Title

Behavioral model of itch, alloknesis, pain and allodynia in the lower hindlimb and correlative responses of lumbar dorsal horn neurons in the mouse

Authors

Akiyama, T
Nagamine, M
Carstens, MI
et al.

Publication Date

2014-04-01

DOI

10.1016/j.neuroscience.2014.02.005

Peer reviewed

Published in final edited form as:
Neuroscience. 2014 April 25; 266: 38–46. doi:10.1016/j.neuroscience.2014.02.005.

Behavioral model of itch, alloknesis, pain and allodynia in the lower hindlimb and correlative responses of lumbar dorsal horn neurons in the mouse

Tasuku Akiyama, **Masaki Nagamine**, **Mirela Iodi Carstens**, and **E. Carstens**
University of California, Davis, Department of Neurobiology, Physiology & Behavior, 1 Shields Avenue, Davis, California 95616

Abstract

We have further developed a behavioral model of itch and pain in the lower hindlimb (calf) originally reported by LaMotte et al. (2011) that allows comparisons with responses of lumbar dorsal horn neurons to pruritic and noxious stimuli. Intradermal (id) microinjection of the pruritogens histamine, SLIGRL-NH2 (agonist of PAR-2 and MrgprC11) and choloroquine (agonist of MrgprA3) into the calf of the lower limb elicited significant biting and a small amount of licking directed to the injection site, over a 30-min time course. Following id injection of histamine, low-threshold mechanical stimuli reliably elicited discrete episodes of biting (alloknesis) over a longer time course; significantly less alloknesis was observed following id injection of SLIGRL-NH2. Capsaicin injections elicited licking but little biting. Following id injection of capsaicin, low-threshold mechanical stimuli elicited discrete hindlimb flinches (allodynia) over a prolonged (>2 hr) time course. In single-unit recordings from superficial lumbar dorsal horn neurons, low-threshold mechanically-evoked responses were significantly enhanced, accompanied by receptive field expansion, following id injection of histamine in histamine-responsive neurons. This was not observed in histamine-insensitive neurons, or following id injection of saline or SLIGRL-NH2, regardless of whether the latter activated the neuron or not. These results suggest that itch-responsive neurons are selectively sensitized by histamine but not SLIGRL-NH2 to account for alloknesis. The presently-described “calf” model appears to distinguish between itch- and pain-related behavioral responses, and provides a basis to investigate lumbar spinal neural mechanisms underlying itch, alloknesis, pain and allodynia.

Keywords

itch; alloknesis; pain; allodynia; scratching; dorsal horn neuron

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.

CONFLICT OF INTERST none

1. INTRODUCTION

Chronic itch frequently accompanies many dermatological and systemic diseases (Ikoma et al., 2006).. The associated scratching exacerbates the skin inflammation, leading to a vicious itch-scratch cycle (Wahlgren, 1999) that reduces the quality of life (Hundley et al., 2006). A potential mechanism is sensitization of itch-signaling pathways, manifested by spontaneous itch and scratching, hyperknesis (enhanced itch), and alloknesis (touch-evoked itch)(Hosogi et al., 2006; Ikoma et al., 2006 for recent review, see Akiyama & Carstens, 2013). Itch can be elicited by innocuous touching of normal skin around a site of histamine-evoked itch (Bickford, 1937; Graham et al., 1951; Heyer et al., 1997; Simone et al., 1991). In chronic itch patients low-threshold mechanical stimuli, such as clothes contacting the skin, can initiate the itch-scratch cycle (Bendsoe et al., 1987; Heyer & Hornstein, 1999; Mason, 2008; Ricci et al., 2006; Wahlgren, 1991).

To develop mechanisms-based treatments for itch, animal models are required. Scratching behavior in rodents is commonly used to assess itch (Carstens & Kuraishi, 2004). This traditionally involves counting hindlimb scratch bouts directed toward a pruritogen injection in rostral back skin. A drawback is that hindlimb scratching does not discriminate between itch and pain since it is the only available response. We developed a novel model of alloknesis in which touch near a site of intradermal (id) injection of histamine and certain other pruritogens, or at the edge of an area of dry skin, reliably elicited hindlimb scratches (Akiyama et al., 2012). In a recent variant, id pruritogen injection in the rodent cheek elicited primarily hindlimb scratching, while algogens elicited mainly forelimb wiping (Shimada & LaMotte, 2008). Scratching was reduced by systemic administration of μ-opioid antagonists whereas wiping was reduced by systemic administration of morphine (Akiyama et al., 2010a; Spradley et al., 2012b). Furthermore, this model allows direct comparisons of behavior with pruritogen- and algogen-evoked responses of first- and second-order trigeminal neurons (Akiyama et al., 2010b; Klein et al., 2011a). For decades, however, much information about itch and pain has come from recordings of lumbar spinal neurons with input from the hindlimb. Moreover, it is important to recognize differences in trigeminal vs. spinal processing of itch and pain (e.g., Spradley et al., 2012b). Thus, a behavioral model that distinguishes between itch and pain from the lower hindlimb is desirable.

Hindpaw injection of 5-HT elicited naloxone-sensitive paw-biting accompanied by licking, while hindpaw injection of algogens only elicited paw-licking (Hagiwara et al., 1999). Mice with chronic dry hindlimb skin exhibited spontaneous paw-biting (Nojima et al., 2004) that was sensitive to naltrexone but not morphine (Akiyama et al., 2010c). Lumbar dorsal horn neurons ipsilateral to the dry skin-treated hindpaw exhibited elevated spontaneous activity (Akiyama et al., 2011a, 2011b). A minor drawback of the hindpaw behavioral model is that weight-bearing and locomotion may interfere with itch-related behaviors. It was recently reported that id injection of histamine into the mouse calf elicited biting whereas injection of capsaicin elicited licking (LaMotte et al., 2011). In the present study, we have further developed and validated this calf model. We additional developed a novel model of alloknesis and allodynia, whereby low-threshold mechanical stimuli reliably elicit biting or hindlimb flinches, respectively, following id injection of histamine or capsaicin. Finally, in electrophysiological experiments we tested whether id injection of histamine enhances the

mechanosensitivity of pruritogen-responsive lumbar superficial dorsal horn as a possible mechanism underlying alloknesis.

2. EXPERIMENTAL PROCEDURES

Experiments were conducted using adult C57BL/6 mice (Harlan, Oxnard CA) (19–32 g body weight) under a protocol approved by the UC Davis Animal Care and Use Committee.

2.1. Behavior

The fur on the calf was shaved and mice were habituated to a Plexiglas recording arena with a transparent cover one week prior to testing. On the test day, the animal was restrained by hand and the skin on one hind limb was mildly stretched. An id microinjection of 10 μl was made in the calf of one of the following: vehicle (isotonic saline), histamine (50 μg; Sigma-Aldrich, St. Louis MO), SLIGRL-NH2 (50 μg; Quality Controlled Biochemicals, Hopkinton, MA), chloroquine (50 μg; Sigma), capsaicin (10 μg; Sigma), or 7% Tween-80 (vehicle for capsaicin). The calf id microinjection was made id using a 30-G needle attached to a Hamilton microsyringe by PE-50 tubing. Immediately following the id microinjection, mice were placed into a clear glass arena containing mirrors set at angles to allow multiple views of the animal that were captured with a high-definition videocamera. Investigators left the room during videotaping. The videocamera was set at high definition and slow motion capture modes to facilitate the assessment of biting and licking behaviors directed to the injected calf in a frame-by-frame video playback conducted offline by two investigators blinded as to the experimental treatment. The duration of biting, licking and flinching episodes were timed at 5-min intervals over the 50-min recording period. A bite is defined as direct contact of the incisors with calf skin. Biting was accompanied by relatively high-frequency low-excursion head movements which were used as an adjunct measure. The duration of each biting action was timed with an original program which allows us to check the movie frame by frame, and individual bite durations were summed to provide the cumulative biting time. The cumulative duration of licking, defined as direct contact of the calf skin by tongue protrusion, was also measured. Licks were accompanied by head movements that were of lower frequency and larger excursion compared to those associated with biting, and were also used as an adjunct measure of licking.

In a separate experiment, the animal was habituated to a recording arena for 1 hr and then tested for alloknesis and allodynia at 5-min intervals, starting 5 min post- histamine or capsaicin injection. Alloknesis and allodynia were assessed as follows. At 5-min intervals, the mouse received 3 separate innocuous mechanical stimuli delivered using a von Frey filament (bending force: 0.7 mN). Stimuli were delivered 2 mm or further distal to the edge of the visible bleb formed by the id injection of histamine or other agents. The 0.7 mN von Frey filament was selected because it never elicited behaviors (e.g. biting, licking and flinching) in naïve mice, and was the minimum strength to elicit biting when delivered to skin surrounding the site of histamine injection. The mice were videotaped with a high-speed recording feature that turns 3 seconds of video into 12 seconds of slow motion video. The presence or absence of a positive response, i.e., biting, licking and flinching directed to the site of mechanical stimulation, was confirmed by video playback. The behavior score

was the total number of positive responses elicited by the three stimuli, i.e., 0, 1, 2 or 3. The sequence was repeated at 5-min intervals out to 60 min post-injection, and again at 90 and 120 min post-injection time points. In experiments with histamine and capsaicin, an overall behavior score per 60 min and 120 min, respectively, was calculated as the sum of individual behavior scores.

2.2 Electrophysiological recording

The mouse was anesthetized with sodium pentobarbital (60 mg/kg ip) and prepared for single-unit recording from the lumbar spinal cord as previously detailed (Akiyama et al., 2009b). A tungsten microelectrode was driven into the superficial lumbar dorsal horn and single extracellularly-recorded units were isolated using mechanical touch and pressure stimuli delivered to the ipsilateral hindpaw. In a few experiments we recorded from units that responded to mechanical stimulation of the ipsilateral calf. However, we decided to focus on units with hindpaw receptive fields because data from such units may be compared with data from our previous electrophysiological studies (Akiyama et al., 2009a,b, 2011 a,b), and it was more difficult to accurately map receptive field dynamics on the calf compared to hindpaw. Recording depths were restricted to <300 μm below the surface as in our previous study. Unit activity was amplified, digitized and displayed on computer using a Powerlab (AD Instruments, Colorado Springs CO) interface. Once a mechanosensitive unit was isolated, we tested the unit responsiveness to light brushing with a cotton wisp, followed by pinching using forceps. Units were classified as wide dynamic range (WDR) if they responded at higher firing rate to pinch than light touch. They were classified as high-threshold (HT) if they responded to pinch but not light touch. The areas of mechanosensitive receptive fields were mapped by determining the perimeter along which application of the same von Frey filament used in behavioral studies (0.7 mN) evoked a response on 50% of application trials. Since some units did not respond, a series of von Frey filaments of progressively stronger bending forces ranging up to 760 mN was used to map the receptive field area. Each unit was retested with the 0.7 mN von Frey filament at least 3 times to establish a baseline response level, which for some units was zero. Five minutes later, either histamine, SLIGRL-NH2 (both 50 μg/μl in saline), or saline, was microinjected id (1 μl volume) within the mechanosensitive receptive field. Unit receptive field areas were redetermined 5 min postinjection, and at 5-min intervals thereafter out to 30 min postinjection, and again 45-min and 60 min postinjection. Unit responses to application of the 0.7 mN on Frey filament at the same sites tested preinjection were also determined at the same time intervals. At the conclusion of the recording, an electrolytic lesion was made. The spinal cord was postfixed in 10% buffered formalin and cut in 50 μm frozen sections to identify the lesion sites under the light microscope.

Unit activity was usually quantified as number of action potentials/sec or min. Responses to von Frey stimuli were summed over a 20-sec period and responses to histamine over successive 60-sec periods. A positive response was defined as a 30% or greater increase in the total number of action potentials per 20 or 60 sec post-stimulus compared to the same time interval before the stimulus. Averaged responses to von Frey stimuli before and after application of the pruritogen (or saline) were compared by paired *t*-test with $P < 0.05$ set as significant.

3. RESULTS

3.1. Chemically-evoked biting and licking behavior

Following id injection of histamine into the calf, mice exhibited biting directed toward the injection site, with significantly less licking (Fig. 1A, F). Biting peaked within the first 10 min post-injection and declined over the ensuing 30 min (Fig. 1A). Biting was characterized by contact of the incisors with the skin in a fairly high-frequency and low-excursion motion of the head. In contrast, licking was characterized by repeated protrusions of the tongue toward the skin over a longer excursion and lower frequency that could be readily distinguished from biting. The cumulative duration of biting was significantly greater following id injection of histamine compared to saline controls ($p<0.05$, unpaired t-test). A similar time course of biting, with significantly less licking ($p<0.05$, t-test), was elicited by id injection of SLIGRL-NH2 (Fig. 1B, F). Similarly, id injection of chloroquine elicited biting and significantly less licking (Fig. 1F) over a similar time course. In contrast, id injection of capsaicin injection in the calf elicited licking behavior that peaked 20 min post-injection, with significantly less biting (Fig. 1C, F). Neither id injection of saline (vehicle for histamine and SLIGRL) nor 7% Tween-80 (vehicle for capsaicin) elicited any significant biting or licking behavior (Fig. 1D–F, respectively).

3.2. Alloknesis and allodynia

A series of 3 innocuous von Frey mechanical stimuli was applied at 5-min intervals following id chemical injections to assess touch-evoked biting, licking or flinching behaviors. Prior to chemical injection, von Frey stimulation never elicited any behavioral responses. Figure 2A shows the time-course for touch-evoked behaviors following id injection of histamine. Robust touch-evoked biting (alloknesis) peaked immediately following id injection of histamine and slowly declined over the ensuing 45 min. The cumulative score for biting was significantly greater ($p<0.05$, unpaired t-test) following id injection of histamine (Fig. 2A) compared to the saline control group (Fig. 2F, open bars). The very low levels of licking and flinching did not differ from saline controls (Fig. 2F). Following id injection of SLIGRL-NH2 (Fig. 2B), touch-evoked biting was significantly less compared to histamine ($p<0.05$, unpaired t-test) but was significantly greater ($p<0.05$, unpaired t-test) compared to the saline control group (Fig. 2F). This was accompanied by very low levels of licking and flinching (Fig. 2B) that did not differ from saline controls (Fig. 2F).

Following id injection of capsaicin (10 μg), there was a rapid increase in touch-evoked flinching (allodynia) over the initial 30 min that persisted for at least 2 hr (Fig. 2C). The cumulative score for flinching was significantly greater ($p<0.05$, unpaired t-test) following id injection of capsaicin (Fig. 2C) compared to vehicle (7% Tween 80) controls (Fig. 2F, gray bars). The low levels of biting and licking following id injection of capsaicin (Fig. 2C) were not different from vehicle controls (Fig. 2F).

3.3. Electrophysiology

Recordings were made from a total of 56 units in 38 mice. Twenty-four were classified as WDR and 32 as HT. Eight of 22 units tested responded to id injection of histamine, and 6 of

24 units tested responded to id injection of SLIGRL-NH2. None of 10 units tested responded to id injection of saline. All units were located in the superficial dorsal horn at a mean depth of 137.4 μm +/− 11.6 below the surface. Histologically recovered lesion sites were located in the superficial dorsal horn (Fig. 4F).

In histamine-responsive units, mean mechanically-evoked responses were significantly greater following id injection of histamine compared to pre-injection. The example of Fig. 3 shows an increased response to the von Frey stimulus following id injection of histamine, which elicited a prolonged increase in firing of this lamina I cell. This was accompanied by an expansion of the mechanosensitive receptive (black area in figurine drawings of hindpaw).

Fig. 4A plots mean peak mechanically-evoked responses vs. time for the histamine-sensitive units, using the same von Frey stimulus that was used in the behavioral studies (■; 0.7 mN). Mean ongoing activity is also plotted (O, dashed line) to show increased firing during the first 15 min post-histamine. Prior to id injection of histamine, only 1/8 units responded to the weakest von Frey stimulus, while all 8 units responded at 5 min post-histamine. The mean mechanically-evoked response was significantly greater than the pre-injection response (−5 in Fig. 4A) out to 45 min post-histamine ($p<0.05$; paired *t*-test). The failure of the 0.7 mN stimulus to initially excite most histamine-responsive units precluded out ability to map receptive field areas. A stronger (55 mN) von Frey stimulus initially excited all 8 histamine-sensitive units, and elicited a significantly greater mean response at 10 and 15 min post-histamine (data not shown). This was accompanied by a significant ($p<0.01$, paired t-test) expansion of receptive field area by an average of 260.4%.

Responses elicited by the same 0.7 mN von Frey stimulus were not affected following id injection of histamine in histamine-insensitive units (Fig. 4B), or following id injection of SLIGRL-NH2 that either did (Fig. 4D) or did not increase neuronal firing (Fig. 4E). Similarly, id saline injection did not affect mechanically-evoked responses (Fig. 4C). In addition, there were no consistent changes in mechanosensitive receptive field areas under any of these treatment conditions.

4. DISCUSSION

4.1. Discrimination between itch and pain

The present results indicate that biting at a site of pruritic stimulation on the lower hindlimb reflects itch, whereas licking at a site of capsaicin injection reflects pain. Intradermal injection of the pruritogens histamine, SLIGRL-NH2 and chloroquine all elicited biting with little licking, whereas id injection of capsaicin evoked licking and little biting, consistant with a recent report (LaMotte et al., 2011) and with the idea that hindpaw biting reflects itch. Presumably, biting or gnawing at an itchy site on the hindlimb serves the same antipruritic function as scratching, since quadrupeds apparently do not scratch an itchy hindlimb with the contralateral paw.

We interpret the hindlimb licking behavior following id injection of capsaicin to reflect pain. Intraplantar injection of capsaicin elicited licking behavior in rats (Klein et al., 2011a), and

hindpaw injection of formalin elicited licking in mice (Hagiwara et al., 1999). We previously reported that systemic administration of morphine suppressed forelimb wiping, but not hindlimb scratching, elicited by id cheek injections of capsaicin or mustard oil in mice and rats, consistent with the notion that forelimb wiping reflects pain (Akiyama et al., 2010c; Spradley et al., 2012a). Presumably, licking of the hindpaw or forelimb wiping directed to the cheek represent the biomechanically available nocifensive responses to ameliorate chemogenic pain in these two different body areas. Licking and wiping may be akin to rubbing an injury to relieve pain.

4.2. Alloknesis and allodynia

Following id calf injection of histamine, low-threshold mechanical stimuli reliably elicited biting (Fig. 2A). Neither naïve mice nor control mice receiving id injection of saline exhibited any significant touch-evoked biting of the calf. We previously reported that following id injection of histamine in the rostral back, low-threshold mechanical stimuli reliably elicited hindlimb scratch bouts over a prolonged time course in a manner that was significantly reduced by systemic administration of naltrexone (Akiyama et al., 2012a). We thus interpret these touch-evoked behaviors to reflect alloknesis. Interestingly, following id injection of SLIGRL-NH2, which itself elicited significant biting (Fig. 2B), low-threshold mechanical stimuli elicited a low incidence of biting that was significantly greater than vehicle controls, but significantly less compared to the group receiving id injection of histamine. This is consistent with our previous study, in which touch stimuli failed to elicit any significant scratching following id injection of SLIGRL-NH2 in the rostral back (Akiyama et al., 2012a). In this latter study, reliable touch-evoked scratching (alloknesis) was observed following id injection of histamine, 5-HT, the PAR-4 agonist AYPGKF-NH2, and the MrgprC11 agonist BAM8–22, but not following SLIGRL-NH2 or the MrgprA3 agonist chloroquine (Akiyama et al., 2012a). Collectively, these data indicate that alloknesis is associated with itch elicited by certain, but not all, pruritogens.

While both SLIGRL-NH2 and BAM8–22 are agonists of MrgprC11 (Liu et al., 2011), it was curious that only BAM8–22 elicited alloknesis (Akiyama et al., 2012a). We previously reported that the histamine H1 receptor antagonist terfenadine attenuated scratching and alloknesis elicited by histamine, but not that elicited by 5-HT, the PAR-4 agonist or BAM8–22 (Akiyama et al., 2012a). This is consistent with current evidence for histamine-dependent and - independent types of itch (Johanek et al., 2007). Separate populations of primary afferents (Johanek et al., 2008; Namer et al., 2008) and primate spinothalamic tract neurons (Davidson et al., 2007, 2012) respond to histamine vs. cowhage, which elicits itch via PAR-2 and -4 receptors (Reddy et al., 2008). In mice, the majority of histamine-responsive dorsal horn neurons also responded to other non-histaminergic pruritogens such as SLIGRL-NH2, chloroquine and 5-HT (Akiyama et al., 2009a, 2009b, 2012b). However, cowhage was not tested in these studies, nor were other non-histaminergic pruritogens such as chloroquine tested in the primate spinothalamic tract studies. It thus remains uncertain to what extent different histaminergic and non-histaminergic itch mediators excite separate or overlapping populations of spinal neurons in these two species. Recent evidence indicates that natriuretic polypeptide B (Nppb) and gastrin releasing peptide (GRP) are essential for both histamine-dependent and –independent itch transmission in mice (Mishra & Hoon, 2013; Sun et al.,

2009), implying that pruriceptors expressing various combinations of transduction molecules (e.g., Mrgrps, PARs, 5-HT2, histamine H1,4, etc.) converge onto a common population of itch-signaling spinal neurons. However, cowhage was not tested in these studies either, leaving open the possibility of an additional itch-signaling pathway. More studies are needed to address the issue of multiple itch-signaling pathways, and why some itch mediators elicit alloknesis while others do not.

A novel finding was that following id injection of capsaicin, light touch reliably elicited hindlimb flinches (Fig. 2C). We previously reported finching behavior following intraplantar injection of capsaicin (Klein et al., 2011b), and flinching is often used as a behavioral readout in the formalin pain assay. We therefore interpret touch-evoked flinching to reflect allodynia.

4.3. Sensitization of itch-signaling pathways

One potential mechanism underlying alloknesis is the sensitization of histamine-dependent itch-signaling neurons. We previously showed that subpopulations of neurons in the superficial doral horn respond to id injection of pruritogens over a time course consistent with scratching behavior (Akiyama et al., 2009a, 2009b), representing candidate itch-signaling neurons. In the present study, we similarly recorded from lumbar dorsal horn neurons with receptive fields on the hindpaw. Most unit receptive fields were in glabrous hindpaw skin, thus not matching exactly the site of pruritic stimulation on furry calf skin in the behavioral studies. Despite the drawback of an imperfect match between behavioral and neurophysiological stimulus loci, it is advantageous that the present neuronal population is otherwise comparable with unit populations reported in our previous studies (Akiyama et al., 2009a,b 2011a,b). The present histamine-responsive dorsal horn neurons exhibited an enhancement of low-threshold mechanically-evoked responses following id injection of histamine, over a time course matching that of behavioral alloknesis. Units unresponsive to id injection of histamine, and units tested with id injection of saline, did not exhibit enhanced mechanically-evoked responses. Moreover, id injection of SLIGRL-NH2 had no significant effect on low-threshold mechanically evoked responses, regardless of whether it activated the dorsal horn neuron or not (Fig. 4 D,E). This is consistent with the finding that the behavior score for touch-evoked biting was significantly lower following id injection of SLIGRL-NH2 compared to that following id injection of histamine (Fig. 2A,B). Therefore, we believe that these similar findings justifiy our present use of lumbar dorsal horn units with hindpaw receptive fields as a correlative neuronal model for the behavioral studies conducted using furry calf skin.

In histamine-responsive units, increased mechanosensitivity following id injection of histamine was manifested by (a) response to the lowest-threshold stimulus in units that did not respond to this stimulus pre-histamine, (b) increased response to suprathreshold mechanical stimuli following histamine, and (c) receptive field expansion. Application of histamine sensitized responses of visceral polymodal nociceptors to mechanical stimulation (Koda & Mizumura, 2002), while SLIGRL-NH2 sensitized cutaneous C-fiber polymodal nociceptors to noxious heat but not mechanical stimuli (Ding-Pfennigdorff et al., 2004). These data are consistent with our observation that id injection of histamine enhanced the

responses of histamine-sensitive dorsal horn neurons to low-threshold mechanical stimuli, whereas such responses were not enhanced following id injection of SLIGRL-NH2 in dorsal horn units sensitive to this pruritogen (Fig. 4). Moreover, morphine, which induces itch, was recently reported to enhance pruritogen-evoked responses, as well as responses to innocuous mechanical stimulation, of trigeminothalamic tract neurons (Moser & Giesler, 2013). Histamine was also reported to activate inward calcium currents in Merkel cells (Boulais et al., 2009), and increased skin temperature due to histamine-evoked vasodilation could increase activity in low-threshold mechanoreceptors (Duclaux & Kenshalo, 1972). These observations provide potential mechanisms for histamine to peripherally sensitize low-threshold mechanoreceptors.

Alternatively, central sensitization could account for the enhanced low-threshold mechanosensitivity and expanded receptive field area of histamine-sensitive dorsal horn neurons. Many histamine-sensitive dorsal horn neurons respond to low-threshold mechanical stimuli, and a high percentage of these neurons also responded to id injection of SLIGRL-NH2 (Akiyama et al., 2009a, 2009b). However, low-threshold mechanically-evoked responses were presently enhanced by id injection of histamine but not SLIGRL-NH2. If central sensitization plays a role, our findings suggest that the dorsal horn neurons would be differentially sensitized by input from histamine- but not SLIGRL-NH2-sensitive primary afferents. If so, the cellular mechanism underlying this differential sensitization remains to be elucidated in future studies.

Alloknesis has also been observed in an experimental model of chronic dry skin pruritus on the rostral back and hindpaw (Akiyama et al., 2011a, 2012a). The onset of alloknesis was delayed relative to the onset of spontaneous scratching, and was associated with a significant increase in Fos expression in the superficial dorsal horn (Nojima et al., 2004). Lumbar superficial dorsal horn neurons recorded ipsilateral to a dry-skin-treated hindpaw exhibited significantly heightened spontaneous activity compared to neurons ipsilateral to the vehicle (water)-treated side, or to an untreated hindpaw (Akiyama et al., 2011a). Curiously, in the latter study we did not observe enhancement of mechanically-evoked responses of neurons ipsilateral to the dry skin-treated hindpaw (Akiyama et al., 2011a). However, this may be because alloknesis was only elicited by stimulation at the border of the dry skin-treated area (Akiyama et al., 2012a), while in the electrophysiological study (Akiyama et al., 2011a) the neuronal receptive fields were within the hindpaw dry skin area where mechanical stimulation would not be expected to elicit enhanced responses.

4.4. Conclusions

Intradermal injection of pruritogens including histamine, SLIGRL-NH2 and chloroquine into the calf primarily elicited biting behavior (with little licking), while id injection of capsaicin elicited licking and little biting behavior. Following id injection of histamine, innocuous mechanical stimuli reliably elicited biting directed to the stimulus site, interpreted as alloknesis. In contrast, following id injection of capsaicin light touch elcited hindlimb flinches, interpreted as allodynia. In electrophysiological studies, histamine-responsive single units in the superficial lumbar dorsal horn exhibited enhanced mechanically-evoked responses and receptive field expansion, possibly reflecting peripheral and/or central

sensitization that may underlie alloknesis. The present behavioral model thus appears to distinguish between itch- and pain-related behavioral responses, and provides a basis to investigate lumbar spinal neural mechanisms underlying itch, alloknesis, pain and allodynia.

Acknowledgments

Supported by grants DE021183 and AR057194 from the National Institutes of Health (to EC) and grant AR063228 from the National Institutes of Health (to TA).

REFERENCES CITED

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

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. 2009a; 102(4):2176–2183. [PubMed: 19625538]

Akiyama T, Carstens MI, Carstens E. Differential itch- and pain-related behavioral responses and micro-opoid modulation in mice. Acta dermato-venereologica. 2010a; 90(6):575–581. [PubMed: 21057739]

Akiyama T, Carstens MI, Carstens E. Facial injections of pruritogens and algogens excite partly overlapping populations of primary and second-order trigeminal neurons in mice. J Neurophysiol. 2010b; 104(5):2442–2450. [PubMed: 20739601]

Akiyama T, Carstens MI, Carstens E. Spontaneous itch in the absence of hyperalgesia in a mouse hindpaw dry skin model. Neuroscience letters. 2010c; 484(1):62–65. [PubMed: 20709155]

Akiyama T, Carstens MI, Carstens E. Enhanced responses of lumbar superficial dorsal horn neurons to intradermal PAR-2 agonist but not histamine in a mouse hindpaw dry skin itch model. J Neurophysiol. 2011a; 105(6):2811–2817. [PubMed: 21430273]

Akiyama T, Carstens MI, Ikoma A, Cevikbas F, Steinhoff M, Carstens E. Mouse model of touch-evoked itch (alloknesis). J Investigat Dermatol. 2012a; 132(7):1886–1891.

Akiyama T, Iodi Carstens M, Carstens E. Transmitters and pathways mediating inhibition of spinal itch-signaling neurons by scratching and other counterstimuli. PloS one. 2011b; 6(7):e22665. [PubMed: 21818363]

Akiyama T, Merrill AW, Carstens MI, Carstens E. Activation of superficial dorsal horn neurons in the mouse by a PAR-2 agonist and 5-HT: potential role in itch. J Neurosci. 2009b; 29(20):6691–6699. [PubMed: 19458238]

Akiyama T, Tominaga M, Carstens MI, Carstens EE. Site-dependent and state-dependent inhibition of pruritogen-responsive spinal neurons by scratching. Europ J Neurosci. 2012b; 36(3):2311–2316.

Bendsoe N, Bjornberg A, Asnes H. Itching from wool fibres in atopic dermatitis. Contact dermatitis. 1987; 17(1):21–22. [PubMed: 3652686]

Bickford RG. Experiments relating to the itch sensation, itc peripheral mechanism and central pathways. Clin Sci. 1937; 3:377–386.

Boulais N, Pennec JP, Lebonvallet N, Pereira U, Rougier N, Dorange G, Chesne C, Misery L. Rat Merkel cells are mechanoreceptors and osmoreceptors. PloS one. 2009; 4(11):e7759. [PubMed: 19898622]

Carstens, E.; Kuraishi, Y. Animal Models of itch: Scratching Away at the Problem. In: Yosipovitch, G.; Greaves, MW.; Fleischer, AB.; McGlone, F., editors. Itch: Basic Mechanisms and Therapy. Monticello, NY: Marcel Dekker; 2004. p. 35-50.

Davidson S, Zhang X, Khasabov SG, Moser HR, Honda CN, Simone DA, Giesler GJ Jr. Pruriceptive spinothalamic tract neurons: physiological properties and projection targets in the primate. J Neurophysiol. 2012; 108(6):1711–1723. [PubMed: 22723676]

Davidson S, Zhang X, Yoon CH, Khasabov SG, Simone DA, Giesler GJ Jr. The itch-producing agents histamine and cowhage activate separate populations of primate spinothalamic tract neurons. J Neurosci. 2007; 27(37):10007–10014. [PubMed: 17855615]

Ding-Pfennigdorff D, Averbeck B, Michaelis M. Stimulation of PAR-2 excites and sensitizes rat cutaneous C-nociceptors to heat. Neuroreport. 2004; 15(13):2071–2075. [PubMed: 15486484]

Duclaux R, Kenshalo DR. The temperature sensitivity of the type I slowly adapting mechanoreceptors in cats and monkeys. J Physiol. 1972; 224(3):647–664. [PubMed: 4627267]

Graham D, Goodell H, Wolff H. Neural mechanisms involved in itch, itchy skin, and tickle sensations. J Clin Investigat. 1951; 30(1):37–49.

Hagiwara K, Nojima H, Kuraishi Y. Serotonin-induced biting of the hind paw is itch-related response in mice. Pain Res. 1999; 14:53–59.

Heyer G, Dotzer M, Diepgen TL, Handwerker HO. Opiate and H1 antagonist effects on histamine induced pruritus and alloknesis. Pain. 1997; 73(2):239–243. [PubMed: 9415511]

Heyer GR, Hornstein OP. Recent studies of cutaneous nociception in atopic and non-atopic subjects. J Dermatol. 1999; 26(2):77–86. [PubMed: 10091477]

Hosogi M, Schmelz M, Miyachi Y, Ikoma A. Bradykinin is a potent pruritogen in atopic dermatitis: a switch from pain to itch. Pain. 2006; 126(1–3):16–23. [PubMed: 16842920]

Hundley JL, Carroll CL, Lang W, Snively B, Yosipovitch G, Feldman SR, Jorizzo JL. Cutaneous symptoms of dermatomyositis significantly impact patients' quality of life. J Am Acad Dermatol. 2006; 54(2):217–220. [PubMed: 16443050]

Ikoma A, Steinhoff M, Stander S, Yosipovitch G, Schmelz M. The neurobiology of itch. Nature Rev Neurosci. 2006; 7(7):535–547. [PubMed: 16791143]

Johanek LM, Meyer RA, Friedman RM, Greenquist KW, Shim B, Borzan J, Hartke T, LaMotte RH, Ringkamp M. A role for polymodal C-fiber afferents in nonhistaminergic itch. J Neurosci. 2008; 28(30):7659–7669. [PubMed: 18650342]

Johanek LM, Meyer RA, Hartke T, Hobelmann JG, Maine DN, LaMotte RH, Ringkamp M. Psychophysical and physiological evidence for parallel afferent pathways mediating the sensation of itch. J Neurosci. 2007; 27(28):7490–7497. [PubMed: 17626210]

Klein A, Carstens MI, Carstens E. Facial injections of pruritogens or algogens elicit distinct behavior responses in rats and excite overlapping populations of primary sensory and trigeminal subnucleus caudalis neurons. J Neurophysiol. 2011a; 106(3):1078–1088. [PubMed: 21653727]

Klein AH, Sawyer CM, Zanotto KL, Ivanov MA, Cheung S, Carstens MI, Furrer S, Simons CT, Slack JP, Carstens E. A tingling sanshool derivative excites primary sensory neurons and elicits nocifensive behavior in rats. J Neurophysiol. 2011b; 105(4):1701–1710. [PubMed: 21273322]

Koda H, Mizumura K. Sensitization to mechanical stimulation by inflammatory mediators and by mild burn in canine visceral nociceptors in vitro. J Neurophysiol. 2002; 87(4):2043–2051. [PubMed: 11929922]

LaMotte RH, Shimada SG, Sikand P. Mouse models of acute, chemical itch and pain in humans. Exp Dermatol. 2011; 20(10):778–782. [PubMed: 21929688]

Liu Q, Weng HJ, Patel KN, Tang Z, Bai H, Steinhoff M, Dong X. The distinct roles of two GPCRs, MrgprC11 and PAR2, in itch and hyperalgesia. Science Signaling. 2011; 4(181):ra45. [PubMed: 21775281]

Mason R. Fabrics for atopic dermatitis. J Family Health Care. 2008; 18(2):63–65.

Mishra SK, Hoon MA. The cells and circuitry for itch responses in mice. Science. 2013; 340(6135): 968–971. [PubMed: 23704570]

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(14):6093–6101. [PubMed: 23554490]

Namer B, Carr R, Johanek LM, Schmelz M, Handwerker HO, Ringkamp M. Separate peripheral pathways for pruritus in man. J Neurophysiol. 2008; 100(4):2062–2069. [PubMed: 18562548]

Nojima H, Cuellar JM, Simons CT, Carstens MI, Carstens E. Spinal c-fos expression associated with spontaneous biting in a mouse model of dry skin pruritus. Neuroscience Letters. 2004; 361(1–3): 79–82. [PubMed: 15135898]

Reddy VB, Iuga AO, Shimada SG, LaMotte RH, Lerner EA. Cowhage-evoked itch is mediated by a novel cysteine protease: a ligand of protease-activated receptors. J Neurosci. 2008; 28(17):4331–4335. [PubMed: 18434511]

Ricci G, Patrizi A, Bellini F, Medri M. Use of textiles in atopic dermatitis: care of atopic dermatitis. Current Problems in Dermatol. 2006; 33:127–143.

Shimada SG, LaMotte RH. Behavioral differentiation between itch and pain in mouse. Pain. 2008; 139(3):681–687. [PubMed: 18789837]

Simone DA, Alreja M, LaMotte RH. Psychophysical studies of the itch sensation and itchy skin ("alloknesis") produced by intracutaneous injection of histamine. Somatosens Motor Res. 1991; 8(3):271–279.

Spradley JM, Davoodi A, Carstens MI, Carstens E. Opioid modulation of facial itch- and pain-related responses and grooming behavior in rats. Acta dermato-venereol. 2012a; 92(5):515–520.

Spradley JM, Davoodi A, Gee LB, Carstens MI, Carstens E. Differences in peripheral endocannabinoid modulation of scratching behavior in facial vs. spinally-innervated skin. Neuropharmacol. 2012b; 63(4):743–749.

Sun YG, Zhao ZQ, Meng XL, Yin J, Liu XY, Chen ZF. Cellular basis of itch sensation. Science. 2009; 325(5947):1531–1534. [PubMed: 19661382]

Wahlgren CF. Itch and atopic dermatitis: clinical and experimental studies. Acta dermato-venereol Suppl. 1991; 165:1–53.

Wahlgren CF. Itch and atopic dermatitis: an overview. J Dermatology. 1999; 26(11):770–779.

Highlights

1. Intradermal injection of pruritogens in the calf elicits biting behavior
2. After histamine, touch elicited biting behavior (alloknesis)
3. Capsaicin elicited licking; postcapsaicin touch elicited flinching (allodynia)
4. Histamine-responsive dorsal horn neurons exhibit enhanced response to touch
5. Model is useful to study spinal mechanisms of alloknesis and allodynia

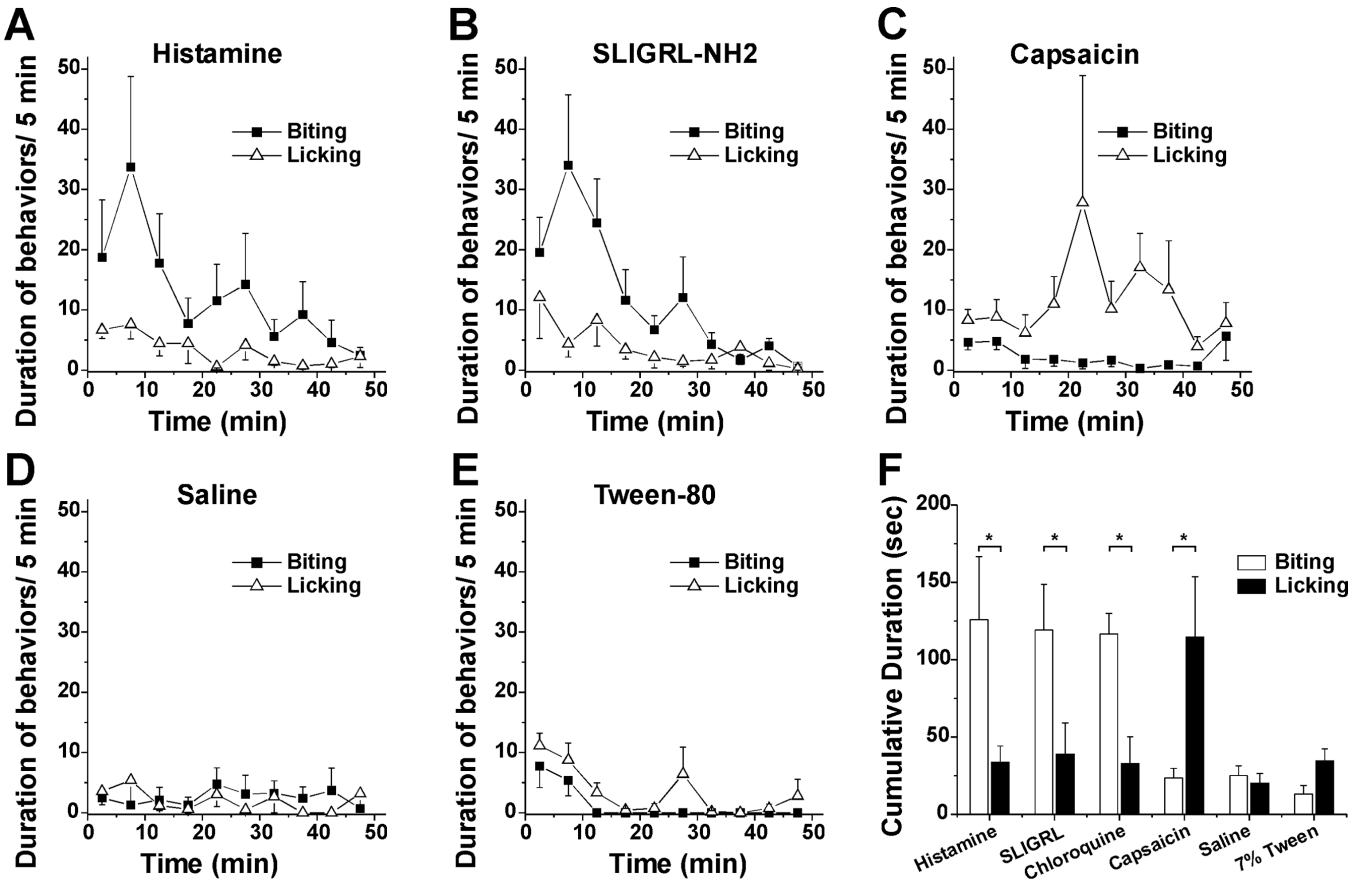

Fig. 1.

Time course of biting and licking elicited by histamine, SLIGRL and capsaicin. A. Cumulative duration of biting (■) or licking (Δ) behaviors vs. time following id injection of histamine (50 μg/10 μl) in the calf skin of the hindlimb. Error bars: S.E.M. (n = 6). B. Same as in A for SLIGRL-NH2 (50 μg/10 μl). C. Capsaicin (10 μg/10 μl). D. Saline. E. 7% Tween 80. F. Mean cumulative duration of biting and licking elicited by each agent. *: significant difference between bite and lick durations (p<0.05, paired t-test; n=6/group).

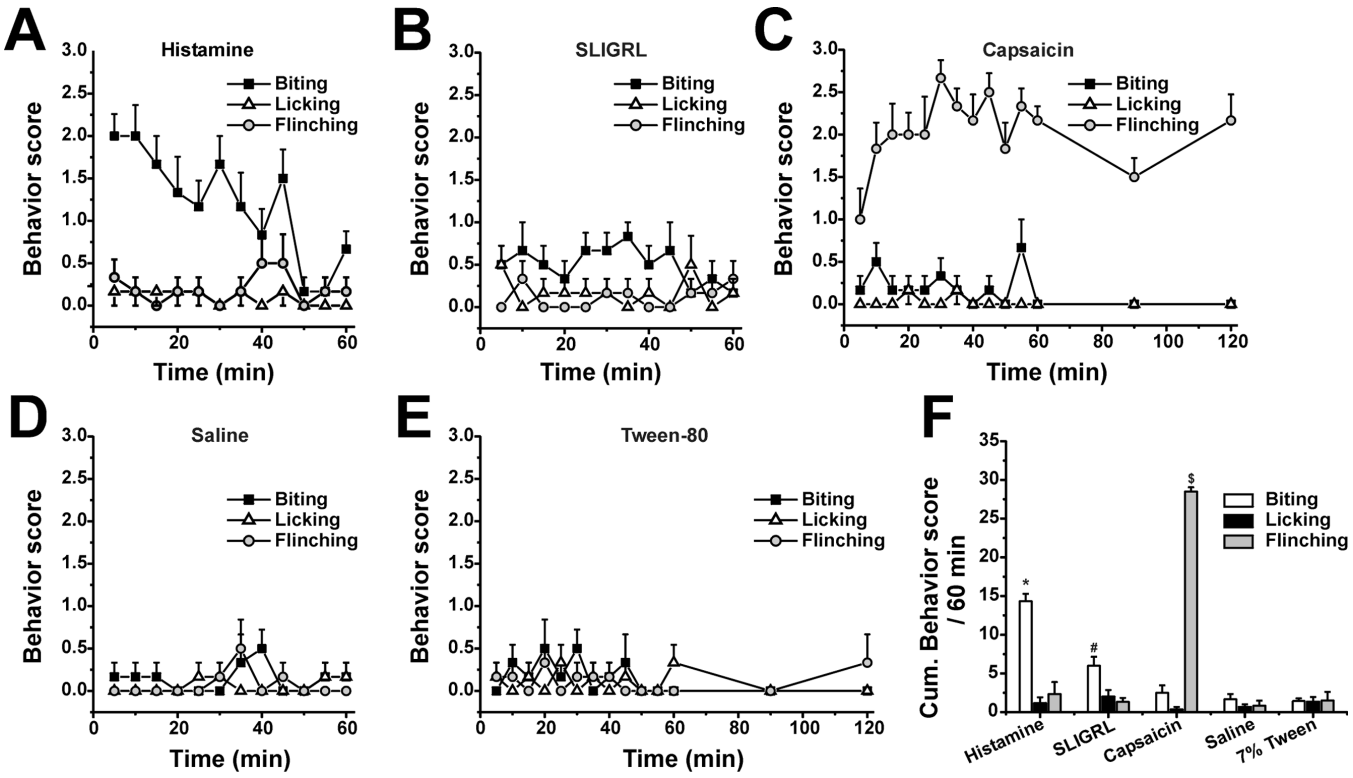

Fig. 2.

Time courses for alloknesis elicited by histamine and SLIGRL, and for allodynia elicited by capsaicin. A. At 5-min intervals after id injection of histamine (50 μg/10 μl) in calf skin, 3 von Frey stimuli (bending force 0.7 mN) were sequentially applied at separate sites 1–2 mm from the histamine injection. Presence or absence of biting (■), licking (Δ) or flinching (●) immediately after each stimulus was used to calculate a behavior score (maximum value=3). Error bars: S.E.M. (n = 6). B. As in A for SLIGRL-NH2 (50 μg/10 μl). C: Capsaicin (10 μg/10 μl). D. Saline. E. 7% Tween 80. F. Cumulative behavior scores for von Frey stimulus-evoked biting, licking and flinching following id injections. *, #: significantly different from saline-evoked biting (p< 0.05, unpaired t-tests, n=6/group). $: significantly different from 7% Tween-80-evoked flinching (p< 0.05, unpaired t-test, n=6/group).

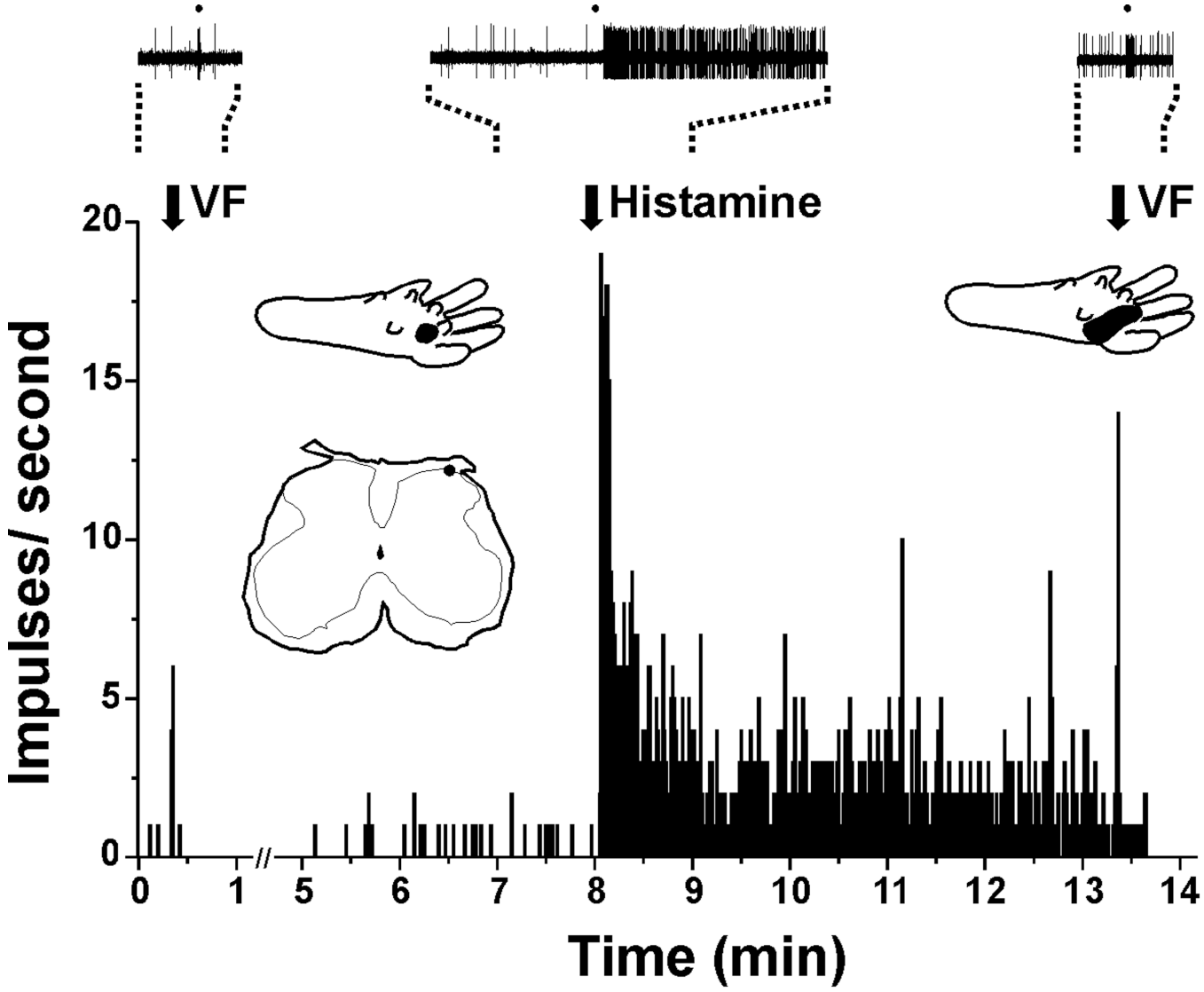

Fig. 3.

Enhancement of mechanically-evoked response of dorsal horn neuron after histamine. Peristimulus-time histogram (bins: 1 sec) of unit's responses to application of von Frey filament (VF, ↓, 0.7 mN),) before and after id injection of histamine (↓). Raw spike traces are shown above for portions of the response (dashed lines); dots show time of VF stimuli. Upper left inset: mechanosensitive receptive field (blue) on hindpaw. Lower left inset: lamina I recording site (●) on spinal section. Upper right inset: mechanosensitive receptive field expanded following histamine.

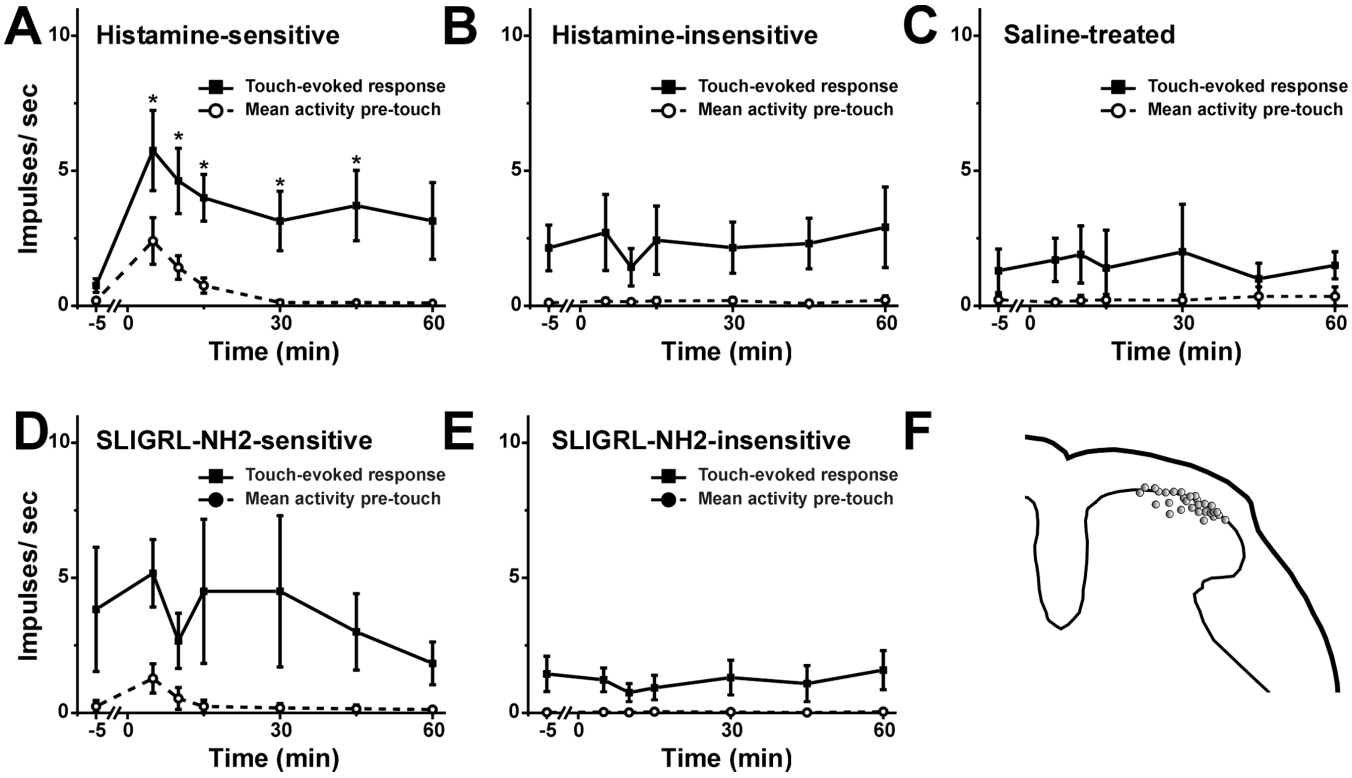

Fig. 4.

Enhancement of mechanosensitivity in histamine-sensitive dorsal horn neurons. A. Histamine-sensitive units (n=8). Graphs plots mean peak responses to innocuous mechnanical von Frey stimulus (0.7 mN; ■, solid line), and mean unit firing rate over a 20 sec just prior to the von Frey stimulus (O, dashed line), vs. time following id injection of histamine (at time 0). Pre-histamine responses are shown at the −5 min timepoint. Error bars: S.E.M. *: significant difference compared to peak response before histamine ($p < 0.05$; paired t-test). B: graph as in A for 14 histamine-insensitive units. C: As in A for 10 units following id injection of saline (vehicle control). D: as in A for 6 units responsive to id injection of SLIGRL-NH2. E: as in A for 18 units insensitive to SLIGRL-NH2. F: 29 histologically recovered recording sites (dots) compiled on representative lumbar section.