization and hyperalgesia include nerve injury,[19] joint or paw inflammation,[20] and the application of chemical irritants to the skin.[21] Mustard oil is a widely used chemical irritant that activates nociceptive fibers and produces long-lasting spinal sensitization in animals[22] and persistent pain and hyperalgesia in humans.[23]

Although recent studies on withdrawal reflexes in NK1 receptor knockout mice[24] indicated that NK1 receptors are involved in encoding noxious stimuli at high intensities and wind-up, the role of NK1 receptors in the dorsal horn neurons in encoding of nociceptive inputs and central sensitization is still not clear. Therefore, we have characterized the neuronal responses in the dorsal horn to acute graded mechanical, thermal, electrical, and chemical stimuli and their responses during central sensitization in NK1 receptor knockout and wild mice.

Materials and Methods

The experiments were performed on 10 NK1 receptor knockout and 13 wild-type BalbC mice of both sexes. Separate breeding stocks of NK1 knockout and wild mice were provided by Dr. Norma Gerard (Perlmuter Laboratory, Children's Hospital, Boston, MA). Animals aged 9–54 weeks and weighing 18–32 g (equally matched between groups) were used. Mice deficient in NK1 receptors were generated by gene targeting as described previously.[25] Briefly, deleting a portion of exon 1 of the *NK-1* gene and replacing it with a cassette

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incorporating a marker gene for neomycin resistance generated knockouts. Embryonic stem cells were used to obtain germ-line transmission; thus, all tissues in the resulting mice were deficient in the *NK-1* receptor gene. The genomic status was periodically monitored by polymerase chain reaction to confirm the presence of the knockout gene marker.[26] Colonies were established in our laboratory and maintained in microisolator cages. The mice received food and water *ad libidum* and were kept in a 12-h day–night cycle. The NK1 receptor knockout mice were fertile, and their appearance and behavior were not distinguishable from wild-type mice. All procedures were reviewed and approved by the Johns Hopkins University Animal Care and Use Committee as consistent with the National Institutes of Health Guide for the Use of Experimental Animals that ensured minimal animal use and discomfort.

Surgical Preparation

Animals were anesthetized by intraperitoneal injection of sodium pentobarbital (initial dose, 70–80 mg/kg) with additional doses given as necessary to maintain suppression of blink, withdrawal, and autonomic reflexes (heart rate) to noxious stimuli. The trachea was cannulated, and the electrocardiogram was monitored. No muscular relaxants were given. The mouse was mounted in a stereotaxic frame, and a laminectomy was performed to expose spinal segments L3–S1. The dura mater was incised and retracted, and the spinal cord was covered with warm mineral oil. The adjacent vertebrae were clamped to stabilize the spinal cord. A feedback-controlled heat lamp was used to maintain core body temperature in the normal range (36.0–37.0°C).

Recording of Dorsal Horn Neuron Activities

Neurons in dorsal horn segments L4–L5 were recorded extracellularly with tungsten microelectrodes (resistance, 1.4–1.8 MΩ) advanced using a hydraulic microdrive. Neural activity was amplified, filtered (high pass 300 Hz, low pass 50 KHz), digitized, and stored for later analysis. Spontaneously active neurons or neurons driven by natural cutaneous search stimuli (brush, tapping, pinch) were isolated for study. Once isolated, the receptive field was mapped with a calibrated pinch and drawn on a standardized map of the mouse hind paw. A single site in the center (most sensitive site) of the receptive field was then chosen for application of the mechanical and thermal stimuli, while sites adjacent to this were chosen for delivery of the electrical stimuli. In a subset of cells, a chemical (mustard oil) stimulus was applied to an adjacent digit within the receptive field but away from the digit to which mechanical or electrical stimuli were delivered. No further cells were studied in any mice after application of the mustard oil.

The natural mechanical stimuli included brushing with a small camel-hair brush, pinch with a calibrated forceps (contacting area, 1 mm^2; force, 6 N), and indentation of the skin with a set of von Frey filaments (0.49, 0.94, 1.46, 2.21, 4.48 bars). Thermal stimuli were applied by preheated brass probes (diameter, 2.5 mm) at 48°C and 51°C, and by application of a drop of acetone for cooling. Each mechanical stimulus was applied for 5 s with an interstimulus interval of 10 s. Heat stimuli were applied for 10 s with an interstimulus interval of 20 s. A wooden probe at room temperature (22°C) with same contact area as the brass probe was used as control for the thermal stimuli.

The electrical stimuli were applied through a pair of fine needles inserted subcutaneously at two points, one just lateral and a second just medial to the central receptive field site used for delivery of the mechanical stimuli. Electrical stimuli were delivered in steps of 0.03–1.0 mA to determine the thresholds for A- and C-fiber activation. Sixteen pulses suprathreshold for C-fiber activation (2 ms, 5 mA)[27] were then applied at intervals of 1, 0.2, and 0.1 Hz.

The responses to chemical stimulation by cutaneous application of mustard oil were examined in a subset of nociceptive neurons. A small piece of cotton compress (1.5 × 3 mm) soaked with 100% mustard oil was applied for 5 s onto a digit within the receptive field but adjacent to that on which the mechanical, thermal, and electrical stimuli were applied. The acute responses to mustard oil were counted as the number of action potentials for the first minute after application. Ten minutes after the mustard oil, the responses to the natural stimuli were retested as described above.

Histology

The mice were killed at the conclusion of each experiment by an overdose of sodium pentobarbital (300 mg intraperitoneally) followed by exsanguination and perfusion with 4% paraformaldehyde. The spinal cord was removed and the recorded segment was defined by dissection and stored in 4% paraformaldehyde. The tissue was later cut into 60-μm slices on a freezing microtome and stained with thionin. The distance between the recorded site in the spinal cord and stimulation sites was measured. The recording sites were localized based on the coordinates below the surface of the spinal cord and the medial lateral position with respect to the midline and the dorsal roots.

Statistical Analysis

The rates of neuronal responses to all stimuli were measured offline. Spikes were discriminated from background (and other units if present) by use of threshold windows and/or template-matching software. The numbers of spikes evoked during stimulus application were summed, and the rate of spontaneous activity, if present, was subtracted. After-discharges were observed in some cells but were uncommon in the cell populations studied as a whole; therefore, discharges in intervals after the

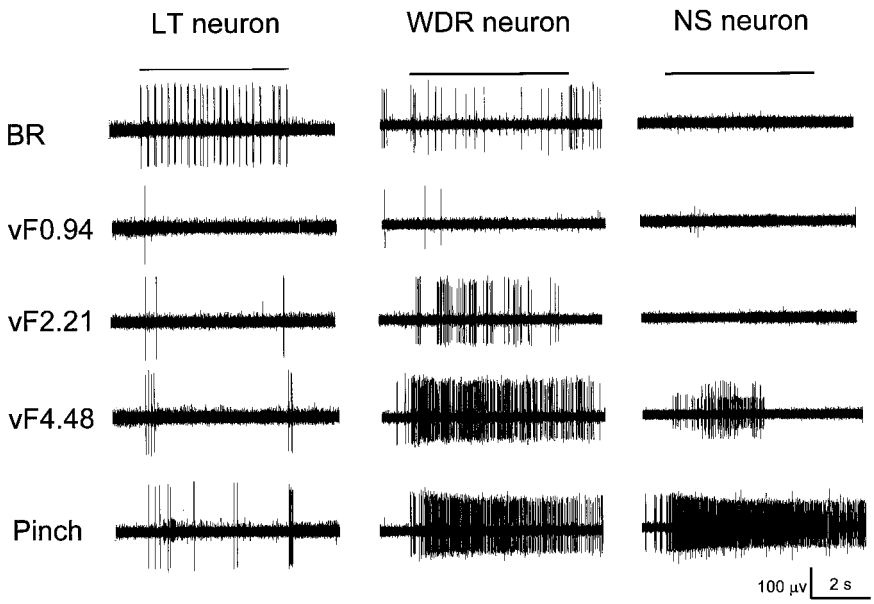

Fig. 1. Analog recordings of neurons in murine dorsal horn. Representative responses of a low-threshold (LT), wide dynamic range (WDR), and two nociceptive-specific (NS) neurons (each was analyzed separately) to calibrated mechanical stimuli in neurokinin-1 (NK1) receptor knockout mice are shown. Comparable neurons were found in the dorsal horn of wild-type mice. The stimuli used in each trace are indicated to the left. Bars on the top indicate the period of stimulation. Calibration bars for duration and amplitude are at the bottom right. BR = brush; vF(number) = von Frey filament (bending force in bars).

stimuli were not analyzed in detail. Neurons were grouped into low-threshold, wide dynamic range (WDR), and nociceptive specific (NS) based on their responses to mechanical stimuli.[28] Low-threshold neurons responded only to innocuous stimuli, and WDR neurons responded to both innocuous and noxious stimuli, with increased response to the noxious stimuli. NS neurons responded to noxious stimuli alone. Responses to electrical stimuli were divided into an early component evoked by A-fiber inputs and a late component evoked by C-fiber inputs. The numbers of action potentials from onset of the electrical stimuli to the beginning of the C-fiber component were combined as a single value for the A-fiber responses, whereas the number of spikes from the onset of the C-fiber component to the end of the first second after the electrical stimuli were counted as the C-fiber response. For analysis of wind-up, the A- and C-fiber components in the response to the first stimulus for each train of the frequencies tested (0.1, 0.2, and 1.0 Hz) were combined to give a single baseline mean response. All subsequent responses were then normalized to this baseline, and the normalized responses among cells were combined to yield the group values.

One- to three-way analysis of variance followed by Tukey *post hoc* analysis for repeated measures were used. *P* values of less than 0.05 were considered significant. All data are presented as the mean ± SEM unless otherwise indicated.

Results

Responses to Natural Stimulation in Wild-type and Knockout Mice

Similar proportions of low-threshold, WDR, and NS neurons were found in the dorsal horn of both NK1 knockout and wild-type mice (fig. 1). Detailed analysis focused on 35 nociceptive neurons (34 WDR, 1 NS) in NK1 knockout and 33 nociceptive neurons (32 WDR, 1 NS) in wild-type mice with receptive fields covering the glabrous skin of the hind paw. The recording depths in knockout mice ranged from 366 to 638 μm below the spinal surface (mean, 502 ± 23 μm), whereas recording depths in wild-type mice ranged from 377 to 651 μm (mean, 514 ± 24 μm). These depths corresponded to locations in spinal lamina III–V for both groups (fig. 2).

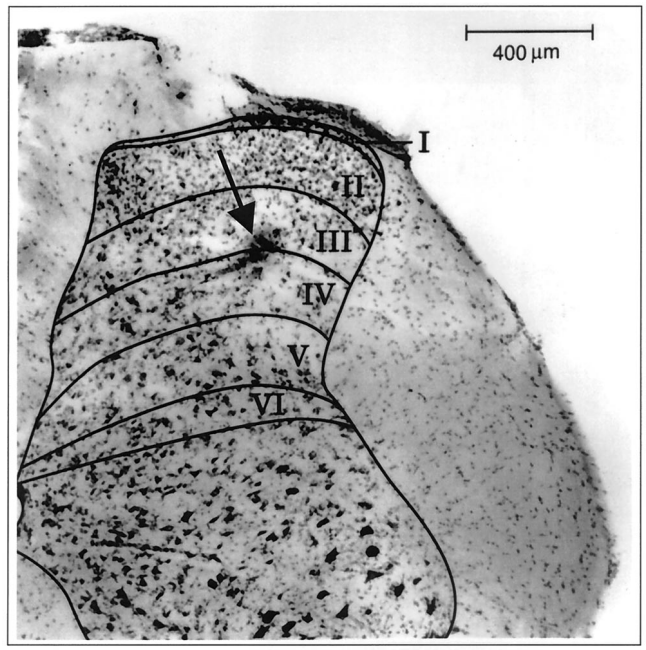

Fig. 2. Mouse spinal cord morphology. The histologic section from the L4 segment of the spinal cord from a neurokinin-1 knockout mouse shows a representative recording location (indicated by the arrow). The laminar organization of the murine dorsal horn is delineated based on the criteria used previously.[45]

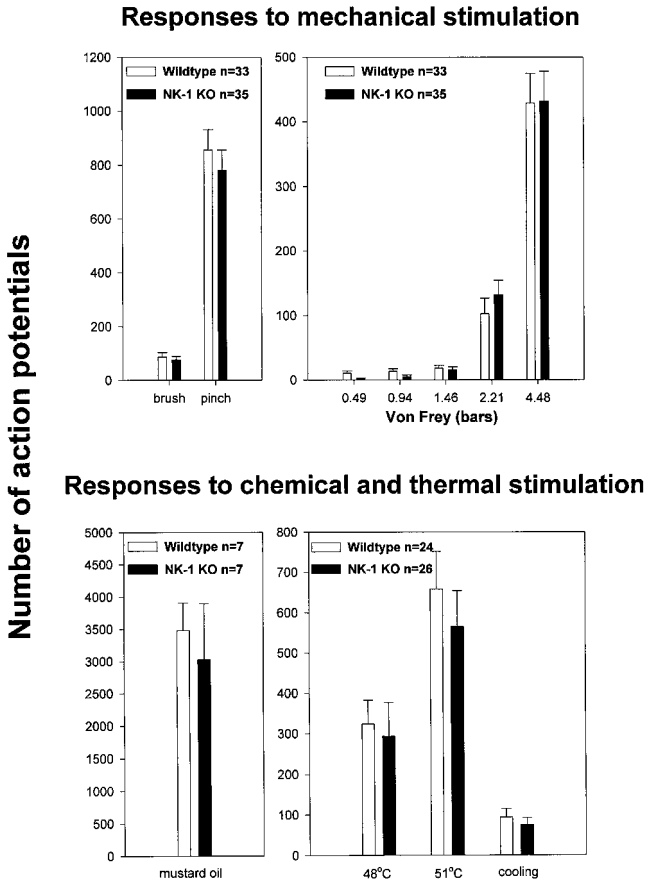

Fig. 3. Responses to cutaneous stimuli in knockout and wild-type mice. The bar graphs show the mean number of action potentials (and SE) evoked by the brush, pinch, von Frey filaments, heat, cooling, and mustard oil stimuli in wild-type (open bars) and neurokinin-1 knockout (NK-1 KO) animals (filled bars). No differences were observed between the acute responses of neurons in wild-type and NK-1 KO mice to mechanical, thermal, or the chemical stimuli.

Spontaneous activity was absent or only very infrequent in cells of both groups of mice. Similarly, the overall pattern and discharge frequency of cells in both groups of mice to all natural mechanical and thermal stimuli and the acute responses to mustard oil were statistically identical (fig. 3). After-discharges were equally frequent in both groups. Finally, the receptive field sizes in the knockout mice were not statistically different from those in the wild-type mice.

Responses to Subcutaneous Electrical Stimulation in Wild-type and Knockout Mice

A- and C-fiber–initiated response components to electrical stimuli applied in the receptive field defined by threshold for activation and latency of responses were observed in both groups of mice (fig. 4).

The electrical thresholds to activate both A- and C-fiber response components in the NK1 knockout and wild-type mice were statistically identical (fig. 5). The latency of the early component was less than 2 ms for both wild-type and NK1 knockout mice. The latency of the late C-fiber component was slightly longer but not significantly different in wild type (94.5 ± 4.4 ms) *versus* NK1 knockout mice (92.6 ± 2.9 ms). The conduction velocity of the late responses was less than 1.0 m/s in all cells of both the wild-type and knockout mice.

Neurons displaying late C-fiber responses were further tested for the ability to show wind-up to repeated electrical stimuli (5 mA, 2 ms) delivered at varying frequencies. A representative example of the responses from wild-type and NK1 knockout mice to repeated C-fiber stimuli delivered at 1.0 Hz is shown in figure 4. The A-fiber responses at all stimulus frequencies and the C-fiber responses delivered at low frequency remained constant in both the NK1 knockout and wild-type mice. However, with stimulation frequencies at 0.2 and 1 Hz, the C-fiber responses progressively increased in wild-type mice, confirming wind-up. The wind-up at 1 Hz was more pronounced than that at 0.2 Hz in the wild-type mice (fig. 6). In contrast, the same stimulation parameters in NK1 knockout mice resulted in significantly less frequency-dependent increases compared with that shown in the wild-type mice (figs. 4 and 6).

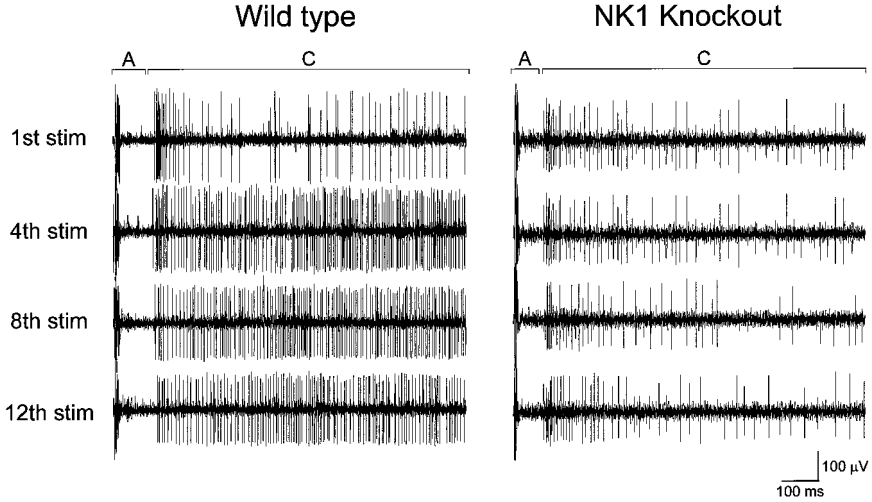

Fig. 4. Analog recordings of wind-up in knockout and wild mice. Analog recordings of representative responses of a neuron from a wild-type (*left*) and a NK1 knockout mouse (*right*) to the first (top line) C-fiber intensity electrical stimulus (5 mA, 2 ms) (top line) and then to later stimuli when repeated at 1.0 Hz (lines 2–4). The top line shows the responses divided into an early latency, A-fiber, and a longer latency, C-fiber, response components. Increased numbers of spikes, or wind-up, is observed for later responses in the series for the representative wild-type neuron, but is not seen in the neurokinin-1 (NK1) receptor knockouts.

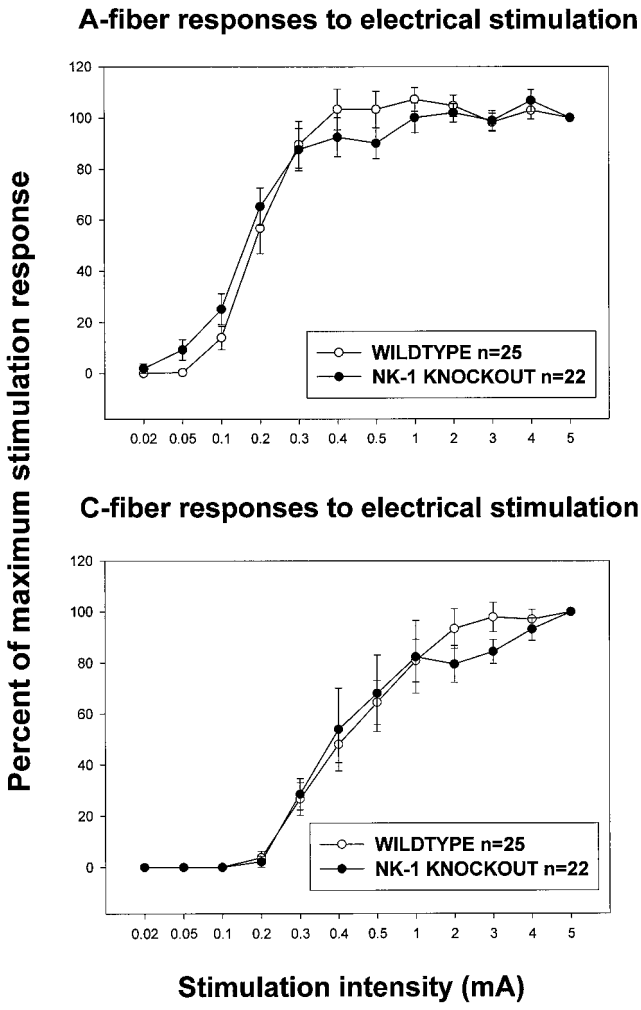

Fig. 5. Population response functions to single electrical stimuli. Symbol and line plots are used to show the mean responses (and SE) to graded electrical stimuli (DC, 2-ms width) in wild-type (open symbols) and neurokinin-1 (NK-1) knockout (filled symbols) mice. A- and C-fiber responses in NK-1 receptor knockout mice were similar to those in wild-type mice. Responses of each neuron were normalized as a percentage of the response to 5-mA current stimuli (mA).

Central Sensitization Induced by Mustard Oil

In 14 WDR neurons with receptive fields covering at least two digits of the plantar surface of the hind paw (7 knockout and 7 wild type), the responses to natural stimuli applied to one digit were examined before and then 10–15 min after mustard oil was applied to the adjacent digit (fig. 7). As previously noted, the spontaneous activity and baseline responses of neurons in wild-type and knockout mice to all stimuli, including the acute responses to mustard oil, were comparable (fig. 3). However, all seven wild-type neurons but only one of seven knockout neurons showed increased responses to punctate stimuli after mustard oil was applied (fig. 7). Two of seven wild-type neurons also showed an expansion of receptive field size to include previously insensitive digits after application of mustard oil, whereas none of the cells showed this in the NK1 knockout mice. Action potential responses to brush, noxious pinch, and thermal stimuli (48 and 51°C, cooling) did not change after mustard oil application in either the wild-type or NK1 knockout mice (fig. 7).

Discussion

There were three main findings in the present study. First, NK1 receptors do not play an important role in the responses of nociceptive neurons in the deep spinal dorsal horn of NK1 knockout mice to acute noxious mechanical, thermal, or chemical stimuli. Second, NK1 receptors play a key role in wind-up observed with repeated C-fiber inputs in the murine dorsal horn. Finally, NK1 receptors are needed for generation of the central sensitization that is normally induced by topical application of mustard oil in mice.

Role of Neurokinin-1 Receptors in the Intensity Coding of Noxious Stimuli

The data in this study indicate that NK1 receptors do not play a major role in the coding of acute noxious stimuli in the deep spinal cord dorsal horn. Action potential responses of nociceptive neurons to graded mechanical, thermal, and electrical stimuli were similar in NK1 knockout to those in wild-type mice. In addition, the acute responses to mustard oil were comparable between the two different genotypes. These findings are consistent with previous physiologic studies showing little effect of NK1 antagonists applied to the deep spinal cord dorsal horn on the responses of primate spinothalamic tract neurons to acute noxious mechanical or thermal stimuli[8] and behavioral studies indicating little effect of NK1 antagonists in acute pain models.[12] Our findings are also consistent with observations in SP–NKA and NK1 knockout mice that behavioral responses to acute noxious mechanical and heat stimuli were similar to those in wild-type animals.[24,29,30] Although these results might suggest that the acute noxious stimuli used in this study, particularly those to rapid heating of the skin, had predominantly Aδ- *versus* C-fiber–mediated transmission,[31] this explanation is unlikely in regard to the responses to the high-intensity electrical volleys or mustard oil.

Nevertheless, our results are not in line with all previous studies on withdrawal reflexes in NK1 receptor knockout mice that suggested the responses to high-intensity noxious stimuli are indeed attenuated by loss of these receptors.[29,30] In this study, we focused on the responses of neurons located at laminae III–V in the spinal cord dorsal horn.

Immunohistochemical studies have suggested that lamina I NK1 receptor–bearing neurons are involved in intensity coding of acute cutaneous pain, whereas deep lamina NK1 cells are involved in mediation of joint pain

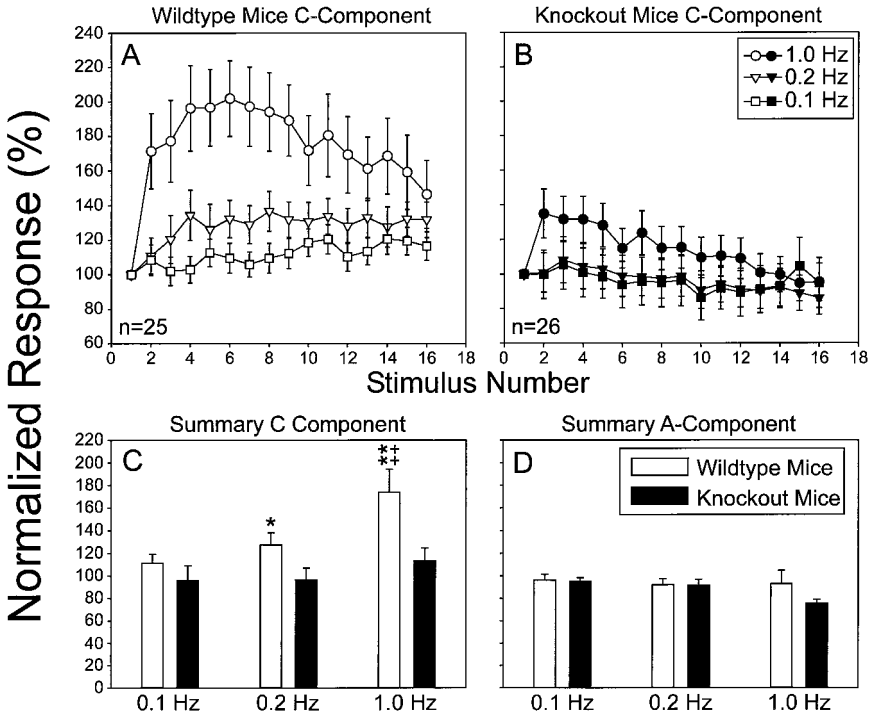

Fig. 6. Population responses to repeated electrical stimuli. Symbol and line plots (*A* and *B*) show the mean (and SE) C-fiber responses of cells in wild-type (open symbols in *A*, *left*) and neurokinin-1 knockout mice (filled symbols in *B*, *right*) to a train of 16 electrical stimuli (5 mA, 2 ms) at different frequencies (1.0, 0.2, and 0.1 Hz; *B*, *top right*). Responses of each neuron were normalized as a percentage of the mean response to the first electrical stimulus in each series. The bar graphs (*C* and *D*) show mean total responses (and SE) at C-fiber (*C*) and A-fiber latencies (*D*) for cells in wild-type (open bars) and neurokinin-1 knockout (filled bars) mice. +*P* < 0.05, ++*P* < 0.01 compared with 0.1-Hz stimulation; **P* < 0.05, ***P* < 0.01, wild-type *versus* knockout mice.

and inflammatory hyperalgesia.[32] Similarly, NK1 receptor internalization showed an intensity-dependent increase in lamina 1 neurons after noxious thermal stimuli, whereas there was no NK1 receptor internalization in the deep laminae after acute noxious stimulation.[33] Taken together, these results may suggest that SP–NK1 receptors might be involved in the intensity coding of acute noxious stimuli only for cells in laminae I–II of the spinal cord, whereas these receptors have a less prominent role in the intensity coding for acute stimuli in nociceptive neurons located in deeper spinal laminae, as tested here.

Role of Neurokinin-1 Receptors in Wind-up

Repeated C-fiber stimulation induces a frequency-dependent enhancement of responses in nociceptive dorsal horn neurons, or wind-up, in many species.[18,34] Our results confirm wind-up, and the important role of NK1 receptors in this phenomenon, in the dorsal horn of mice.[24] Wind-up is considered to model the initial trigger events that lead to central sensitization.[17] Notably, wind-up was shown in mice at a somewhat lower interstimulus frequency (0.2 Hz) than has been common in other studies (0.3Hz).[35] This observation may be related to the overall higher frequency of neuronal discharges to all cutaneous stimuli observed in mice compared with that previously experienced by us for rats[36] and primates.[8,10] C-fiber stimulation produces long-lasting synaptic potentials consisting of NMDA[15] and neurokinin receptor-mediated components,[16] the temporal summation of which after C-fiber stimulation leads to wind-up.[18] NMDA and neurokinin receptor antagonists attenuate these long-lasting synaptic C-fiber potentials and wind-up in rat, cat, and primate dorsal horn.[15,16] The data from the present study are thus in agreement with many lines of previous work.

Finally, it should be noted that although few, nevertheless some individual neurons in knockout animals were encountered that expressed significant response facilitation. This finding may suggest that wind-up can be induced in the absence of NK1 receptors either through the activity of NMDA receptors alone or perhaps through NMDA receptors plus neurokinin-2 receptors. Alternatively, because the developmental consequences of embryonic loss of NK1 receptors are unknown, other neurochemical systems may partially assume the role previously mediated by NK1 receptors. Of these possibilities, wind-up through NMDA receptors alone seems the most likely since hyperalgesia can be provoked by nonnoxious thermal stimulation,[37] presumably below threshold for neuropeptide release.

Role of Neurokinin-1 Receptors in the Central Sensitization Induced by Mustard Oil

Mustard oil selectively activates nociceptors,[22] causes neurogenic inflammation,[38] primary (afferent) and secondary (central) sensitization of neurons in experimental animals,[22,39] and cutaneous hyperalgesia in humans.[23] In the current study, mustard oil applied topically to one digit within the receptive field produced sensitization of neuronal responses to mechanical but not thermal stimuli applied to an adjacent digit within the same receptive field in wild-type mice. Units were observed to respond to lower-threshold von Frey filaments than in the base-

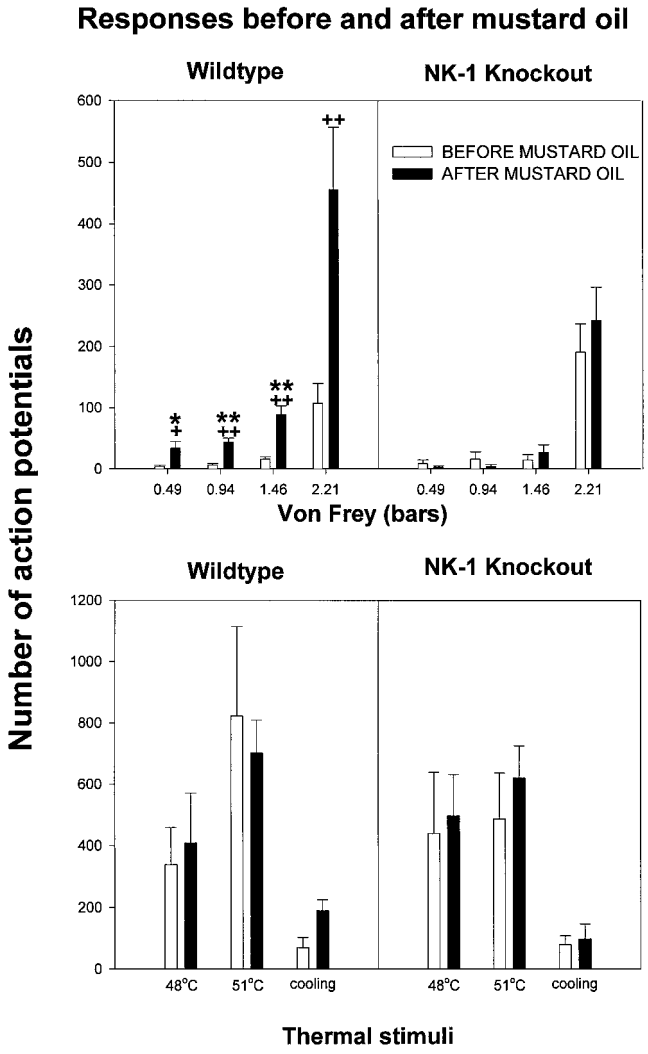

Fig. 7. Population responses to mustard oil. The bar graphs show the mean responses (and SE) to mechanical and thermal stimuli before (open bars) and then 10–15 min after mustard oil application (filled bars) in wild-type (n = 7, *left*) and neurokinin-1 (NK-1) knockout mice (n = 7, *right*). +P < 0.05, ++P < 0.01 compared with pre–mustard oil values; *P < 0.05, **P < 0.01, wild-type *versus* knockout mice.

line recording, and the responses to the suprathreshold stimuli were also enhanced. Two of seven neurons in wild-type animals showed an expansion in their receptive field size after mustard oil application. In summary, our results in wild-type mice are consistent with the generation of spinal sensitization as shown in other species.[40]

In contrast, neurons in NK1 knockout mice did not consistently show enhancement of responses to mechanical or thermal stimuli after mustard oil application. Only one of the seven WDR neurons in NK1 knockout mice tested with mustard oil showed lowering of von Frey threshold, and none of the neurons showed an expansion of receptive field margins. These data are consistent with that of other investigators, showing that SP and NK1 receptor antagonists reduce mechanical hypersensitivity in models of pathologic pain,[8] and with previous behavioral studies in NK1 knockout mice[30] and in rats treated for cytotoxic injury to SP receptor–expressing dorsal horn cells that failed to develop postinjury cutaneous hypersensitivity.[41]

It is remarkable that selective deletion of the NK1 receptor alone would result in such robust attenuation of hyperalgesic responses and neuronal sensitization in the knockout mice. The majority of NK1 receptor–containing cells in the rodent dorsal horn were suggested to be neurons that send projections to rostral forebrain targets.[42] It is likely that our sample included both projection and nonprojection cell types. The reduced wind-up and mustard oil sensitization we observed thus suggests a global distortion of dorsal horn processing of nociceptive signals by loss of a single gene in a select population of cells. Only 33% of the lamina III–V spinothalamic cell population previously shown to encode well for postinjury mechanical hypersensitivity[21] express NK1 receptor immunoreactivity.[42] Although as high as 77% of lamina I spinothalamic neurons expressed NK1 receptor signal,[42] this immunoreactivity is confined to the multipolar and fusiform neuron types[43] that have not been shown to sensitize after peripheral injury in a manner that might account for secondary hyperalgesia. Yet the question that naturally arises is whether projection cells, thought previously to be dedicated to the forebrain signaling of dorsal horn output, might also have a fundamental regulatory impact on the activity of other local circuit dorsal horn cell types. If so, given the widespread impact of such a cell population, it is notable that the functions would not be reconstituted by another of the remaining cell types or by another receptor in these same neurons, such as the neurokinin 2 receptors, assuming the previous role of NK1 receptors. It thus appears that NK1 receptors have a very highly conserved and specific role across species, even though these receptors show a fair degree of pharmacologic heterogeneity among species.[44]

In summary, the data from the current study indicate that NK1 receptors are not involved in the intensity coding of acute noxious stimuli in the deep spinal cord dorsal horn. However, NK1 receptors play important role in the wind-up induced by repeated C-fiber inputs and in the central sensitization after neurogenic inflammation. The specific role of NK1 receptor in the mechanisms of central sensitization, characteristic of various pathologic pain states, makes it highly relevant target for developing drug therapies for patients suffering from neuropathic pain.

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