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Site- and state-dependent inhibition of pruritogen-responsive spinal neurons by scratching

Tasuku Akiyama, Mitsutoshi Tominaga, Mirela Iodi Carstens, and E. Carstens

University of California, Davis, Department of Neurobiology, Physiology & Behavior, 1 Shields Avenue, Davis, California 95616

Abstract

The relief of itch by scratching is thought to involve inhibition of pruritogen-responsive neurons in the spinal cord. We presently recorded responses of superficial dorsal horn neurons in mice to intradermal injection of the pruritogens chloroquine and histamine. Scratching within an area 5–17 mm distant from the injection site, outside of the units' mechanoreceptive fields (off-site), significantly inhibited chloroquine- and histamine-evoked responses without affecting capsaicin-evoked firing. This is consistent with observations that scratching at a distance from a site of itch is antipruritic. In contrast, scratching directly at the injection site (within the receptive field; on-site) had no effect on chloroquine-evoked neuronal firing, but enhanced the same neurons' responses to intradermal injection of the algogen, capsaicin. Moreover, neuronal responses to histamine were enhanced during on-site scratching, followed by suppression of firing below baseline levels after termination of scratching. Scratching thus inhibits pruritogen-responsive neurons in a manner that depends on the input modality (i.e., pain vs. histamine-dependent or histamine-independent itch) and skin location.

Keywords

itch; pain; histamine; mice; superficial dorsal horn neurons

Introduction

Itch is an unpleasant sensory experience associated with the desire to scratch. Scratching usually provides relief from itch (Yosipovitch *et al.*, 2005), although excessive scratching of chronic itchy skin can induce damage, disrupting the skin barrier and initiating a vicious itch-scratch cycle (Wahlgren, 1999). Scratching affords relief from acute itch at least partly via inhibition of spinal pruritogen-responsive neurons. Monkey spinothalamic tract neurons are modulated in a state-dependent manner by cutaneous scratching; scratching inhibited

Corresponding Author: Prof. E. Carstens, University of California, Davis, Department of Neurobiology, Physiology & Behavior, 1 Shields Avenue, Davis, California 95616, Tel.: 1-530-752-7767 (lab), Fax: 1-530-752-5582, eecarstens@ucdavis.edu.

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responses elicited by the pruritogen histamine but did not inhibit responses of the same neurons to the algogen, capsaicin (Davidson *et al.*, 2009). In a mouse model of chronic dry skin itch, the elevated spontaneous activity of superficial dorsal horn neurons was significantly inhibited by cutaneous scratching via spinal interneurons that release the inhibitory neurotransmitters glycine and GABA; inhibition was mediated by both direct segmental and indirect suprasegmental pathways (Akiyama *et al.*, 2011).

In the present study, we investigated if murine superficial dorsal horn neurons are excited by intradermal (id) injection of the pruritogen, chloroquine and histamine, and if such responses are inhibited by cutaneous scratching. Chloroquine is an anti-malarial drug that elicits itch in humans and scratching behavior in mice via the molecular receptors mrgprA3 and TRPA1 co-expressed in sensory nerve fibers (Liu *et al.*, 2009; Wilson *et al.*, 2011). Chloroquine-evoked scratching behavior is significantly attenuated in mice lacking TRPA1 (Wilson *et al.*, 2011) or with neurotoxic ablation of spinal neurons expressing the gastrin releasing peptide receptor (Sun *et al.*, 2009). We identified superficial dorsal horn neurons that responded to chloroquine and histamine; these neurons also responded to capsaicin. Cutaneous scratching induced state-dependent effects, inhibiting chloroquine- and histamine- but facilitating capsaicin-evoked activity. Moreover, we show for the first time that effects of scratching are also site-dependent: Scratching off-site (5–17 mm from the injection site) inhibited chloroquine- and histamine- but not capsaicin-evoked firing. In contrast, scratching on-site (directly at the injection site) facilitated capsaicin-evoked firing, had no effect on chloroquine-evoked firing, and facilitated histamine-evoked activity followed by inhibition post-scratching.

Materials and Methods

Experiments were performed using 15 adult male C57BL/6 mice (Simonsen, Gilroy, CA; 20–32 g) under a protocol approved by the UC Davis Animal Care and Use Committee. The single-unit recording from the lumbar spinal cord was conducted as previously detailed (Akiyama *et al.*, 2009a; Akiyama *et al.*, 2009b). Anesthesia was induced by sodium pentobarbital (60 mg/kg ip) and maintained by supplemental injections (10–20 mg/kg/hr). A tungsten microelectrode recorded single-unit activity in the lumbar spinal cord. A chemical search strategy (Akiyama *et al.*, 2009a; Akiyama *et al.*, 2009b) was used to identify and isolate chloroquine-responsive units. Our search strategy was intended to maximize the chance of isolating a chloroquine- or histamine-responsive neurons; we assume that such neurons either gave rise to ascending projections, or served as interneurons in segmental scratch-reflex circuitry, and no attempt was presently made to distinguish between these possibilities. Briefly, a small (~0.25 µl) intradermal (id) microinjection of chloroquine (100 µg/µl; Sigma-Aldrich, St. Louis MO) or histamine (50 µg/µl; Sigma) was made in the ventral hindpaw and a spontaneously active unit in the superficial lumbar dorsal horn (depth < 300 µm) was isolated. After the spontaneous activity had waned, the identical chemical in a volume of 1 µl was injected again at the same site through the same needle. Only units exhibiting an increase of >30% in firing to this second test microinjection were selected for further study. Responses were usually recorded for at least 30 min, although in many cases unit firing declined over a shorter period.

During the time that the unit exhibited a relatively stable level of firing following id injection of chloroquine or histamine (usually 3–5 min post-injection), scratch stimuli were successively delivered directly at the injection site (on-site), or at a site 5–17 mm away from the injection site (off-site). Scratching was accomplished by moving a brush bristle approximating the size of a mouse toenail in a back-and-forth motion across the hindpaw skin at a constant frequency of 2 Hz, excursion of 3 mm, force of 300 mN, and duration of 20 sec. The scratch stimulus was then applied for 20 sec, on- or off-site. At least 60 sec after

the end of the first scratch stimulus, the same scratch stimulus was applied again at the other site, with the order of site of scratching (on- or off- site) counterbalanced across experiments.

The scratch stimuli were tested against pruritogen-evoked firing that was constantly declining. Thus, it was usually only possible to accurately test the effects of two scratch stimuli (on- and off- site) before firing declined to too low a level. In a few cases, effects of scratching were assessed against unit firing that was elicited by an additional id injection of the same pruritogen tested initially.

When unit firing to the second pruritogen injection declined and reached a steady level, the unit's mechanosensitive receptive field was determined. The perimeter of the mechanical receptive field was mapped using a von Frey filament (55 mN bending force) by determining sites at which the unit either did (within receptive field) or did not (outside receptive field) respond to at least 3 of 5 repeated applications. The rationale for choosing this bending force is that it was sufficient to map the maximum extent of the mechanical receptive field as assessed by comparison of receptive field sizes mapped using a range of von Frey stimuli (0.7 mN: $1.2 \pm 0.6 \text{ mm}^2$, 6.9 mN: $3.7 \pm 0.9 \text{ mm}^2$, 55mN: $9.9 \pm 1.4 \text{ mm}^2$, 758 mN: $10.3 \pm 1.4 \text{ mm}^2$). Receptive field perimeters were transferred to scale drawings of the mouse hindpaw. Drawings of receptive fields were scanned and their areas measured using Elements software (Nikon Instruments Inc., Melville, NY). Units were classified as wide dynamic range (WDR) –type if they responded in a graded manner to innocuous mechanical stimulation (brushing, cotton wisp) and noxious pinch, or nociceptive-specific (NS) if they responded to noxious pinch (and to the 55 mN von Frey stimulus) but not to the cotton wisp or brush stimuli. The properties are similar to those of WDR and NS units shown in previous studies to respond to histamine, serotonin, or a PAR-2 agonist (Akiyama *et al.*, 2009a; Akiyama *et al.*, 2009b).

Following tests of scratching on pruritogen-evoked activity, additional chemical stimuli were injected id within the same receptive field at a different location via a separate injection cannula. The order of subsequent chemical injections was saline (vehicle for chloroquine and histamine), 7% Tween 80 in saline (vehicle for capsaicin) and capsaicin (30 μg ; Sigma). Most (9/10) chloroquine-responsive units also responded to capsaicin. Following the id capsaicin injection, we tested the effects of scratching on- and off-site, at approximately the same sites and in the same manner as described above for chloroquine and histamine.

Action potentials were recorded to a computer and counted using Chart software (AD Instruments, Colorado Springs CO). Unit activity was usually quantified as number of action potentials/ sec and displayed in peristimulus-time histogram (PSTH) format with 1 sec bins. Mean firing rate was calculated over a 1 min period pre- and again 1 min immediately post-injection of chloroquine, histamine, capsaicin or vehicles. Ongoing responses elicited by chloroquine, histamine or capsaicin were summed at 20-sec intervals before, during and after on- or off-site scratching, and compared by repeated-measures analysis of variance (ANOVA) followed by post-hoc Dunnett's test, with $p < 0.05$ set as significant. At the end of each experiment, an electrolytic lesion was made at the spinal cord recording site. The spinal cord was postfixed in 10% buffered formalin, cut in 50 μm frozen sections, and examined under the light microscope to identify lesions.

Results

Chloroquine

Chloroquine-responsive neurons (6 NS, 4 WDR) were located in the superficial dorsal horn at a mean depth of $50.1 \mu\text{m} \pm 20.7$ (SEM) below the surface. All units had mechanosensitive receptive fields on the ipsilateral hind paw, and responded to id microinjection of chloroquine within the receptive field. Mean unit firing immediately post-chloroquine injection was significantly greater compared to the pre-injection level or following vehicle injection (Fig. 1).

Scratching off-site (5–17 mm from the chloroquine injection site) inhibited ongoing activity elicited by chloroquine. Fig. 2 shows an example of a superficial dorsal horn unit that responded to id chloroquine (upper left-hand peristimulus-time histogram=PSTH) and capsaicin (upper right-hand PSTH). Scratching was delivered to the skin either on-or off-site. When unit activity was quiescent, scratching on-site, but not off-site, evoked a response (Fig. 2, lower middle PSTH). During the response to chloroquine, off-site scratching inhibited ongoing firing while on-site scratching did not (Fig. 2, lower left-hand PSTH).

Overall, off-site scratching inhibited each of the 10 units (Fig. 3A,B). Analysis of raw spike counts before, during and after scratching revealed an overall significant effect ($F(2, 27)=13.1$, $p=0.000308$), with post-hoc tests revealing that the mean firing rate during off-site scratching was significantly lower (to a mean of 1.99 ± 0.56 Hz; 48.1%, Dunnett's test: $p<0.05$) compared to the immediate 20-sec pre-scratch level (3.70 ± 0.67 Hz, Fig. 3B), and remained significantly lower (2.59 ± 0.35 Hz; 77.1%, Dunnett's test: $p<0.05$) post-scratching (Fig. 3B).

In contrast, on-site scratching had no significant effect on firing rate (Fig. 3C, D). One-half of the units exhibited increased firing during on-site scratching, but the overall effect (124.3% of pre-scratch level) was not statistically significant (Fig. 3D). The inset diagram in Fig. 3B shows each unit's receptive field (dashed outlines) on a drawing of the hindpaw. For each unit, the location of off-site scratch stimuli was outside the receptive field, while the on-site location of scratching was within each unit's receptive field. The inset to Fig. 3F shows histologically-reconstructed recording sites of chloroquine-responsive units in the superficial dorsal horn.

Nine of the 10 chloroquine-responsive units responded to capsaicin injected id within the same unit's mechanical receptive field at least 40 min later. The mean response to capsaicin was comparable to that elicited by chloroquine, and significantly greater than pre-injection baseline or vehicle-evoked levels (Fig. 1). In contrast to the inhibitory effect of off-site scratching on chloroquine-evoked activity, off-site scratching failed to consistently affect capsaicin-evoked activity (Fig. 2, left half of lower right-hand PSTH; Fig. 3E, F). However, on-site scratching increased activity in 6 of the 9 units, resulting in a significant overall increase in firing during on-site scratching (to 326.4%, main effect of on-site scratching on capsaicin-evoked firing: $F(2, 24) = 4.75$, $p=0.0240$) compared to the pre-scratch baseline (Fig. 2, right half of lower right-hand PSTH; Fig. 3G, H). Post-scratch firing decreased to the pre-scratch level (Fig. 3H). Mean on-site scratch-evoked activity during the response to capsaicin was also significantly higher compared to the mean on-site scratch-evoked firing rate prior to capsaicin (7.0 ± 1.8 Hz vs. 2.8 ± 0.6 Hz, $p=0.0441$).

Histamine

Histamine-responsive neurons (1 NS, 5 WDR) were located in the superficial dorsal horn (Fig. 4F) at a mean depth of $68.5 \mu\text{m} \pm 26.9$ (SEM) below the surface. All units had mechanosensitive receptive fields on the ipsilateral hind paw, and responded to id

microinjection of histamine within the receptive field. Mean unit firing immediately post-histamine injection was significantly greater compared to the pre-injection level or following vehicle injection (Fig. 1).

Off-site scratching inhibited each of the 6 units (Fig. 4A, B, main effect of off-site scratching on histamine-evoked firing: $F(2, 15) = 4.45$, $p = 0.0416$). Off-site scratching significantly reduced the mean firing rate during scratching compared to the immediate 20-sec pre-scratch level (Fig. 4B, Dunnett's post-hoc test: $p < 0.05$), followed by recovery to the pre-scratch level (Fig. 4B).

In contrast, on-site scratching increased the firing rate during scratching compared to the pre-scratch level (Fig. 4C, D). Upon cessation of scratching, the mean firing rate decreased significantly below the pre-scratch baseline level (Fig. 4C, D, effect of on-site scratching on histamine-evoked firing: $F(1, 10) = 21.3$, $p = 0.00577$), consistent with a previous report (Davidson *et al.*, 2009).

Each of the histamine-responsive units responded to capsaicin injected id within the same unit's mechanical receptive field at least 40 min later. The mean response to capsaicin was comparable to that elicited by histamine, and significantly greater than pre-injection baseline or vehicle-evoked levels (Fig. 1). Data for the mean peak response to capsaicin were pooled in Fig. 1, since there was no significant difference in the mean capsaicin-evoked firing rates between histamine-responsive and chloroquine-responsive spinal neurons.

Similar to chloroquine-responsive spinal neurons, off-site scratching failed to consistently affect capsaicin-evoked activity (Fig. 4E, F). However, on-site scratching increased activity in all of the 5 units, resulting in a significant overall increase in firing during on-site scratching (to 3122.4%, main effect of on-site scratching on capsaicin-evoked firing: $F(2, 12) = 6.60$, $p = 0.0203$) compared to the pre-scratch level (Fig. 4G, H). After the cessation of scratching, the firing rate returned to the pre-scratch baseline level (Fig. 4H).

Discussion

Cutaneous scratching has state- and site-dependent effects on the activity of pruritogen-sensitive neurons in the superficial dorsal horn. Our results are consistent with a recent report that primate spinothalamic tract neuronal responses are modulated in a state-dependent manner; histamine-evoked responses were inhibited whereas capsaicin-evoked responses were not inhibited by scratching (Davidson *et al.*, 2009). Importantly, the present findings extend this by showing that effects of scratching are also site-dependent. Scratching away from the injection site (off-site) inhibited chloroquine- and histamine-evoked activity. Scratching directly at the injection site (on-site) did not affect chloroquine-evoked firing, but facilitated histamine-evoked activity, followed at the end of the scratch period by a decline in firing to a level significantly below the pre-scratching baseline. These results are consistent with a previous study in which most primate spinothalamic tract neurons similarly exhibited elevated levels of histamine-evoked firing during scratching, followed by a decline in firing post-scratching (Davidson *et al.*, 2009) that subsequently recovered to pre-scratch levels within approximately 30 sec as observed presently. Additionally, the present findings have revealed differential effects of scratching on neuronal activity elicited by histamine compared to chloroquine that acts independently of histamine receptors (Wilson *et al.*, 2011). While neuronal firing elicited by both histamine and chloroquine was suppressed during off-site scratching, firing elicited by histamine, but not chloroquine, was suppressed after the termination of on-site scratching.

Our finding that off-site scratching inhibited pruritogen-evoked activity was unexpected, and seems counter-intuitive, since scratches are presumably directed to the site of itch to remove

the causal stimulus such as an insect or plant spicule. Indeed, in a human study, localization of the site of itch evoked by histamine iontophoresis was accurate to within approximately 7 mm and matched the accuracy of localization of a painful stimulus (Koltzenburg *et al.*, 1993). Our present result is supported by older studies reporting that itch elicited by cutaneous insertion of spicules from cowhage (*Mucuna pruriens*) is suppressed by pin-prick applied within a large region surrounding the punctuate source of itch, and can be far as 24 cm away but within the same dermatome (Graham *et al.*, 1951; Chapmann *et al.*, 1960). Acupuncture stimulation delivered within the same dermatome more effectively suppressed histamine-evoked itch than acupuncture delivered extrasegmentally (Lundeberg *et al.*, 1987). Consistent with this, scratch marks on the skin tend to be aligned longitudinally along dermatomal lines, particularly in the arms and legs (Savin *et al.*, 1999), with the length of scratch marks correlated with two-point discrimination thresholds (Cornbleet, 1953). All of these observations indicate that scratches delivered within the same dermatome, but not necessarily directly to the site of itch, effectively relieve itch.

Chloroquine-evoked firing was reduced during the period of off-site scratching, and remained significantly lower than the pre-scratch firing rate 20–40 sec after the end of scratching (Fig. 2A, B). The failure of the firing rate to return to the pre-scratch level is due to the time-dependent decay in chloroquine-evoked firing, possibly accompanied by inhibition that outlasted the period of scratching. Histamine-evoked firing was reduced during the period of off-site scratching and immediately after on-site scratching (Fig. 4C, D), followed by recovery of firing. Our data are consistent with those of Davidson *et al.* (2009), who reported that most spinothalamic tract neurons exhibited an increase in firing rate during scratching within the receptive field, followed by an inhibition of firing post-scratching that recovered within approximately 30 sec. In mice, scratching phasically inhibited spontaneous activity of dorsal horn neurons receiving input from an area of experimental dry skin (Akiyama *et al.*, 2011). These results are consistent with a recent report that histamine- or cowhage-evoked itch was suppressed by scratching, with itch sensation returning within 6–12 sec (Kosteletzky *et al.*, 2009). However, older studies have reported that relief of cowhage-evoked itch by scratching sometimes persisted for several minutes (Chapmann *et al.*, 1960). Neural mechanisms underlying prolonged scratch-evoked suppression of itch are currently not known.

Inhibitory interneurons are thought to play an important role in the inhibition of itch-signaling spinal neurons by releasing glycine and/or GABA as neurotransmitters. Spinal application of glycine and GABA-A and GABA-B receptor antagonists attenuated or abolished scratch-evoked inhibition of spontaneous activity in dorsal horn neurons with input from dry skin (Akiyama *et al.*, 2011). Moreover, these spinal inhibitory interneurons may exhibit a low level of basal activity, based on evidence that decreased activity in, or deletion of, inhibitory spinal interneurons leads to enhanced itch. Loss of a population of inhibitory interneurons in the superficial dorsal horn of knockout mice lacking the transcription factor *Bhlhb5* (Ross *et al.*, 2010), or knockout of the glutamate transporter VGLUT2 in Nav1.8-expressing nociceptors (Lagerstrom *et al.*, 2010; Liu *et al.*, 2010), both resulted in excessive scratching behavior. These findings suggest that a reduction in nociceptive input decreases spinal inhibition to disinhibit itch transmission.

In addition to segmentally-organized inhibitory mechanisms activated by scratching near the site of pruritogenic stimulation, there may be a more global network, possibly similar to Diffuse Noxious Inhibitory Controls (DNIC) and involving suprasegmental pathways, that contributes to the suppression of itch by noxious counterstimuli delivered at sites remote from the source of itch (e.g., (Murray & Weaver, 1975)). The involvement of suprasegmental pathways is supported by our observation that scratch-evoked inhibition of spontaneously firing dorsal horn neuron in mice with dry skin was reduced by

approximately 50% following cold-block or transection of the upper cervical spinal cord (Akiyama *et al.*, 2011).

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Abbreviations

WDR	wide dynamic range
NS	nociceptive-specific
PSTH	peristimulus-time histogram

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Fig. 1.

Increased firing of spinal neurons following chloroquine, histamine and capsaicin. Bar graphs show, from *left to right*, responses to id microinjection of vehicle saline (vehicle for chloroquine; $n=8$), chloroquine (CQ, 100 $\mu\text{g}/\mu\text{l}$, $n=10$), histamine (His, 50 $\mu\text{g}/\mu\text{l}$, $n=6$), 0.7% Tween 80 (vehicle for capsaicin; $n=8$), and capsaicin (Cap, 30 $\mu\text{g}/\mu\text{l}$, $n=14$). Mean firing rate was calculated over a 1 min period pre- and again 1 min immediately post-injection. Error bars: SEM. Mean firing post-chloroquine and -capsaicin injections was significantly greater compared to mean firing pre-injection as well as mean firing following vehicle injections ($*p < 0.01$ for both; paired t -test and unpaired t -test, respectively).

Fig. 2.

Individual example. Upper left peristimulus-time histogram (PSTH; bin: 1 sec) shows response of superficial dorsal horn unit to id injection of chloroquine (CQ; at arrow; 100 $\mu\text{g}/\mu\text{l}$), and effects of off-site and on-site scratching. Gray area shows expanded portions of the PSTHs above; duration of scratching is indicated by large horizontal bars above lower PSTHs. Figurine drawings of hind paw show injection site (X) and location of scratching (double-headed arrows). Upper and lower middle PSTHs shows unit's response to brush, pinch and scratching within (on-site) and outside the receptive field (off-site), after CQ-evoked firing had waned. In middle figurine drawings of hind paw, location of mechanosensitive receptive field indicated by gray area. Upper and lower right PSTHs shows same unit's response to id capsaicin (30 $\mu\text{g}/\mu\text{l}$) and scratch stimuli.

Fig. 3.

State- and site-dependent inhibition of activity of chloroquine-responsive superficial dorsal horn neurons. A: Averaged PSTH (bins: 1 sec) of activity following id chloroquine (CQ) in 10 superficial dorsal horn units, before, during and after scratching on- or off-site. Horizontal arrows indicate duration of scratching. Error bars: SEM.

B: Graph plots individual (thin lines) and mean (thick line $\pm$ SEM) responses of superficial dorsal horn units following id chloroquine, before (pre), during, and after (post) off-site scratching. Responses normalized to pre-scratch firing rate. *: significantly different compared to the pre-scratch baseline ($p < 0.05$ during; $p < 0.05$ post, Dunnett's test). #: significantly different compared to during ($p < 0.05$; paired t -test). Inset figurine shows perimeters of units' mechanical receptive fields (dashed lines) on drawing of ipsilateral hindpaw.

C: PSTH of same units in A, in which scratching was delivered on-site during the unit's response to chloroquine.

D: Graph as in B showing chloroquine-evoked activity, pre-, during, and post- on-site scratching. One-half of the units exhibited increased firing during scratching, but the overall effect (124.3%) was not statistically significant. There was no significant correlation between the chloroquine-evoked firing rate and the degree of on-site scratch-evoked inhibition ($r = 0.545$, $p = 0.103$, Pearson's product moment correlation).

E: PSTH as in A showing lack of effect of off-site scratching on mean response to capsaicin.

F: Graph as in B showing capsaicin-evoked activity, pre-, during, and post- off-site scratching. Inset shows histologically-recovered recording sites in superficial dorsal horn of units tested with CQ.

G: PSTH as in C showing facilitatory effect of on-site scratching during the response to capsaicin.

H: Graph as in D showing capsaicin-evoked activity, pre-, during, and post- on-site scratching. *: significantly different compared to pre ($p < 0.05$; Dunnett's test).

Fig. 4.

State- and site-dependent inhibition of activity of histamine-responsive superficial dorsal horn neurons. A: Averaged PSTH (bins: 1 sec) of activity following id histamine (His) in 6 superficial dorsal horn units, before, during and after scratching on- or off-site. Horizontal arrows indicate duration of scratching. Error bars: SEM.

B: Graph plots individual (thin lines) and mean (thick line $\pm$ SEM) responses of superficial dorsal horn units following id histamine, before (pre), during, and after (post) off-site scratching. Responses normalized to pre-scratch firing rate. *: significantly different compared to the pre-scratch baseline ($p < 0.05$ during; Dunnett's test). Inset figurine shows perimeters of units' mechanical receptive fields (dashed lines) on drawing of ipsilateral hindpaw (two receptive fields spanned the entire hindpaw).

C: PSTH of same units in A, in which scratching was delivered on-site during the unit's response to histamine.

D: Graph as in B showing histamine-evoked activity, pre-, during, and post- on-site scratching. *: significantly different compared to the pre-scratch baseline ($p < 0.05$ post, Dunnett's test).

E: PSTH as in A showing lack of effect of off-site scratching on mean response to capsaicin.

F: Graph as in B showing capsaicin-evoked activity, pre-, during, and post- off-site scratching. Inset shows histologically-recovered recording sites in superficial dorsal horn of units tested with histamine.

G: PSTH as in C showing facilitatory effect of on-site scratching during the response to capsaicin.

H: Graph as in D showing capsaicin-evoked activity, pre-, during, and post- on-site scratching. *: significantly different compared to pre ($p < 0.05$; Dunnett's test).