Research Paper

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Conditioned pain modulation in rodents can feature hyperalgesia or hypoalgesia depending on test stimulus intensity

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Abstract

The counterirritation phenomenon known as conditioned pain modulation, or diffuse noxious inhibitory control in animals, is of increasing interest due to its utility in predicting chronic pain and treatment response. It features considerable interindividual variability, with large subsets of pain patients and even normal volunteers exhibiting hyperalgesia rather than hypoalgesia during or immediately after receiving a conditioning stimulus. We observed that mice undergoing tonic inflammatory pain in the abdominal cavity (the conditioning stimulus) display hyperalgesia, not hypoalgesia, to noxious thermal stimulation (the test stimulus) applied to the hindpaw. In a series of parametric studies, we show that this hyperalgesia can be reliably observed using multiple conditioning stimuli (acetic acid and orofacial formalin), test stimuli (hindpaw and forepaw-withdrawal, tail-withdrawal, hot-plate, and von Frey tests) and genotypes (CD-1, DBA/2, and C57BL/6 mice and Sprague-Dawley rats). Although the magnitude of the hyperalgesia is dependent on the intensity of the conditioning stimulus, we find that the direction of effect is dependent on the effective test stimulus intensity, with lower-intensity stimuli leading to hyperalgesia and higher-intensity stimuli leading to hypoalgesia.

Keywords: CPM, DNIC, Stimulus intensity, Parametrics, Strain differences

1. Introduction

The psychophysical phenomenon currently known as conditioned pain modulation (CPM),[59] or heterotopic noxious conditioning stimulation, is the human correlate of the diffuse noxious inhibitory control (DNIC) phenomenon first demonstrated in rats in 1979 by Le Bars et al.[28,29] The observation that pain inhibits pain is quite old, but the laboratory demonstration in humans of such counterirritation has become increasingly popular as an experimental measure of the "capacity" of endogenous pain inhibitory mechanisms.[22] Evidence has been amassed suggesting that deficits in CPM predict the development of chronic pain[30] and poor physical functioning,[13] and treatment response to duloxetine,[60] and are restored after successful treatment with tapentadol.[45] The magnitude and even direction of the phenomenon in healthy volunteers are, in fact, highly variable[49,50] and dependent on various parameters (modality, intensity, and body area) of both the conditioning and the test stimuli.[21,42,50]

Diffuse noxious inhibitory control has been studied almost exclusively in rats; we were able to identify 2 articles observing DNIC in cats[40,41] and just 5 in mice.[14,15,25,31,32] In a study performed in outbred CD-1 mice, we observed that after an intraperitoneal injection of acetic acid, mice displayed increased sensitivity to noxious thermal heat on the plantar hindpaw (Ref. 26, see Fig. 4A). This apparent thermal hyperalgesia—the exact opposite of what DNIC theory would predict—was quite robust, representing an ≈4- to 5-second decrease in paw-withdrawal latencies (from an ≈20-second baseline) sustained over the 30-minute duration of acetic acid–induced abdominal constriction behavior. Given the surprising direction of this effect, and the increasingly prominent role of mice in pain research,[33] we performed a parametric analysis of CPM/DNIC in the laboratory mouse using acetic acid and orofacial formalin as conditioning stimuli. We find here that, in mice and rats, the direction of CPM/DNIC is dependent on the intensity of the test stimulus, with lower intensity stimuli—of the sort in most common use in modern algesiometry—associated with hyperalgesia, not hypoalgesia.

2. Methods

2.1. Animals

Naive, young adult (7-12 weeks of age) mice of both sexes were used, in approximately equal numbers.[34] In all experiments except one, mice were outbred, whereas CD-1 (ICR; Crl) mice were bred in our laboratory (J.S.M.) from breeders obtained from Charles River Laboratories (St. Constant, QC). In one study, inbred DBA/2J and C57BL/6J mice, bred in our laboratory from breeders obtained from The Jackson Laboratory (Bar Harbor, ME), were used. All mice were housed with their same-sex littermates (2-4 animals per cage) in standard shoebox cages,

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maintained in a temperature-controlled (20 ± 1°C) environment (14:10 hours light/dark cycle), and fed (Envigo Teklad 8604, Lachine, QC, Canada) and watered ad libitum. All experiments were approved by a local animal care and use committee and conform to Canadian Council on Animal Care guidelines.

In one study, male Sprague-Dawley rats (300-375 g) were used. Rats were purchased from Charles River Laboratories and were acclimatized to the vivarium for 7 days before the start of experiments. Rats were housed 2 to a cage and were maintained on a 12:12-hour light/dark cycle, with food (Envigo Teklad 2920X) and water available ad libitum.

Mice were assigned to conditions using within-cage randomization where possible. Because experiments involved the comparison of withdrawal latencies before and after the injection of acetic acid, which caused obvious behavioral changes, in most cases, experimenters could not be blinded to condition. However, many experiments were performed by laboratory personnel unfamiliar with the hypothesis. In addition, the hypothesis itself derived from a chance observation of data collected for a completely different purpose and was entirely unexpected.

2.2. Algesiometry

Assays and measures were performed as previously described in more detail.[35] Brief descriptions are as follows:

2.2.1. Acetic acid abdominal constriction

Mice received an intraperitoneal injection of acetic acid diluted to concentrations ranging from 0.1% to 1.2%, in a volume of 10 mL/kg. In general, the resulting abdominal constrictions, known to last for 30 to 40 minutes after injection, were observed but not quantified because acetic acid was used as a conditioning stimulus.

2.2.2. Radiant heat paw-withdrawal test

Mice were placed on a 0.5-cm-thick glass floor within small Plexiglas cubicles (9 × 5 × 5 cm high), and a focused high-intensity halogen lamp beam (Sylvania OSRAM FCS 64640; 150 W; 0.6-mm^2 area; Ontario, Canada) was shone from below onto the midplantar surface of the hindpaw, or in some experiments, the forepaw. The commercial device (IITC Model 336; Stoelting, Woodland Hills, CA) was set to 1 of 7 intensity levels, ranging from 20% to 95% of the available heat intensities of the device, corresponding to power outputs ranging from ≈35 to 165 W/mm^2. Thermocouple measurements at 5-second intervals of glass temperature produced by these settings are shown in supplementary Figure 1 (available at http://links.lww.com/PAIN/A699). In all other experiments using this assay, the lowest stimulus intensity (20% of maximum; 35 W/mm^2) was used.

Latency to withdraw from the stimulus was measured to the nearest 0.1 seconds. All reported measurements at every time point (see graphs) represent the averaged withdrawal latency of the right and left hindpaw. In one experiment, an observer blinded to acetic acid concentration was asked to subjectively score whether she considered the withdrawal response to be "normal" or "exaggerated," featuring longer and/or more pronounced lifting, lifting and/or shaking of the hindpaw in addition to mere withdrawal from the stimulus.

In the rat experiment, a different device of the same make and model was set to an intensity corresponding to withdrawal latencies of 10 to 15 seconds. This experiment was also performed in a different laboratory (T.C.).

2.2.3. Hot water tail-withdrawal test

While lightly restrained in a cloth/cardboard holder, the distal half of the mouse's tail was dipped into a bath of water maintained at 46.0 to 52.0 (±0.1°C). Latency to respond to the heat stimulus by vigorous flexion of the tail was measured at 5 time points each before and after acetic acid injection, separated by 5-minute intervals.

2.2.4. Hot-plate test

Mice were gently placed on a metal surface maintained at 53.0 ± 0.2°C (IITC Model PE34MHC; Stoelting) within a transparent Plexiglas cylinder (15-cm diameter; 22.5 cm high). The latency to either lick, lift or shake either hindpaw was measured with a stop watch as a nocifensive endpoint, once immediately before acetic acid injection and once 15 minutes after injection.

2.2.5. Von Frey test

The up–down method of Dixon[8] was used. Mice were placed on a perforated metal floor (with 5-mm diameter holes placed 7 mm apart) within small Plexiglas cubicles as described above, and a set of 8 calibrated von Frey fibers (Stoelting Touch Test Sensory Evaluator Kit #2 to #9; ranging from ≈0.015 g to ≈1.3 g of force) were applied to the plantar surface of the hindpaw until the fibers bowed and then held for 3 seconds. Reported measurements represent the averaged withdrawal threshold of the right and left hindpaw tested 3 times in succession, immediately before acetic acid injection and 15 minutes after injection.

2.2.6. Orofacial formalin test

The method described by Luccarini et al.[31] was used. Ten microliter of 2.5% formalin was injected into the left upper lip. This produces marked rubbing behavior of the lip over the next 45 minutes, which was observed but not quantified since orofacial formalin was used as a conditioning stimulus. In this experiment paw-withdrawal latencies were measured at 6 time points each before and after acetic acid injection, separated by 5-minute intervals.

2.2.7. Spared nerve injury

Spared nerve injury (SNI) was performed under isoflurane/oxygen anaesthesia as described previously.[51] Mice were tested for mechanical sensitivity before and 7 days after surgery (both before and after acetic acid injection) using the von Frey test as described above, except that the "spared" sural region was targeted.

3. Results

3.1. Long-lasting thermal hyperalgesia during and after acetic acid injection

Mice of both sexes were tested for baseline thermal nociception 5 times at 5-minute intervals on the radiant heat paw-withdrawal test (**Fig. 1A**). Female mice trended towards higher pain sensitivity, but no statistically significant sex differences were observed ($F_{1,38} = 1.0$, $P = 0.32$), and latencies were fairly stable (repeated measures [time]: $F_{4,152} = 2.1$, $P = 0.08$). After intraperitoneal injection of 0.9% acetic acid—in CPM/DNIC-relevant terms, the "conditioning stimulus"—mice were tested

again for thermal nociception (the "test stimulus") at 5-minute intervals for 30 minutes, and all postinjection latencies were robustly hyperalgesic (**Fig. 1A**). Overall, a highly significant thermal hyperalgesia—that is, "anti-DNIC"—was demonstrated (**Fig. 1B**) equally in both sexes ($F_{1,40} = 174.5$, $P < 0.001$; sex × time: $F_{1,40} = 0.8$, $P = 0.36$). The presence of paw-withdrawal testing did not in turn affect abdominal constriction behavior caused by acetic acid (with testing: 18.2 ± 1.8 writhes; without testing: 25.5 ± 3.7 writhes; $t_6 = 1.8$, $P = 0.13$).

To replicate the finding and establish its duration, a separate group of mice was tested—by a different experimenter—at 5-minute intervals before and after acetic acid or saline injection, at 1-minute intervals for 10 minutes and 20-minute intervals thereafter for 6 hours (**Fig. 1C**). Repeated-measures analysis of variance revealed a highly significant condition × time interaction ($F_{33,462} = 2.7$, $P < 0.001$), with a significant time effect observed in the acetic acid group ($F_{33,231} = 3.8$, $P < 0.001$) but not the saline group ($F_{33,231} = 0.8$, $P = 0.77$). Although abdominal constriction behavior ceased in all mice by 40 minutes after injection, significant hyperalgesia emerged at 5 minutes after injection and persisted until 70 minutes after injection. At no time point was there any evidence of analgesia. Baseline latencies were lower in this experiment, which was conducted by a female experimenter possibly reflecting the lack of initial stress-induced analgesia.[53]

3.2. Hyperalgesia is dependent on the conditioning stimulus intensity

To establish the influence of the intensity of the conditioning stimulus on the magnitude of hyperalgesia, we varied the concentration of acetic acid. The concentration groups did not differ significantly in their baseline-withdrawal latencies ($F_{4,24} = 1.7$, $P = 0.19$). As shown in **Figure 2A**, acetic acid concentration strongly affected hyperalgesic magnitude and statistical significance (concentration × time: $F_{4,24} = 7.8$, $P < 0.001$), with higher concentrations producing more hyperalgesia. Furthermore, postinjection paw-withdrawal responses were much more likely to be judged by an experimenter blinded to concentration as "exaggerated" (**Fig. 2B**), and this likelihood was also highly dependent on acetic acid concentration (concentration × time: $F_{4,24} = 17.8$, $P < 0.001$).

3.3. Hyperalgesia is observed using different body parts, test stimuli, and conditioning stimuli

To test whether the presence of hyperalgesia instead of hypoalgesia was due to the particular pairing of acetic acid as a conditioning stimulus and the radiant heat paw-withdrawal test as the test stimulus, we conducted new experiments using different assays. First, we examined whether switching

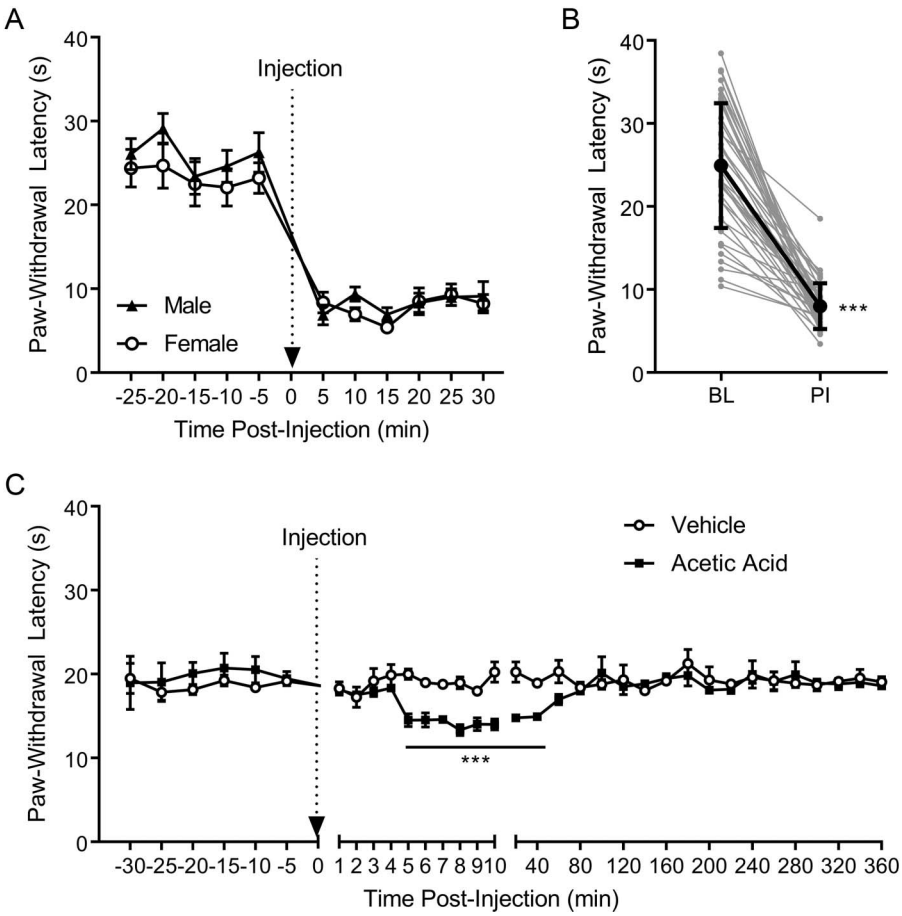

Figure 1. Increased hindpaw thermal pain sensitivity (thermal hyperalgesia) of mice after intraperitoneal injection of acetic acid. (A) Time course data. Symbols represent mean ± SEM paw-withdrawal latency (seconds); n = 25 male and 17 female mice. (B) Data collapsed into preinjection (baseline; BL) and postinjection (PI) averages. Dark symbols represent mean ± SEM paw-withdrawal latency; light symbols represent individual data points. (C) Results of a replication experiment with an extended (6-hour) time course conducted by a different experimenter. Symbols as in graph A; n = 8 mice/condition. ***$P < 0.001$ compared with BL (or averaged preinjection latencies).

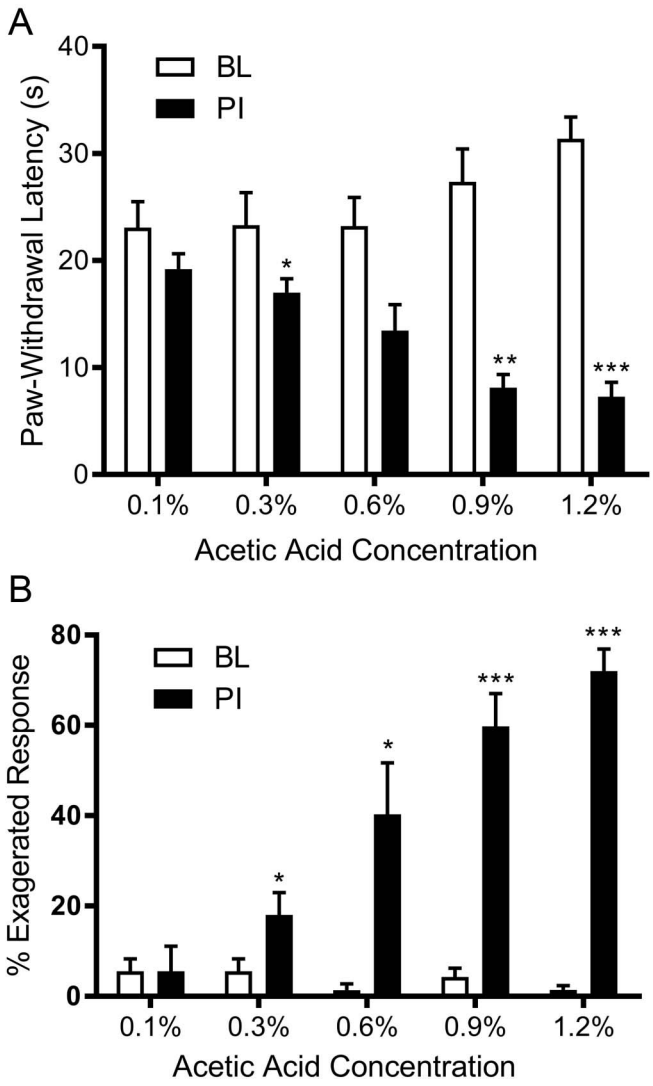

Figure 2. Thermal hyperalgesia increases as a function of conditioning stimulus intensity, that is, acetic acid concentration. (A) Hindpaw-withdrawal latencies within 30 minutes prior (BL) and 30 minutes after (PI) injection acetic acid of concentrations ranging from 0.1% to 1.2%. Bars represent mean ± SEM paw-withdrawal latency averaged over multiple time points as in Fig. 1A; n = 5-6 mice/concentration. (B) Percentage of withdrawal responses judged by a blinded observer to be "exaggerated" (see Methods). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with corresponding BL.

from the hindpaw to the forepaw affected our findings. As shown in **Figure 3A**, hyperalgesia was observed in forepaws tested on the radiant heat paw-withdrawal assay ($F_{11,77} = 8.7$, $P < 0.001$). A 6-hour long experiment testing the forepaws was also performed, with significant hyperalgesia emerging at 3 minutes after injection and lasting for 40 to 60 minutes; no analgesia was observed at any time point (see supplementary Figure 2, available at http://links.lww.com/PAIN/A699). Hyperalgesia was also observed when nociception was assessed before and after acetic acid injection on the 49°C hot water tail-withdrawal test ($F_{9,135} = 2.7$, $P = 0.006$) (**Fig. 3B**), the 53°C hot-plate test ($F_{1,25} = 17.4$, $P = 0.01$) (**Fig. 3C**), and the von Frey test ($F_{1,11} = 10.4$, $P = 0.008$) (**Fig. 3D**). Furthermore, if acetic acid was replaced by orofacial formalin as the conditioning stimulus, hyperalgesia was also observed using the radiant heat paw-withdrawal test ($F_{11,176} = 4.8$, $P < 0.001$) (**Fig. 3E**).

3.4. Hyperalgesia is strain-dependent in mice and can be observed in rats

To assess the possible strain dependence of the phenomenon, we tested a new cohort of CD-1 mice tested alongside mice of 2 other mouse strains, DBA/2J and C57BL/6J. A significant strain × time interaction was observed ($F_{2,21} = 11.6$, $P < 0.001$). As shown in **Figure 4** (left), significant time effects were seen in the CD-1 ($F_{1,8} = 132.8$, $P < 0.001$) and DBA/2J ($F_{1,7} = 10.4$, $P = 0.01$) strains, but not in C57BL/6J ($F_{1,6} = 4.3$, $P = 0.08$). Of interest (see below) is the fact that C57BL/6 mice displayed significantly lower baseline latencies than the other strains ($F_{2,21} = 7.0$, $P = 0.005$).

To assess whether hyperalgesia is specific to mice, we also tested Sprague-Dawley rats in a similar fashion. As shown in **Figure 4** (right), they too displayed hyperalgesia, not hypoalgesia, after acetic acid injection ($F_{1,10} = 11.6$, $P = 0.007$).

3.5. Hyperalgesia is dependent on the test stimulus intensity

The magnitude of the observed hyperalgesia was larger in the experiment shown in **Figure 1A** compared to that shown in **Figure 1C** and also larger to that observed previously,[26] but the baseline paw-withdrawal latencies were also higher, suggesting that effective test stimulus intensity might affect the magnitude of the phenomenon. To test this, a new experiment was performed (by a female experimenter) in which paw-withdrawal test stimulus intensities were varied over a large range (see Materials and Methods), leading to baseline-withdrawal latencies ranging from 2.3 to 18.8 seconds. As shown in **Figure 5A**, at low stimulus intensities—corresponding to baseline thermal latencies >10 seconds—we again observed robust hyperalgesia after acetic acid. Using a moderate stimulus intensity—corresponding to baseline latencies of ≈7 seconds—neither hyperalgesia nor significant hypoalgesia was observed. However, high stimulus intensities—corresponding to baseline latencies <5 seconds—resulted in statistically significant hypoalgesia or DNIC. This reversal from hyperalgesia to hypoalgesia based on test stimulus intensity was also demonstrated on the forepaws (intensity × time: $F_{1,14} = 184.7$, $P < 0.001$) (**Fig. 5B**) in the tail-withdrawal test (temperature × time: $F_{1,16} = 318.6$, $P < 0.001$) (**Fig. 5C**) and using orofacial formalin as the conditioning stimulus (intensity × time: $F_{1,14} = 189.5$, $P < 0.001$) (**Fig. 5D**).

3.6. Hyperalgesia becomes hypoalgesia after nerve injury

In in vivo electrophysiological experiments in rats, DNIC is abolished after spinal nerve ligation,[1,2] (but see Ref. 12), a nerve injury associated with symptoms of neuropathic pain. To assess whether nerve injury can alter the observed hyperalgesia, baseline von Frey thresholds were measured and mice were then subjected to SNI or sham surgery (**Fig. 6**). As expected, 7 days after surgery, SNI-operated mice but not sham-operated mice were profoundly allodynic on the ipsilateral hindpaw (surgery × time [Pre-Surgery to Post-Surgery BL]: $F_{1,27} = 11.6$, $P = 0.002$). Acetic acid injection did not cause further hyperalgesia in SNI-treated mice, rather robust hypoalgesia (DNIC) was observed (surgery × time [Post-Surgery BL to Post-Surgery PI]: $F_{1,27} = 17.3$, $P < 0.001$).

4. Discussion

The major finding of this article is that, when using certain test stimulus parameters, following the application of 1 of 2 tonic

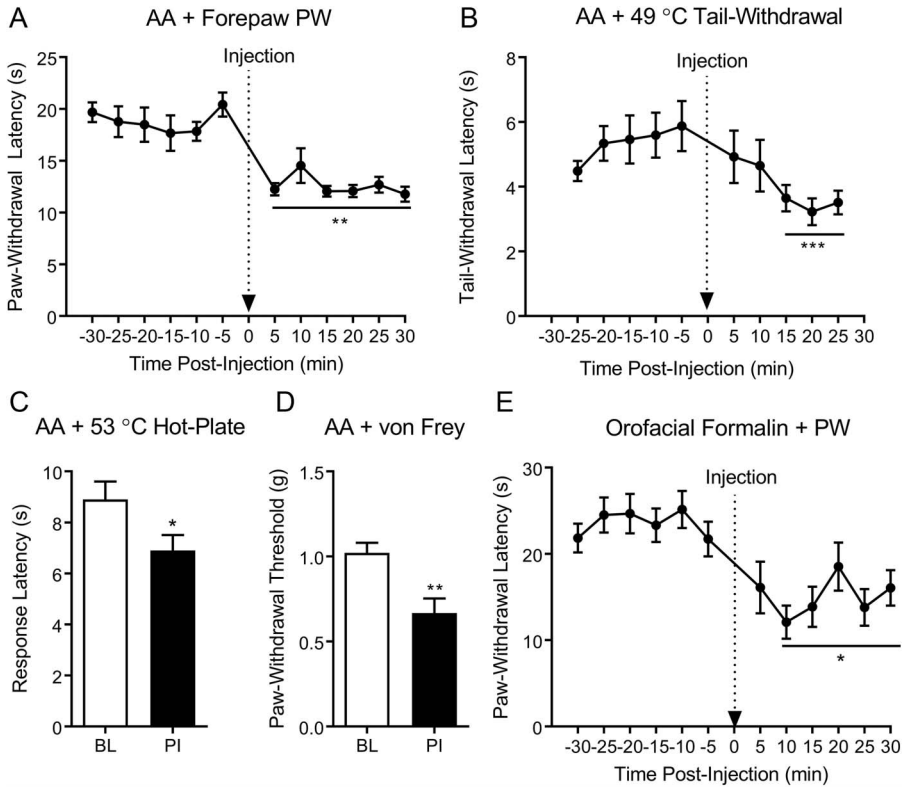

Figure 3. Hyperalgesia is observed despite changing the tested paw (A), test stimulus (B–D), or conditioning stimulus (E). In each graph, the conditioning stimulus (0.9% acetic acid [AA] or orofacial formalin) is listed first; the test stimulus (paw-withdrawal [PW] or others) is listed second. Symbols represent mean ± SEM forepaw-withdrawal latency (A; n = 8), tail-withdrawal latency (B; n = 17), or hindpaw-withdrawal latency (E; n = 20) at time points before and after acetic acid injection or injection of formalin into the lip. Bars in (C and D) represent mean ± SEM latency to nocifensive response (lick, shake, or lift; C; n = 26) or hindpaw-withdrawal threshold (g; D; n = 12) before (BL) and after (PI) injection with acetic acid. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with corresponding BL or averaged preinjection latencies.

(conditioning) pain stimuli—acetic acid or orofacial formalin—mice (and rats) may display hyperalgesia rather than hypoalgesia. This "anti-DNIC" is observed in both sexes, multiple rodent genotypes (although in a genotype-dependent manner), and using multiple conditioning and test stimuli. We find that the magnitude of the hyperalgesia is dependent on the intensity of the conditioning stimulus. More importantly, we find that the direction of the effect—that is, DNIC or "anti-DNIC"—is dependent on the intensity of the test stimulus.

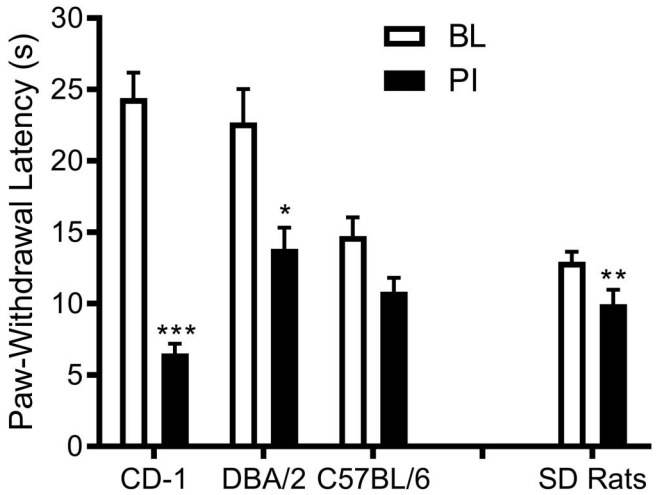

Figure 4. Hyperalgesia is strain-dependent and can be observed in rats. Bars represent mean ± SEM hindpaw-withdrawal latency (seconds) before (BL) and after (PI) acetic acid injection (n = 7-9 mice/strain; n = 12 rats). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with corresponding BL.

4.1. Why has this phenomenon never been reported before?

Since the discovery of DNIC by Le Bars et al. in 1979,[28,29] most investigations of this phenomenon have been on the studies of rat electrophysiology. We are unaware of any study ever observing an increase in firing rates of neurons in the DRG or dorsal horn associated with conditioning stimulation. Furthermore, modern studies of electrophysiological DNIC in rats have demonstrated that firing rates of wide dynamic range neurons in laminae V-VI of the dorsal horn decrease after ear pinch over a broad range of test stimulus intensities (application of von Frey fibers ranging from 8 to 60 g).[1,2] However, the independence of noxious stimulus–evoked activity of spinal cord neurons and withdrawal behavior has been previously demonstrated.[37]

We can identify a total of 22 published behavioral studies of DNIC (see supplementary Table 1, available at http://links.lww.com/PAIN/A700), all performed in male Sprague-Dawley rats,[3–5,7,10,18,23,24,37–39,43,44,47,57,58] save 1 study in Sprague-Dawley rats of both sexes[11] and 5 studies in male mice.[14,15,25,31,32] In 9 of the extant studies, the rats were anesthetized. A variety of conditioning stimuli and test stimuli were used in these investigations; in some of these, the intensity of the conditioning stimuli were varied, but we are unaware of any

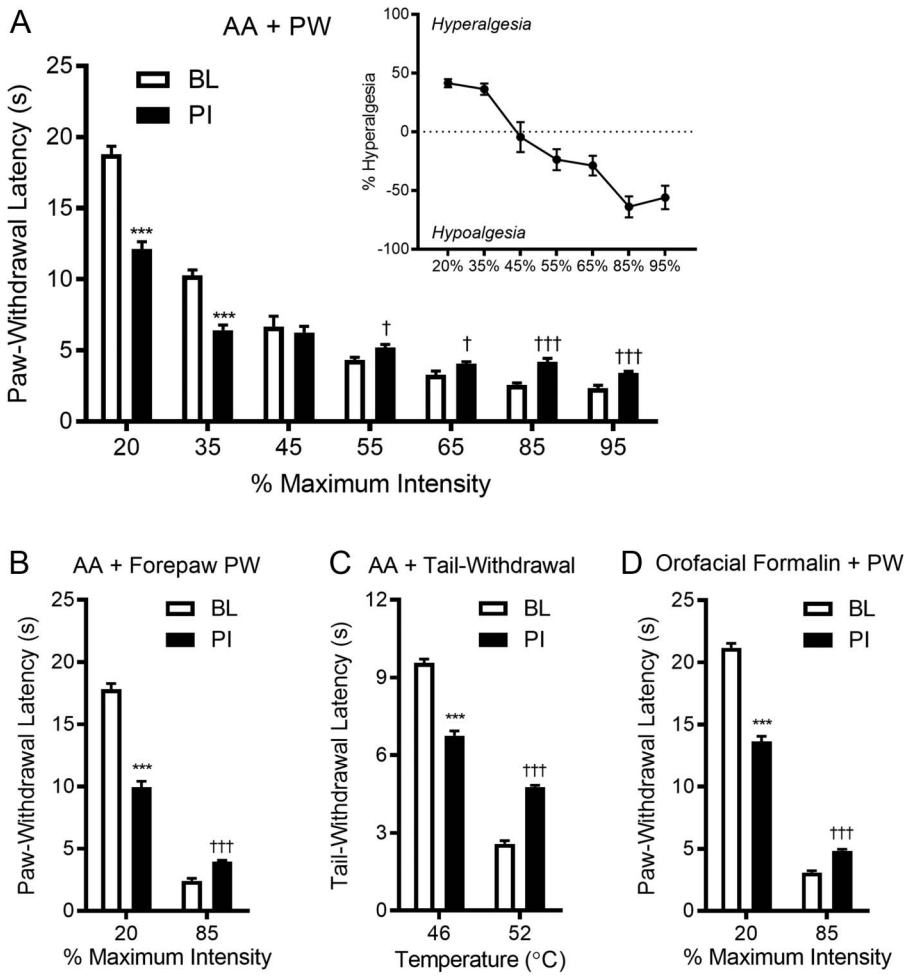

Figure 5. The presence of hyperalgesia or hypoalgesia depends on test stimulus intensity. In each graph, the conditioning stimulus (0.9% acetic acid [AA] or orofacial formalin) is listed first; the test stimulus (paw withdrawal [PW] or tail withdrawal) is listed second. Bars in (A) represent mean ± SEM hindpaw-withdrawal latency (seconds) before (BL) and after (PI) acetic acid injection. Symbols in the inset represent mean ± SEM percent hyperalgesia, according to the formula: [(BL − PI)/BL] × 100. At all time points, different groups of mice were tested using a radiant heat device set from 20% to 95% of maximal intensity, producing maximal glass temperatures ranging from 27.7 to 53.5°C (see Supplementary Fig. 1, available at http://links.lww.com/PAIN/A699); n = 11 to 13 mice/intensity setting. Bars in (B) represent mean ± SEM forepaw-withdrawal latency, at 2 different test stimulus intensities, before and after acetic acid injection; n = 8 mice/intensity. Bars in (C) represent mean ± SEM tail-withdrawal latency before and after acetic acid injection at 2 different water temperatures; n = 8 to 10 mice/temperature. Bars in (D) represent mean ± SEM hindpaw-withdrawal latency, at 2 different test stimulus intensities, before and after orofacial formalin injection; n = 8 mice/intensity. ***$P <$ 0.001 decrease from BL; †$P < 0.05$, †††$P < 0.001$ increase from BL.

study in rodents that has ever varied the intensity of the test stimuli. In all these studies but two, analgesia was observed. Of particular interest is the investigation of Morgan et al.,[38] which simultaneously observed hypoalgesia using hindpaw immersion in 50°C water as the conditioning stimulus and tail flick from radiant heat as the test stimulus, but hyperalgesia using tail immersion in 50°C water as the conditioning stimulus and hindpaw flick from radiant heat as the test stimulus (see below).

We speculate that the apparent novelty of the current observations might be explained by noting that, in existing behavioral investigations of DNIC, test stimulus intensities were high enough to lead to reflex withdrawals in 10 seconds or less (in the 11 articles where these were reported; see supplementary Table 1, available at http://links.lww.com/PAIN/A700), with a median of 5 seconds. Modern experiments tend to use lower stimulus intensities; in 26 rodent studies published in the journal *Pain* in 2017 in which thermal-withdrawal latencies of the hindpaw or tail were reported, the median was 13.5 seconds (see supplementary Table 2, available at http://links.lww.com/PAIN/A700).

4.2. Magnitude and direction of conditioned pain modulation/diffuse noxious inhibitory control depends on effective stimulus intensity

We show here that the direct manipulation of test stimulus intensity can determine whether DNIC or "anti-DNIC" is observed. We interpret other findings in this study using similar logic. For example, the larger degree of hyperalgesia observed in **Figure 1A** (≈17 seconds decrease) vs **Figure 1C** (≈7 seconds decrease) might be explained by the higher baseline latencies (and thus, lower effective stimulus intensity) in the former, due either to experimenter effects[9,53] or differences in calibration of the equipment over time. The reduced and nonsignificant ($P =$ 0.08) hyperalgesia displayed by C57BL/6 mice in **Figure 4** might be due to higher "effective" (ie, subjective) stimulus intensity due to genetic background; this strain has long been appreciated to represent an outlier with respect to thermal nociception.[27,36] Finally, the behavior of both sham- and SNI-operated mice in **Figure 6** can be similarly interpreted. The slight (nonsignificant; $P = 0.08$) decrease in withdrawal thresholds of

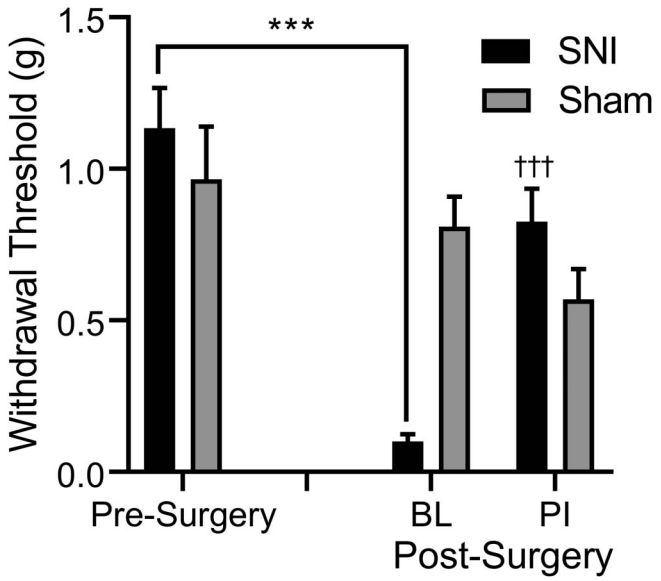

Figure 6. Neuropathic mice display hypoalgesia, not hyperalgesia. Bars represent mean ± SEM hindpaw-withdrawal threshold (g) before surgery (Pre-Surgery), and 7 days Post-Surgery, before (BL) and after (PI) acetic acid injection; n = 13 to 16 mice/condition. ***$P < 0.001$ decrease from Pre-Surgery as indicated; †††$P < 0.001$ increase from BL within surgical condition.

sham-operated mice—perhaps through social transfer[52]—might be responsible for the failure of these mice to exhibit significant hyperalgesia post–acetic acid ($P = 0.11$). More obviously, the extreme hyperalgesia of SNI-operated mice renders their effective stimulus intensity very high, leading to frank hypoalgesia post–acetic acid.

4.3. Other factors affecting the behavioral expression of diffuse noxious inhibitory control

Test stimulus intensity is obviously not the only factor affecting the magnitude and direction of CPM/DNIC. For example, as mentioned above, Morgan et al.[38] observed hypoalgesia or hyperalgesia depending on whether the tail or hindpaw was the site of the test stimulus. Unlike the hindpaw, the tail is a thermoregulatory organ in the rodent. Intriguingly, despite the direction of the behavioral response, evoked activity of spinal cord neurons was always inhibited.[38] The authors suggest that the hyperalgesia might be due to an increase in the excitability of a subset of neurons that are receiving input from primary afferents and have direct control over motoneurons that are responsible for the withdrawal response. In another study by Morgan and Whitney,[39] the failure to observe DNIC in the hindpaw was explained through the conditioning stimuli changing reflex topography from hindlimb flexion to extension. Conditioned pain modulation magnitude in humans is dependent on what body regions are stimulated by the conditioning and test stimuli,[50] suggesting differential homotopic vs heterotopic and segmental vs heterosegmental circuitries.[55]

4.4. Relationship with human conditioned pain modulation

As mentioned in the introduction, the human CPM phenomenon is of increasing interest due to its apparent value in predicting both chronic pain susceptibility and treatment responsiveness. As such, many studies have investigated parametric considerations around its use in both pain patients and healthy volunteers. It has been appreciated for some time that the phenomenon displays robust interindividual variability, dependent in healthy volunteers on age and sex,[13,17] ethnicity,[6] genetic background,[48] testosterone levels,[56] and attention[54] and psychosocial stress[19,20] at the time of testing. The variability observed is not just of CPM magnitude, but also direction. For example, Potvin and Marchand[49] observed that 42% of 96 fibromyalgia patients and 21% of 71 healthy controls exhibited hyperalgesia (what the authors refer to as "facilitatory CPM") rather than hypoalgesia in thermal pain thresholds immediately after immersion of the forearm in 12 °C water for 2 minutes.

A number of human studies have investigated the effect on CPM of parametric considerations, including conditioning and test stimulus modality, body region, intensity, and details of timing and repetition. According to the methodological review of Pud et al.,[50] findings regarding the relationship between the intensity of the conditioning stimulus and the magnitude of CPM have been "mixed". In general, CPM magnitude fades with time after the conditioning stimulus ceases. A more recent methodological review by Kennedy et al.[22] considers parametric values of the test stimulus because they relate to reliability and repeatability, but no mention is made of their effect on CPM magnitude or direction. It seems that a full consideration of the effect of test stimulus intensity on CPM has been hampered by the inability of large proportions of volunteers to tolerate cold water immersion (the most common conditioning stimulus by far in human CPM studies) at low temperatures for long periods. In 2 studies by the same laboratory in which electrical stimulation of the tooth was used as test stimuli and varied from ≈23 to 37 μA, different conclusions were arrived at with respect to the impact of test stimulus intensity.[16,46]

4.5. Nomenclature and future directions

Given that pain in another part of the body can cause either hypoalgesia or hyperalgesia, we would suggest that the term diffuse noxious inhibitory control is at least partially inaccurate, given that inhibitory implies hypoalgesia at the behavioral level. We believe that the increasingly popular term for the phenomenon, CPM, so named after a meeting of interested researchers in 2009,[59] is problematic as well. Conditioning is a well-defined process in psychology involving repeated pairings of stimuli; CPM as commonly implemented involves no "conditioning". Heterotopic noxious conditioning stimulation is problematic for the same reason.

It remains to be determined if the neural circuitry and associated neurochemistry underlying the facilitatory CPM/"anti-DNIC" phenomenon described here is similar or dissociable from that underlying conventional hypoalgesic DNIC. In rats, important roles of monoamines acting on brain stem α_2-adrenoreceptors and serotonin 5-HT_3 and 5-HT_7 receptors have been demonstrated.[1,2] We have been unable to demonstrate any effect on behavioral hyperalgesia using similar pharmacological manipulations (not shown), raising the possibility that this phenomenon has an entirely different neural substrate. Given the common (if not majority) observation of facilitatory CPM in humans, it might be of great clinical relevance to study this further in rodents.

Conflict of interest statement

The authors have no conflict of interest to declare.

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Appendix A. Supplemental digital content

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