SEPARABLE YET INTERACTIVE COMPONENTS OF EXPERIMENTAL POST-INCISIONAL MECHANICAL HYPERESTHESIA

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Abstract

Acute post-operative pain limits function and slows recovery. To better understand the mechanisms underlying acute pain we have further investigated a post-incisional pain model using tactile hypersensitivity as an index of pain. After 1 cm incision of the medial-dorsal skin of male rats, closed with one silk suture, there is a rapidly developing increase in the responsiveness to punctate stimulation using nylon monofilament von Frey hairs (VFH), appearing as the increased contraction of sub-cutaneous muscles, the graded *cutaneous truncii muscle response* (CTMR). Such hypersensitivity in rats represents both the perception of pain in response to previously non-painful stimuli (*allodynia*) and an enlargement of the responses to stimuli that were mildly painful in intact skin (*hyperalgesia*). We found the probability and vigor of CTMR increases with increasing VFH force, described by a complex *Force* vs *Response* (*F-R*) curve. In intact/pre-incisional skin, such F-R data are well fitted by a single sigmoidal function that has one *threshold* (liminal force to produce a statistically "just-detectable" response), and one *mid-point* (force to produce half the maximum response). Four hours after incision, at the peak of post-incisional hypersensitivity, the F-R data are better fit by the sum of two such sigmoidal functions, with post-incisional allodynia accounted for by the emergence of a previously undetected response to VFH forces ten-fold lower than the threshold of intact skin. Interestingly, both the peak amplitude and the duration of these low-threshold, post-incisional allodynic responses are enhanced by "conditioning", when the skin is also stimulated by strong force VFHs (that test hyperalgesia), a phenomenon that does not occur in intact skin. Actions of sub-incisional bupivacaine suggest that allodynia is initiated by local injury and inflammatory processes, while central sensitization accounts for the enhancement of allodynia by conditioning stimulation.

Keywords: Incision, Allodynia, Hyperalgesia, Nociception, Neuronal plasticity

1. Introduction

Post-operative pain affects patients' mental state and physical activity, including deep breathing after thoracotomy, or locomotion, after abdominal or lower extremity surgery. Accelerated post-operative recovery often results from early ambulation, deeper breathing, and physical therapy, so the attenuation of post-operative pain has benefits for healing that extend beyond the immediate relief of suffering.[1]

Movement-related pain arises from the stretching or compression of injured tissues. The perception of pain from mechanical stimuli that are not normally noxious is termed "allodynia", whilst enhanced pain from normally mildly painful stimuli is termed "hyperalgesia".[2] Pain from normal movement or palpation at or adjacent to the incision/suture site is "primary" whereas that at distant locations is "secondary".[2] The objective assessment of pain from controlled stimuli can be described as a continuous, monotonic increase in pain intensity, up to a maximum pain perception, with increasing stimulus intensity. Allodynia and hyperalgesia have been described as resulting from a simple shift of the *stimulus strength* versus *pain intensity* relation to lower strength values, such that the *threshold* for liminal activity is reduced and the responses to previously effective stimuli are enhanced.[3]

Brennan and co-workers established a model of paw incision in the rat that has been a mainstay for much of the research on post-incisional pain.[4] Incision through the skin and fascia resulted in 2-3d of mechanical hypersensitivity to punctate stimulation, with greater sensitization when the underlying muscle was also incised. Both injury-released Nerve Growth Factor (NGF)[5,6] and a lowered pH[7] at the incision site contributed to the sensitivity, which was accompanied by spontaneous discharges of nociceptors innervating the wound area, and local heat hyperalgesia.[8] The incision-induced mechano-sensitization required the pH- and temperature-sensitive transducing receptor TRPV-1,[9,10] and was reversed by morphine, gabapentin and NSAIDs, identifying it as a response to pain.[11]

Because most clinical surgery involves hairy skin, a subsequent model examined mechano-hypersensitivity after incision on the rat's back.[12] Incision through the skin and fascia only caused at least 7d of primary and secondary mechano-sensitivity, which was pre-empted by pre-operative, subcutaneous (s.c.) injection of the local anesthetic bupivacaine.[12] Bupivacaine (0.25%) injected under the locus of incision 30 min before surgery, blunted both the immediate (30 min post-incision) and the prolonged primary allodynia with minimal effects on primary hyperalgesia, and abolished secondary allodynia while suppressing secondary hyperalgesia.[12] In contrast, when bupivacaine was injected at a locus distant from the incision, in order to achieve the same systemic dosing, neither the immediate, and prolonged, primary allodynia nor hyperalgesia were affected, but secondary allodynia was abolished and secondary hyperalgesia was reduced. These differential actions on allodynia and hyperalgesia of local and systemic bupivacaine suggested that they might be caused by different mechanisms and prompted the current investigation.

Here, we examined the tactile Force-Response (F-R) relationship in the rat's hairy skin with higher resolution, using a wider range of lower force VFHs applied to skin before and for one week after an incision, to more carefully examine allodynia and hyperalgesia after the incision.

2. Methods

2.1. Animal Care and Handling

The Standing Committee on Animals of Harvard Medical School approved all procedures, which were consistent with the

NIH Guide for the care and use of laboratory animals.[13] Male Sprague-Dawley rats, 250-300g at the time of surgery (Charles River Laboratories, Cambridge, MA), were housed in cages containing soft bedding, on a 12 h light:dark cycle, with food and water provided *ad libitum*. The ambient room temperature of all experiments was 23-25 ^{o}C. There were a minimum of five preoperative handling sessions, in which the rat was trained to sit still on the experimenter's arm for 5 mins, but was free to move in response to VHF stimulation on the back. Handling sessions, that reduce stress and its associated hypoalgesia, were conducted in the morning over three days, with two handling sessions per day.

2.2. Behavioral testing

Nocifensive responses were evoked by 4 VFH punctate stimulations, at 2 sec intervals, to dorsal skin that had been clipped at the beginning of baseline testing, allowing the underlying muscle contractions (CTMR) to be seen more clearly by the wrinkling of the skin.[14] Monofilament VHF forces of 0.02, 0.3, 0.6, 1, 4, 6, 9, 17, 36, 79, 108, 162 and 337 mN were incrementally applied, perpendicularly to the skin at ~0.5 cm from the incision, until a contraction occurred or the VFH bent, at its maximum force. Each contraction was graded as 0 for no contraction, 0.5 for weak, small contractions and 1.0 for robust, strong contractions. The sum of each score for the 4 stimuli, which would have a maximum of 4.0, was normalized by dividing by 4, resulting in a "Graded Response" that ranged from 0 to 1.0. Graded Responses were assessed in the pre-operative baseline period and then postoperatively over 1 week, at 0.5, 1, 1.5, 2, 3, 4, 8, 24, 48, 72, 96, 122, 140 and 168 hours. Systemic morphine attenuates the post-incisional allodynia, showing its equivalence to pain in this animal model.[12]

Tactile sensitivity was also scored by a "population response", wherein the fraction of rats that responded with any detectable contraction to stimulation by a normally sub-allodynic force, e.g., 18mN, was calculated. Population responses were 0 in intact skin and could rise to 1.0 at the maximum, i.e., all rats responded.

In a second protocol, we tested for possible interactions between "strong" and "weak" force stimulation. "Weak" force stimulation was used alone to elicit a just-detectable Graded Response (score = 0.2) and measure allodynia alone. In the "conditioning" protocol both strong forces (>80mN) and weak forces were applied, but only the response to the weak forces, i.e., allodynia, is reported. The number of stimuli was adjusted so that the total Graded Response was the same for "weak" and "strong" conditioning modes, by using a larger number of "weak" stimulations. Stimuli were applied over 2-168 hours, at 0.5 cm from the incision. Control procedures using sham rats, with clipped skin but no incision, showed no conditioning by "strong" force stimulation on allodynia.

2.3. Drug delivery

Rats were injected 30 min before surgery, near the medial dorsal location during brief anesthesia by sevoflurane (Abbot Laboratories, North Chicago, IL). Four hundred microliters of either 0.25% bupivacaine (made from the HCl salt; Sigma-Aldrich, St. Louis, MO) and dissolved in sterile 10mM HEPES buffered saline, pH 7.4, or the buffer alone (*Vehicle*) were injected, using a 1mL tuberculin syringe with 25 gauge needle (Becton Dickinson, Franklin Lakes, NJ). Animals were allowed to recover from the general anesthesia, which took less than 5 mins, before the subsequent surgery.

2.4. Surgical Procedure

Rats were again anesthetized by sevoflurane inhalation. The skin was swiped with 70% isopropyl alcohol pre-pads (Triad

Disposables, Inc., Brookfield, WI) and a single, 1 cm long incision made, rostro-caudal, through the skin and sparing the underlying muscle, with a No. 10 surgical blade (Harvard Apparatus, Holliston, MA). The incision was closed immediately with a non-absorbable 3-0 silk suture (Harvard Apparatus, Holliston, MA), the incision site swabbed with iodine (Providone; Clinipad Corporation, Rocky Hill, CT). Rats were weighed under anesthesia and then returned in their cage to complete their recovery. In no cases did rats indicate any resting irritation or pain from the incision, by trying to lick, bite or scratch the site.

2.5 Data Analysis and Statistics

The means ± SE of the Graded Response (0-1.0) are graphically presented. Graded Responses at different times were compared to the baseline values using the non-parametric Mann-Whitney test, with repeated measures correction (Statview, Cary, NC). Graded Responses between "weak" and "strong" force conditioning modes were compared to each other with Mann-Whitney test. $P<0.05$ was considered significant.

Force-Response curves were fitted to the data by a logistics function from the following three parameter equation (1):

$$\text{Graded Response} = A\,(F - F_t)^n / [(F - F_t)^n + (F_{0.5} - F_t)^n] \qquad \text{equation (1)}$$

where Graded Response is the normalized response to the applied force F, F_t is the threshold force, below which all responses are zero, $F_{0.5}$ is the curve's midpoint force, at which the Graded Response= 0.5, and n is the power coefficient that characterizes the steepness of the curve. A simple function with one set of parameters was adequate to fit to the data of intact skin, where A=1.0, but after incision the sum of two such functions was required for the best fit to the data; there the total maximum response of 1.0 is fractionated into a relative amplitude for allodynia (A_{PI}) and the balance for hyperalgesia (H_{PI}), each with its separate parameters for threshold, $F_{0.5}$ and n.

Population responses were compared to baseline (0, by definition) using Chi-square statistics.

3. Results

3.1. Time-course of tactile hyper-sensitivity.

Primary post-incisional hypersensitivity occurs after the incision on the rat's back and depends on the location of tactile stimulation and the force of the stimulus.[4] Primary allodynia is demonstrated by the appearance of a Graded Response to stimulation at 0.5cm from the incision by a very weak VFH, 0.3mN, (Figure 1Aa). (The time course of hypersensitivity is shown on a semi-log graph so that the earliest values can be clearly displayed on a scale that covers 24h.) Primary allodynia reaches significance with a delay of ~2h, peaks, with considerable variability, at 3-4h and has resolved to insignificant levels by 8h. Primary hyperalgesia, tested by the response to a much stronger force, 79mN, rises more rapidly, from a pre-operative response of 0.5-0.6 in intact skin to a maximum value of almost 1.0 at about 1h and remains near that high value for at least 24h (Figure 1Ab).

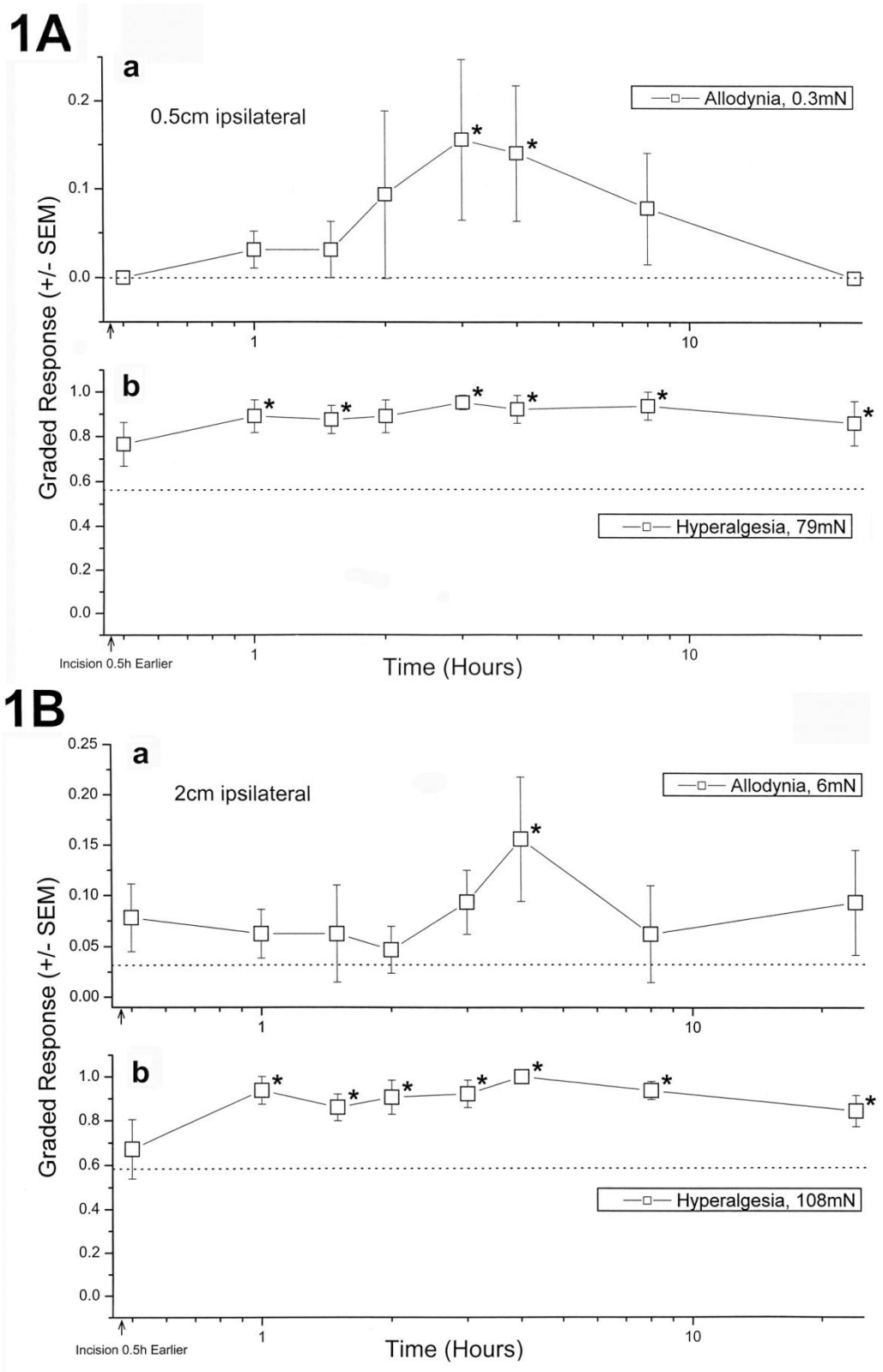
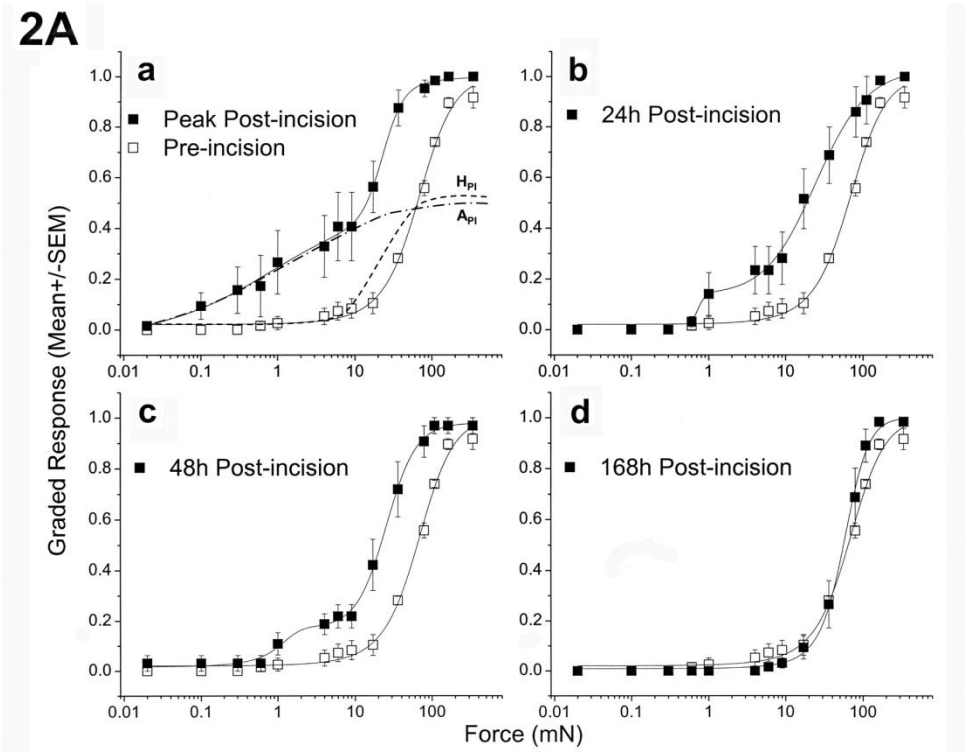


Figure 1. 24 hours time course of (**A**) primary and (**B**) secondary post-incisional hyperesthesia. Graded Responses to weak stimulation with low force von Frey hairs (1Aa, 1Ba) measure allodynia, and strong stimulation with high force VFHs (1Ab, 1Bb) measure hyperalgesia. All rats pre-incisionally injected with vehicle (buffered saline, pH 7.4), at 30 min before the first measurement. (n=8). * $P<0.05$ for comparison with baseline, pre-operative responses, shown by the horizontal broken lines.

Secondary hypersensitivity, measured by stimulation 2cm from the incision site, shows a similar pattern (Figure 1B). (The absolute forces for eliciting allodynia and hyperalgesia are greater at the more lateral dorsal locus, probably because the tissue under the skin is looser and stronger forces are required to compress the skin to the same degree.) These findings are consistent with our earlier report,[4] but extend the allodynic stimulation range to much lower forces.

3.2 Force-Response curves resolve allodynia from hyperalgesia.

In order to better resolve the difference between post-incisional allodynia and hyperalgesia, Graded Responses were assessed over a broad range of VFH forces and at different times after the incision. Full Graded Response *vs* Force curves for stimulation in the primary hypersensitivity location are shown in Figure 2A for intact skin (open squares, in all 4 panels) and for incised skin at different times after surgery (filled squares). The data for intact skin are fitted by a single form of Equation 1 (see Methods), with one threshold value (F_t= 13.1±6.7g), one mid-point value ($F_{0.5}$ = 64.3±2.8g), and an amplitude of 1.0 (by definition, as this marks the upper limit of the Graded Response, see Methods). The threshold force is difficult to determine from curve fitting, due to the very small responses at threshold, and is not further reported in this paper. At the peak of incisional hypersensitivity (3-4h post-operative, Figure 2Aa) the data are better fit by the sum of two such functions, each with amplitude about 0.5, but one with a higher $F_{0.5}$ (22.7±1.5g), that increases steeply in the hyperalgesic range, and one with a 20-fold lower $F_{0.5}$ (0.9± 1.2g) that accounts for allodynia. These separate functions are shown in Figure 2Aa by the dashed-dotted line (allodynia) and dotted line (hyperalgesia). The parameters of $F_{0.5}$ for allodynia and hyperalgesia, and A^a, the fraction of the maximum Graded Response for allodynia, are collected in Table 1, for stimulation at 4 distances from the incision site (spanning primary to secondary loci) and for 4 times, the peak response time (~4h) and 24, 48 and 168h after incision.



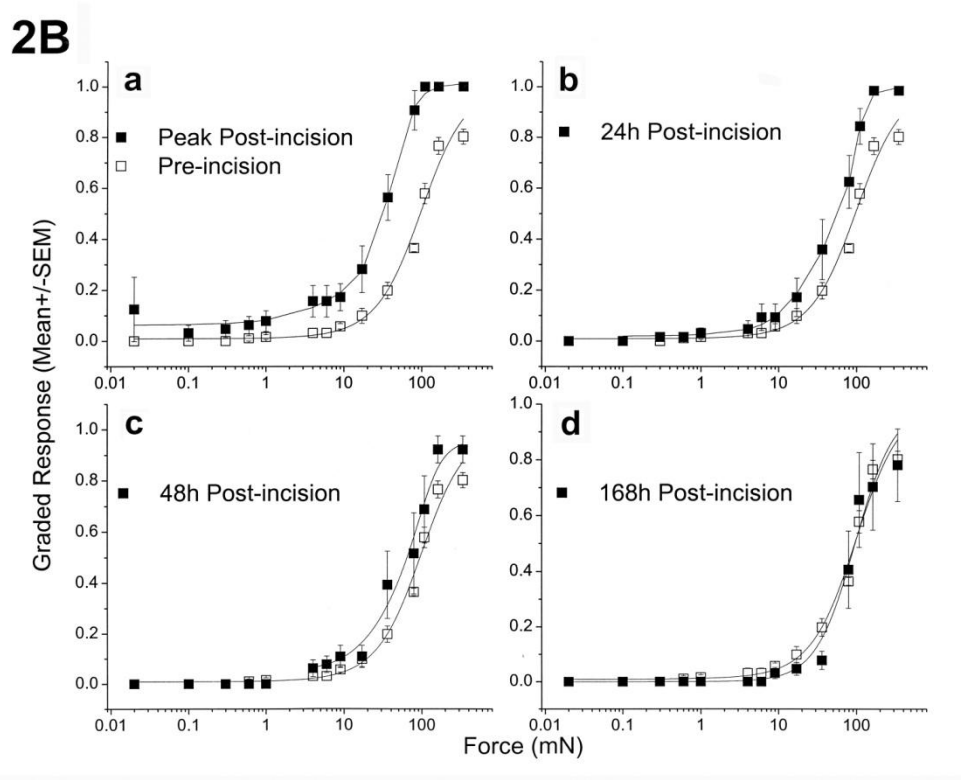


Figure 2. Graded Response *vs* Force data for intact skin (Pre- incision; □) and for skin at different times after the (Post-incision ;■). (**A**) Responses at the primary site, for stimulation at 0.5cm from the wound, measured at Peak (3-4hr), 24, 48 and 168 hours. Intact skin had been pre- injected with vehicle. (**B**) Responses to stimulation at a secondary site, 2cm from the wound. (n=8 for all). The broken line in 2Aa shows the separate logistic function fitted to the Peak Post-incision "hyperalgesia" data, with total amplitude H_{PI}; the dashed-dot line is the logistic function fit to the Peak Post-incision "allodynia" data, with total amplitude A_{PI} (see Methods for functions). Parameters for these fits, and for all other fits (not shown graphically), are listed in Table 1.

The primary allodynic component becomes progressively smaller but its $F_{0.5}$ value remains constant, as does that for primary hyperalgesia, over 4-48hr post-operative time (Figure 2A, Table 1). Seven days (168hr) after the incision the primary F-R curve is indistinguishable from that of intact skin (Figure 2Ad).

Post-Incisional Hyperesthetic Parameters			
Location	**0.5cm Ipsilateral**		
Time	**Allodynia**		**Hyperalgesia**
	$F_{0.5}^{a}$ (mN)	**A^{a}**	**$F_{0.5}^{h}$ (mN)**
Pre-Incisional	ND	0	64.28 ± 2.80
Peak (4h)	0.90 ± 0.00	0.47 ± 0.03	22.72 ± 1.46
24h	0.70 ± 1.12	0.14 ± 1.22	24.93 ± 52.24
48h	0.91 ± 0.18	0.17 ± 0.02	25.46 ± 1.31
168h	ND	0	55.79 ± 1.57
Location	**1cm Ipsilateral**		
Time	**Allodynia**		**Hyperalgesia**
	$F_{0.5}^{a}$ (mN)	**A^{a}**	**$F_{0.5}^{h}$ (mN)**
Pre-Incisional	ND	0	61.49 ± 2.69
Peak (4h)	6.00 ± 0.00	0.57 ± 0.01	30.72 ± 1.70
24h	2.62 ± 2.02	0.50 ± 0.00	36.10 ± 0.00
48h	ND	0	47.85 ± 1.62
168h	ND	0	43.59 ± 1.40
Location	**2cm Ipsilateral**		
Time	**Allodynia**		**Hyperalgesia**
	$F_{0.5}^{a}$ (mN)	**A^{a}**	**$F_{0.5}^{h}$ (mN)**
Pre-Incisional	ND	0	94.77 ± 5.68
Peak (4h)	4.00 ± 0.00	0.26 ± 0.04	40.06 ± 3.52
24h	22.97 ± 1.56	0.50 ± 0.00	90.28 ± 2.61
48h	55.85 ± 22.93	0.50 ± 0.00	70.00 ± 0.00
168h	ND	0	95.74 ± 6.53
Location	**2cm Contralateral**		
Time	**Allodynia**		**Hyperalgesia**
	$F_{0.5}^{a}$ (mN)	**A^{a}**	**$F_{0.5}^{h}$ (mN)**
Pre-Incisional	ND	0	117.65 ± 7.05
Peak (4h)	2.93 ± 16.13	0.13 ± 0.26	83.82 ± 1.60
24h	ND	0	105.60 ± 4.24
48h	ND	0	73.76 ± 0.10
168h	ND	0	98.12 ± 0.72
A^{a} = Amplitude of Allodynia; $F_{0.5}^{a}$ = mid-point force for allodynic component; $F_{0.5}^{h}$ = mid-point force for hyperalgesic component; ND = Not Defined			
15 min. Pre-incisIon Vehicle Injection (400ul, pH=7.4) on the right flank of rats			

Secondary hypersensitivity, measured 2cm from the incision, shows a similar progression of changes in the F-R curve (Figure 2B), but with a smaller relative amplitude for the allodynic component and a smaller shift in the $F_{0.5}$ value of the hyperalgesic component from that in intact skin. On the contralateral side, at 2cm from

the incision, an allodynic component is so variable as to be undetectable and the shifts of $F_{0.5}$ for hyperalgesia are relatively less than those at the primary locus (Table 1). The differences between primary and secondary post-incisional hypersensitivity, although present throughout the post-operative period, are particularly large for the early allodynic component (compare Figures 2Aa and 2Ba), where allodynia makes a larger contribution to the pain stimulated near the incision than that measured at a distance, implying that local factors may be more important for acute allodynia.

3.3. Strong stimulation enhances measured allodynia.

We next investigated the possibility that probing with a strong force, that stimulates the hyperalgesic component of post-incisional pain, can modify the allodynic component detected by weak forces. In intact skin, repeated stimulation by VFHs of force >96 gm did not affect the Graded Response to a VFH of 1-2 gm (data not shown). After incision, however, the addition of strong force stimulation enhanced the allodynic component of the Graded Response, both increasing the magnitude of the immediate post-operative response, from 0.15 to about 0.37, and extending its duration, from 24-48h to 168h (Figure 3A). Similarly, the Population Response was extended. All rats developed some responsiveness to a weak VFH within an hour after surgery, but the addition of strong force stimulation maintained that fraction at ~0.8 whereas only weak force stimulation resulted in a fall to insignificance by 96h (Figure 3C).

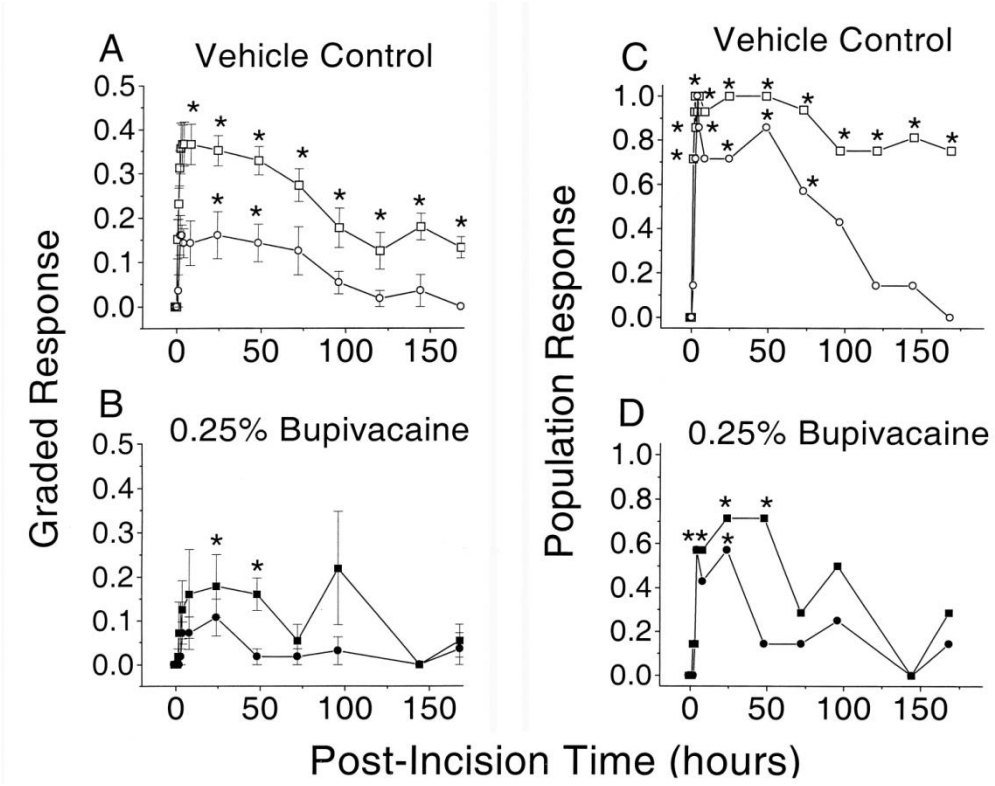


Figure 3. Primary Graded Responses (A,B) and Population Responses (C,D) to stimulation by a weak force alone (○,●) or to a weak force when strong force stimulation has been also applied (□,■). Post-incision responses after pre-incisional vehicle injection are shown by the open symbols (A,C) ; post-incisional responses after pre-incision injection shown by the filled symbols (B,D). n=16 for vehicle, n=8 for bupivacaine. * $P < 0.05$ for comparison with baseline, pre-operative responses.

3.4. Actions of local bupivacaine.

Previous research has shown that local, pre-incisional bupivacaine suppressed primary allodynia but reduced primary hyperalgesia to a lesser extent.[12] Therefore, we examined the ability of locally injected bupivacaine to alter the conditioned sensitization of allodynia by strong force stimulation. Bupivacaine did slow the rise of allodynia, and also reduced its maximum amplitude while shortening its duration (such that the Graded Response for allodynia never reached significance over baseline; Figure 3B). But the conditioned enhancement, due to strong force stimulation, was still present, and of about the same degree, with local bupivacaine as with the vehicle control. Similarly, although bupivacaine lowered the peak Population Response, to 0.6-0.7, the conditioning by strong force stimulation still increased its amplitude and extended its duration (Figure 3D).

4. Discussion

4.1. Summary findings.

The findings of this investigation show that tactile allodynia after incision of the hairy skin appears as a novel hypersensitivity, evoked by punctate stimulation by forces that are at least 10 times smaller than the threshold forces in intact skin. When tested by these low forces alone, allodynia appears more slowly and is shorter lasting than the rapidly developing and longer lasting hyperalgesia that is tested by the larger forces. Thus, allodynia and hyperalgesia are not simply the result of a continuous leftward shift in the force-response function of intact skin[3] and are likely to result from different mechanisms.[2]

In addition, repeated stimulation with strong forces, of the range required to evoke hyperalgesia, is able to both increase the strength of the allodynic response and lengthen its duration after surgery. Anesthesia by local, pre-operative bupivacaine, which is known to suppress primary and to abolish secondary allodynia, without affecting primary hyperalgesia,[12] does not prevent the conditioned enhancement of allodynia by strong force stimulation, showing that the coupling between these two modes of tactile response remains despite the local anesthetic.

4.2. Local mediators of hypersensitivity.

Post-incisional allodynia appears to be a response to local perturbations around the incision site. Allodynia is shorter lasting than hyperalgesia and selectively suppressed by local bupivacaine, whereas primary hyperalgesia is unaffected.[12] Numerous chemicals are released from injured skin and distal nerve as a result of the injury of incision, and the subsequent inflammatory reactions may involve additional substances and also induce hyperactivity in structures not directly affected by the initial injury.[2,15] Prostaglandins (e.g., PGE2),[16] Nerve Growth Factor (NGF),[5] ATP,[17] Endothelin-1(ET-1),[18] bradykinin (BK),[19] Substance-P (SubP)[20] and glutamate[21] are elevated after skin injury and all have been shown to enhance the responsiveness of cutaneous nociceptors. For some chemicals, as with ET-1, this enhancement leads to an unstimulated nociceptor discharge that causes explicit pain, shown, for example, by paw shaking, licking and biting,[22] but for most others no explicit pain occurs but the incised area becomes hypersensitive to thermal or tactile stimulation.[6,23]

This latter situation characterizes the responses of hairy skin after an incision, reported here. Using the same model, we showed previously that post-incisional allodynia was suppressed by pre-operative, local injection of an antagonist of ETA receptors for ET-1.[12] Post-incisional hyperalgesia, in contrast, was unaffected by this treatment, implicating ET-1 in the

pathogenesis of primary allodynia. Injections of ET-1 into the rat paw are known to cause primary (ipsilateral paw) tactile hyper-sensitivity, as well as to sensitize the contralateral paw,[24] this latter effect due to central (spinal) sensitization as well as efferent conduction to the contralateral side. The secondary hypersensitivity after back incision is abolished by a local, peri-incisional ETA receptor antagonist,[18] showing that activation of those afferent fibers that induce central sensitization depends on ET-1 released by injury. It is not possible to attribute the sensitization exclusively to ET-1 acting directly on nociceptor fibers, however, since ET-1 induces release of a variety of algogenic substances from keratinocytes, and potentiates the actions of ATP[25] and sensitizes the paw to the algogenic actions of NGF (A. Khodorova and G. Strichartz, unpublished observations).

4.3. Actions of local anesthetics.

The ability of local bupivacaine to inhibit primary allodynia may be due to direct actions on impulse conduction of nociceptive fibers but also to indirect actions via other sensitizing agents. Local anesthetics like bupivacaine are known to inhibit the direct responses to isolated sensory neurons to Sub P,[26] to ET-1,[27] and to BK (C. Hamaya, T. Barr and G. Strichartz, unpublished observations), all agents known to sensitize nociceptive neurons *in vivo*. That systemic bupivacaine abolishes secondary post-incisional allodynia and hyperalgesia reflects an action of systemic local anesthetics on the processes of central sensitization.[28] Importantly, however, in contrast to our earlier report,[12] and the results shown here (Figure 3), injection of bupivacaine into the plantar skin before the Brennan-type paw incision delayed but did not prevent the behavioral sensitization (as also observed in human subjects for pain from volar forearm incision[29]) or the eventual increase in dorsal horn neuronal sensitization.[30] This difference might explain the effects of a purely local versus a local plus systemic action of bupivacaine.

4.4. Fiber types associated with allo-dynia and hyperalgesia.

Low threshold mechanical activation is most often associated with fast conducting myelinated (Aα,β-) fibers.[31] However, such activation does not normally result in a painful (nocifensive) reaction. The appearance of such responses, like the CTMR evoked by VFHs in the allodynic range after incision reported here, may be due to a change in the mechano-sensitivity of distal nerve endings[32,33] or to changes in central processing of their input, such that latent connections in the dorsal horn become functional.[34,35] In the paw incision model, little to no changes of Aδ- and C-fiber nociceptors occurred after surgery,[8,23] but there was a marked increase in the mechanical sensitivity of previously mechanically insensitive afferents (MIAs),[15,36] which are also known to be recruited to a sensitive state by inflammatory mediators.[32] Recordings from fibers that innervate the rat's paw, following injection of Complete Freunds Adjuvant (CFA),[33] or Prostaglandin E2,[37] which is known to be elevated during inflammation, show increases over controls in both A- and C-fiber discharges responding to mechanical stimulation. The increase in A-fiber discharge after CFA is greatest in the low-intermediate force range, whereas that for C-fibers continues to grow through the highest force range. Force-response curves for C-fiber impulses show a shift in the mid-point force from 75gm to ~10gm after PGE2 injection, similar to the shifts seen in the behavioral responses between intact skin and the post-incisional condition, reported here. These comparisons support the notion that much of post-incisional allodynia can be explained by changes in the responsiveness of peripheral nerves innervating the skin.[2]

Local anesthetics have a differential blocking activity on different types of peripheral nerve fibers.[38] Small myelinated fibers are most susceptible to conduction failure by lidocaine applied to peripheral nerve, whilst the smallest, non-myelinated (C-) fibers are the least susceptible.[38,39] Since hyperalgesia after incision is unaffected by local bupivacaine,[12] whereas allodynia is strongly inhibited, these differential actions are consistent with the preceding supposition, that hyperalgesia requires conduction by non-myelinated C-fibers, likely C-nociceptors, and allodynia selectively involves large diameter A-fibers.

It is also possible, however, that responses of non-myelinated low-threshold mechano receptors are responsible for allodynia.[40] These C-fibers are present in small numbers but make direct connections on the nociceptor receiving neurons of the dorsal horn laminae I and II. In mice lacking these fibers, due to specific knockout (KO), mechano-sensitivity after nerve injury or inflammation is greatly reduced. Notably, after Brennan-type paw incision the normally heightened responses to light and intermediate forces are much smaller in the KO mice,[40] suggesting their important contribution to post-incisional tactile allodynia. In the absence of injury or inflammation, however, there is no indication of a role for these peripheral neurons.

4.5. A role for central sensitization.

Surgical incision is known to alter the responsiveness of spinal cord dorsal horn (DH) neurons. Increased spontaneous ("background") firing, increase in receptive field (RF) size and reductions of threshold for VFH stimulation have all been reported.[41-44] Recordings from high threshold (HT) and wide dynamic range (WDR) neurons, the latter responding to both weak and strong mechanical stimulation of their cutaneous receptive fields, Zahn and Brennan[41] found reduced mechano threshold for about 1/3 of WDR neurons but no change in HT neurons; blunt mechanical stimulation near the wound site excited almost all WDRs and barely any HTs. The intense noxious stimulation of pinch of the paw skin revealed an expansion of the RFs of both types of DH cells. Subsequent studies with this model reported that changes in the withdrawal behavior and expansions of RFs for mild VFH stimulation occurred in WDR neurons far more than in HTs, but both types of cells showed enhanced responses to strong punctate stimulation.[42] Similar differential responses of WDRs and HTs were observed after incision of the hairy rat skin,[43] that included the underlying fascia and muscle, although the duration of these changes were much briefer than the behavioral hypersensitivity reported in the current paper.[44]

In interpreting the findings of the current paper in terms of the spinal cord responses, we propose that hyperalgesia corresponds to a direct input of C- and Aδ-fiber nociceptors to neurons in the superficial DH which project to pain sensing centers in the brain and also interact neurochemically with interneurons (and glial cells) in the spinal cord. Allodynia corresponds to the input of normally non-nociceptive low-threshold (LT) mechanosensitive afferents to WDR neurons, which are also activated by C- and Aδ-nociceptors and change after incision to develop a lower threshold and spontaneous activity that enables their greater excitation by the LT afferents. The elevated input during the conditioning by strong forces, from nociceptors as well as from the now sensitized LT afferents, further shifts the WDRs into a hyper-responsive state, accounting for the enhancement and prolongation of allodynia.

Electrophysiological experiments using different glutamate receptor

antagonists during paw incision suggest that acute hyperalgesia depends on transmission via AMPA receptors and acute allodynia via NMDA receptors.[45] However, long-term changes in spinal signal processing are likely to engage pathways that cause post-translational changes in receptor physiology as well as the transcription and translation that leads to expression and activation of new proteins and pathways.[46] How this variety of changes in response to peripheral incision contributes to the acute and long-term hypersensitivity after surgery remains to be investigated.

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