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Engagement of Descending Inhibition from the Rostral Ventromedial Medulla Protects Against Chronic Neuropathic Pain

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Abstract

A puzzling observation is why peripheral nerve injury results in chronic pain in some, but not all, patients. We explored potential mechanisms that may prevent the expression of chronic pain. Sprague-Dawley (SD) or Holtzman (HZ) rats showed no differences in baseline sensory thresholds or responses to inflammatory stimuli. However, spinal nerve ligation (SNL)-induced tactile allodynia occurred in approximately 85% of SD and 50% of HZ rats, respectively. No apparent differences were observed in a survey of DRG or spinal “neuropathic markers” following SNL regardless of allodynic phenotype. SNL-induced allodynia was reversed by administration of lidocaine within the rostral ventromedial medulla (RVM), a site that integrates descending pain modulation via pain inhibitory (i.e., OFF) and excitatory (i.e., ON) cells. However, in SD or HZ rats with SNL but without allodynia, RVM lidocaine precipitated allodynia. Additionally, RVM lidocaine produced conditioned place preference in allodynic SD or HZ rats but conditioned place aversion in non-allodynic HZ rats. Similarly, RVM U69,593 (kappa opioid agonist) or blockade of spinal α_2 adrenergic receptors precipitated allodynia in previously non-allodynic HZ rats with SNL. All rats showed an equivalent first phase formalin responses. However, HZ rats had reduced second phase formalin behaviors along with fewer RVM OFF cell pauses and RVM ON cell bursts. Thus, expression of nerve-injury induced pain may ultimately depend on descending modulation. Engagement of descending inhibition protects in the transition from acute to chronic pain. These unexpected findings might provide a mechanistic explanation for medications that engage descending inhibition or mimic its consequences.

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Introduction

The dominant focus of research on chronic, and especially neuropathic, pain has been on changes in pain generating or transmitting mechanisms. However, many patients with peripheral nerve pathologies do not develop significant or persistent pain [55]. For example, only approximately 10% to 26% of patients with diabetes develop painful neuropathies [9; 26; 58], and only 7% to 27% of patients with herpes zoster rash develop postherpetic neuralgia [31; 70]. Development of neuropathic pain after endodontic procedures [75] or traumatic nerve injury [71] occurs in less than 5% of individuals. In spite of the considerable variability in the susceptibility of individuals to develop neuropathic pain, the reasons for this variability are unknown [50; 55; 68].

One obstacle to our understanding of the variability of chronic pain resides in the animal models employed for the study of neuropathic pain. The great majority of studies of experimental neuropathic pain commonly employ rodent strains in which peripheral nerve injury consistently (i.e., >90%) produces what appears to be a “painful” state [6]. However, some rodent strains are “resistant” to the development of experimental neuropathic pain [47; 82]. As in humans, reasons for the susceptibility and/or resistance to experimental neuropathic pain in rodents are not understood.

Much evidence suggests that the sensory experience of pain depends on descending pain modulatory circuits arising ultimately from the rostral ventromedial medulla (RVM) [16; 17]. Cells in the RVM project to the spinal dorsal horn and either enhance (i.e., ON cells) or inhibit (i.e., OFF cells) nociceptive traffic [17; 19; 74]. Following experimental nerve injury, facilitatory influences from the RVM can maintain central sensitization and expression of neuropathic pain behaviors [4; 54; 60; 62]. The clinical relevance of descending modulation is supported by the efficacy of drugs used for the treatment of neuropathic pain. Thus, number needed to treat (NNT) analyses consistently demonstrate that the most effective medications are those that engage descending pain inhibitory circuits or that mimic the consequences of descending inhibition [20]. Such compounds include tricyclic antidepressants [42; 46], serotonin–norepinephrine reuptake inhibitors (SNRI's) [36; 42] and norepinephrine (NE) reuptake blockers such as duloxetine, a compound with FDA approval for neuropathic pain, [21]. Spinal clonidine has been shown to be effective in neuropathic patients [14; 43]. Additionally, opiates are efficacious in neuropathic pain [20], and act, in part, by engagement of descending inhibition [15; 17; 33]. Finally, recent evidence suggests the possibility that gabapentinoids may also engage descending inhibition from the locus coeruleus [27; 28].

We observed that two closely related outbred strains [32], Sprague-Dawley (SD) and Holtzman (HZ) rats, showed different incidences of neuropathic pain behaviors after spinal nerve ligation (SNL) injury. Rather than looking for mechanisms that promote pain, here, we took advantage of this dichotomy to explore mechanisms that might protect against the development of a chronic neuropathic pain state. Based on mechanisms of drugs effective in patients, we hypothesized that differences in the expression of experimental neuropathic pain may be a consequence of engagement of descending pain inhibitory mechanisms.

METHODS

All testing was performed in male SD or HZ (outbred) rats from the same supplier (Harlan, Indianapolis, Indiana). These are genetically homogenous outbred strains [32]. All experiments were performed in accordance with the policies of the International Association for the Study of Pain and received approval from the Institutional Animal Care and Use

Committee (IACUC) of the University of Arizona. All behavioral testing was performed by an experimenter blinded to the surgery and to the strain.

Spinal nerve ligation (SNL)

Nerve injury to the L5 and L6 spinal nerves was performed as described by Kim and Chung [39]. Rats were anesthetized with isoflurane and the L5 and L6 spinal nerves were exposed and tightly ligated with 4-0 silk sutures. Rats with motor deficiency were excluded. Sham surgery was identical to SNL but without actual ligation.

Complete Freund's Adjuvant (CFA)-induced Inflammation

Rats received intraplantar injection of complete Freund's adjuvant (CFA) (100 μ l, s.c.; Calbiochem) into the left hindpaw. Control rats received an equivolume saline injection. Rats with CFA developed signs of inflammation that were prominent 4 days after the injection.

Intrathecal catheter placement

Rats were implanted with spinal catheters for intrathecal drug administration [81]. Animals were anesthetized with ketamine (80 mg/kg) and xylazine (12 mg/kg) and the atlanto-occipital membrane was exposed and an incision was made through which 7.5 cm of PE-10 tubing was inserted and secured to the musculature. Any rats exhibiting motor deficiency were discarded from testing. Drug injections were performed in a volume of 5 μ l, followed by a 1 μ l of air and then 9 μ l saline flush to clear the catheter.

RVM Cannulation

Anesthetized rats (ketamine and xylazine) were placed in a stereotaxic headholder. The skull was exposed and a pair of 26-gauge guide cannulae (1.2 mm apart and in a single pedestal; Plastics One Inc., Roanoke, VA) was directed toward the lateral portions of the RVM (AP-11.0 mm from bregma, L \pm 0.6 mm, DV-8.5 mm from the base of skull). The guide cannulae were secured to the skull and a five day recovery period followed. Drug injections were performed by slowly expelling 0.5 μ L of solution through a 33-ga injection cannula protruding an additional 1 mm into fresh brain tissue.

Paw withdrawal threshold

The hindpaw withdrawal thresholds were measured by probing the plantar aspect of the hindpaw with 8 calibrated von Frey filaments (Stoelting, Wood Dale, Illinois) (0.41 g to 15 g). Withdrawal threshold was determined by sequentially increasing and decreasing the stimulus strength and determined by a Dixon non-parametric test [6].

Conditioned place pairing

The single trial conditioned place preference protocol was performed as previously described [41] with conditioning day 10 days following SNL or sham surgeries. Starting 7 days post-SNL/sham surgery, all rats underwent a 3 day pre-conditioning period with behavior recorded on day 3 to verify no pre-conditioning chamber preference. All animals were exposed to the environment with full access to all chambers across 30 min each day. On day 3, behavior was recorded for 15 min and analyzed to verify absence of preconditioning chamber preference. The following day (day 4), rats received the appropriate control (i.e. vehicle) and immediately placed in the appropriate conditioning chamber for 30 min. Four (4) hr later, rats received the appropriate drug treatment and immediately placed in the opposite conditioning chamber for 30 min. Chamber pairings were counterbalanced. On test day 5, 20 hours following the afternoon pairing, rats were placed in the place preference box with access to all chambers and their behavior recorded

for 15 min for analysis for chamber preference. Increased post-conditioning time spent in the drug-paired chamber, as compared to pre-conditioning time, indicates conditioned place preference (CPP). Decreased post-conditioning time spent in the drug-paired chamber, as compared to pre-conditioning time, indicates conditioned place aversion (CPA). No change between time spent in the drug paired chamber, as compared to pre-conditioning time, indicates no conditioned place preference or aversion.

Immunohistochemistry

Spinal tissue was fixed and stained according to previously reported established protocols [24; 56; 77]. Frozen lumbar (20 μ m) and DRG (10 μ m) sections were incubated with antibodies to selected markers.

The following antibodies and dilutions were employed: OX42 (mouse, 1:1,000, Sigma); GFAP (mouse, 1:5,000, Serotec, Raleigh, NC), Nav1.8 (rabbit, 1:1,000, gift of Dr. Sanja Novakovic, Roche Biosciences); Nav1.3 (rabbit, 1:1,000, Alomone Labs); ATF3 (rabbit, 1:5,000, Santa Cruz Biotechnology), CGRP (rabbit, 1:40,000, Peninsula Laboratories), Substance P (rabbit, 1:10,000, Peninsula Laboratories), MOR (rabbit, 1:2,000, Neuromics), IB4 (Ab to lectin; 1:1,000, Vector Laboratories), P2X3 (rabbit, 1:5,000, Neuromics), Galanin (rabbit, 1:10,000, Peninsula), NPY (rabbit, 1:10,000, Peninsula), PKC γ (rabbit, 1:20,000, Santa Cruz Biotechnology). Secondary antibodies were Cy3-conjugated goat anti-rabbit IgG (1:500, Jackson ImmunoResearch Laboratories, West Grove, Pennsylvania) or Alexa Fluor 594-conjugated goat anti-rabbit IgG (1:1,000, Molecular Probes, Eugene, OR). For IB4 histochemistry, the sections were directly incubated in FITC-conjugated lectin IB4 (1:1,000, Vector Labs, Burlingame, California). Fluorescence images of DRG and spinal cord sections were acquired with a Nikon E800 fluorescence microscope and a Hamamatsu C5810 color CCD camera and its proprietary Image Processor software (Hamamatsu Photonic System, Bridgewater, NJ).

Statistical Analysis

Statistical comparisons among means within a treatment group were performed with ANOVA followed by Fisher's Least Significant Difference test. Pair-wise comparisons were made with Student's *t*-test. Comparisons between two treatments were performed by two-factor ANOVA. Significance was set at $P < 0.05$. Examination of frequency distributions for the response thresholds of HZ and SD rats with SNL revealed bimodal distributions, indicated by the presence of two maxima in each distribution [69]. One can be reasonably assured that two populations are present when the means represented by each maximum are separated by more than the sum of the standard deviations associated with each mean, as was the case here [69]. A conservative approach was taken here, where data points within two standard deviation units from the mean, representing 95% of the total sample, were included in the analysis of "allodynic" and "non-allodynic" animals.

For CPP experiments, data were analyzed before conditioning (baseline) and after conditioning using two-factor ANOVA (chambers vs. treatment) followed by Bonferroni test of post-conditioning compared to pre-conditioning time spent in the drug paired chamber to determine conditioned place preference (increase in post-conditioning time vs pre-conditioning time) or conditioned place aversion (decrease in post-conditioning time vs. pre-conditioning time) [41]. If significant conditioned place preference or conditioned place aversion was determined, group differences were analyzed using the difference from baseline scores, which were calculated for each rat using the formula: test time in chamber - preconditioning time spent in chamber. Difference scores from baseline for the drug paired chamber between SNL and sham operated rats were analyzed using paired *t*-tests. Rats were

tested for their response thresholds to von Frey filaments one day before and one day after the place preference experiment to confirm the presence or absence of allodynia.

Electrophysiology

Electrophysiology experiments were performed in male rats (250-300g) anesthetized with 4% isoflurane administered through a trachea cannula. Body temperature was kept at 37 °C with a feedback controlled electric blanket (Harvard Apparatus, MA). Each rat was placed in a stereotaxic apparatus, a hole was drilled in the skull (1.5–2.5 mm caudal to interaural, within 0.5 mm of midline) and the dura mater was removed to allow placement of an electrode in the RVM. The plantar region was placed against a small fixed platform to allow access for mechanical and chemical (5% formalin) stimuli. Responses to noxious radiant heat were measured with a tail flick unit (Ugo Basile, PA). Mechanical responses to a 100g von Frey filament applied to the hindpaw or brief pinch with forceps were determined. Isoflurane concentration was adjusted (0.8-1.0%) until discrete reflexes to noxious pinch (hindpaw) and heat (tail) could be elicited [49] but without causing spontaneous movements or signs of distress. Anesthesia was strictly regulated in order to avoid fluctuations that could affect RVM activity [5].

Extracellular recordings were obtained with tungsten electrodes (9-12 M Ω , Frederick Haer, Brunswick, ME) and the signal was amplified (10k), bandpass filtered (300 Hz to 5 kHz) and digitized (SciWorks, Datawave, CO). Spike waveforms were monitored and stored for off-line analysis. Neuronal activity was recorded continuously for 30 min before the initiation of experimentation. ON-cells showed an increase and OFF-cells a decrease in activity preceding the occurrence of the tail flick [1; 18 and paw withdrawal [5; 80] from a noxious stimulation source. Neutral cells showed no change in activity associated with withdrawals and were excluded from these experiments. Only cells that consistently responded to all stimuli were included. Neurons were isolated and characterized (ON-cell or OFF-cell), and 2 to 3 thermal noxious stimuli were applied at 5 min intervals, followed by s.c. injection of formalin (50 μ L, 5%) into the hindpaw. Formalin produces a distinct biphasic excitatory response in spinal nociceptive-related neurons and RVM ON-cells along with a non-biphasic manner ongoing activity of RVM OFF-cells; these responses last around 60 min [10; 11]. Thermal tests were repeated to confirm presence of ON-cell or OFF-cell activity, and the recording sites were marked with an electrolytic lesion [13; 67]. Neuronal firing was analyzed off-line (SciWorks, Datawave, CO) and measured as spikes/sec with a bin size resolution equal to 1 sec. For ON-cells, the number of spontaneous bursts after formalin injection lasting for more than 10 sec was assessed as well as their duration in sec. For OFF-cells, the number of complete pauses in firing lasting for more than 10 sec, and their duration, were assessed.

Results

The SD and HZ rats were evaluated for behavioral responses to innocuous tactile or noxious thermal stimuli in baseline conditions and after induction of hindpaw inflammation elicited by Complete Freund' Adjuvant (CFA). No differences were observed in baseline tactile (Fig. 1A) or thermal (Fig. 1B) thresholds. Moreover, there were no differences in either the time-course or the magnitude of CFA-induced tactile (Fig. 1A) or thermal hypersensitivity (Fig. 1B). A total of 185 male HZ rats and 150 male SD rats (250-300 g) were subjected to spinal nerve ligation (SNL) injury. An additional 50 rats of each strain were subjected to sham-surgery. All surgeries were performed by the same experimenter (J.O.). Response thresholds to probing the hindpaw with von Frey filaments were determined 10-14 days after surgery in a blinded fashion. A bimodal distribution of response thresholds was observed in both strains (Fig. 2A, 2B). The response thresholds observed in this experimental group of 150 SD rats with SNL presented a mean withdrawal threshold (SD) of 3.7 (1.74 SD) g for

the first distribution and a mean withdrawal threshold of 12.4 (1.74, SD) g for the second distribution. These data were compared to historical data from an additional 633 rats of the same age and at the same time point with SNL performed by the same experimenter (J.O.) in our laboratory. No significant differences in the distributions of response thresholds were observed in either group of SD rats (Supplemental Figure 1) and for this reason, the data were combined and the response threshold distribution for all 783 SD rats with SNL is presented (Fig. 2A). In the SD rats, the mean (and standard deviation) of the first distribution was 3.8 (1.65) g, and that of the second was 13.3 (2.27) g. The same analysis was performed in 185 HZ rats. For the HZ rats, the mean (and standard deviation) of the first distribution was 3.9 (1.10) g and that of the second was 13.7 (1.60) g. On this basis, rats responding at the mean of the first distribution (plus two standard deviations) or lower were defined as “allodynic” while those responding at the mean of the second distribution (minus two standard deviations) or greater were defined as “non-allodynic”. No significant effects were found in sham-operated controls of either strain (data not shown). The data from the SD rats revealed a distribution of approximately 85% “allodynic” and 15% “non-allodynic” animals in the overall sample of 783 SD rats (Fig. 2A); if the analysis was done on the sample size of 150 SD rats, 90% were determined to be “allodynic” and 10% were “non-allodynic” (Supplemental Figure 1). Data from HZ rats showed that 51% of these rats were “allodynic” while 49% were “non-allodynic” (Fig. 2B). Notably, the response thresholds of the “allodynic” HZ and SD rats were not significantly ($p > 0.05$) different. Likewise, the response thresholds of the “non-allodynic” SD and HZ groups also were not significantly different.

A survey of common “markers” associated with peripheral nerve injury in either the spinal cord and/or dorsal root ganglion (DRG) was performed in SD and HZ rats. Following SNL or sham surgery, DRG and spinal cords of allodynic SNL SD rats and non-allodynic HZ rats (or sham-operated controls for each strain) were stained for GFAP (astrocyte marker), OX42 (microglial marker), CGRP, SP, IB4, galanin, NPY, Nav1.3, Nav1.8, PKC γ , MOR, P2X3 and ATF3. Up- or down-regulation of these markers was similar to previous observations [7; 23; 24; 34; 51; 56; 60; 61; 76] and no apparent differences were observed between the two SNL groups (Supplemental Figs 2-5). No differences were observed between shams of either strain (Supplemental Figs 2-5). Hence, the “pain” phenotype of HZ rats did not appear to be related to differences in expression for these histochemical markers often associated with experimental neuropathy.

Previous studies from our laboratory have demonstrated that enhanced sensitivity to evoked stimuli in SD rats following SNL can be reversed by lidocaine-induced inactivation of the RVM, reflecting blockade of descending facilitation [4]. The role of descending facilitation was reinforced by lesion of putative pain facilitatory cells (i.e., presumably ON cells) within the RVM by lesion with dermorphin-saporin [4; 60] suggesting that the observed lidocaine effects were likely to be relevant to descending modulation rather than affecting rostrally projecting fibers of passage. Allodynic and non-allodynic SD and HZ rats received RVM lidocaine and response thresholds to tactile stimulation were monitored. Consistent with previous observations [4], allodynic SD and HZ rats receiving RVM lidocaine showed a time-dependent reversal of response thresholds (Supplemental Fig. 6A, 6B); no effects were seen in sham-operated controls receiving RVM lidocaine. However, in non-allodynic SD and HZ rats, RVM administration of lidocaine produced a time-dependent precipitation of tactile allodynia (Fig. 3A,B). Injuries to peripheral nerves are believed to produce a tonic aversive state that reflects the presence of “spontaneous” (i.e., non-evoked) pain that can be unmasked through negative reinforcement [40; 41; 52; 64]. Thus, we have shown that inactivation of the RVM produces place preference in SD SNL rats with allodynia suggesting the presence of spontaneous “pain” [40; 41; 52; 64]. Allodynic and non-allodynic HZ rats received RVM lidocaine and place conditioning was performed. Consistent with our

previous studies [41], RVM lidocaine produced place preference in allodynic SD rats (data not shown). Similarly, in HZ rats with SNL-induced allodynia, RVM lidocaine produced place preference (Fig 3C,D), revealing the presence of SNL-induced spontaneous pain. In non-allodynic HZ rats, however, RVM lidocaine produced conditioned place aversion (CPA) (Fig. 3C,D). This finding reveals the absence of a tonic aversive pain state in non-allodynic HZ rats consistent with the conclusion that these rats are protected from neuropathic pain.

U69,593, a kappa opioid receptor agonist was used experimentally as a research tool to inhibit the activity of RVM OFF and neutral cells without influencing ON cells [45] as previously reported and to ensure that transient precipitation of allodynia in previously non-allodynic HZ rats by RVM lidocaine reflected tonic descending inhibition rather than non-specific effects on ascending fibers of passage. U69,593 had no effect in sham-operated rats or allodynic HZ rats, but precipitated allodynia in previously non-allodynic rats (Fig. 4A). As descending inhibition is thought to result in an increase in release or activity of norepinephrine in the spinal dorsal horn [59], allodynic and non-allodynic HZ rats received intrathecal (i.th.) administration of yohimbine (α_2 adrenergic antagonist) [2]. No effects were seen in sham-operated controls or in allodynic HZ rats. Consistent with a tonic descending inhibition-dependent protection from neuropathic pain, however, i.th. yohimbine precipitated allodynia in previously non-allodynic HZ rats (Fig. 4B).

We next asked if behavioral and electrophysiological responses in the RVM would be different between SD and HZ rats when C-fiber drive was elicited by formalin injection into the hindpaw. While 1st phase responses to formalin injection were not different between rat strains, 2nd phase behaviors were markedly reduced in HZ rats (Fig. 5A). Recordings were then made of RVM ON and OFF cells [5] between rat strains in lightly anesthetized animals to quantify potential differences in descending modulatory systems. Consistent with 1st phase behavioral responses, ON cell bursts and OFF cell pauses evoked by initial formalin injection were not different between rat strains (Supplemental Fig. 7). In contrast, significant differences were seen in responses over the 60 min post-formalin recording period (Fig. 5B and C), consistent with decreased 2nd phase responses in HZ rats. In HZ rats, following the initial OFF cell pause, we observed a significant reduction in the total number, total duration and average duration of OFF cell pauses compared to SD rats (Fig. 5B, F, G and Supplemental Fig 8). Moreover, the number, total duration and average duration of ON cell bursts were significantly decreased in HZ rats when compared to SD rat ON cells (Fig. 5C,D,E, Supplemental Fig 8). We did not observe two populations of second phase response to formalin in this population of HZ rats.

Discussion

We have shown that both SD and HZ rats show variable expression of experimental neuropathic pain after a common injury protocol. SD and HZ rats are derived from the same SD stock [32] suggesting that observed differences in these closely related rats are likely to be the result of random genetic drift. The similarity between the strains is reflected in similar baseline responses to noxious thermal and light tactile stimuli, as well as in the timecourse of behaviors after CFA-induced peripheral inflammation. Thus, response thresholds prior to injury, or following an inflammatory stimulus, do not predict which individual animal is likely to develop chronic neuropathic pain. Likewise, it is also not clearly known what factors may influence the development of chronic pain in patients [50; 55; 68].

Initial studies in 150 SD rats demonstrated the apparent presence of two populations with the great majority, but not all, animals showing SNL, but not sham-induced hypersensitivity. Data from these rats were combined with historical SD controls from our laboratory since

(a) the surgeries were all performed by the same experimenter in the same manner; (b) the rats were the same approximate age from the same supplier; and (c) sensory thresholds were evaluated on approximately the same post-injury day (i.e., 10-14 days) in a blinded fashion and (d) the current and historical groups did not differ significantly in the mean responses for the allodynic and non-allodynic distributions. An analysis of more than 780 SD rats revealed two populations with the great majority (i.e., approximately 85%) exhibiting neuropathic behaviors. The same analysis on 185 HZ rats with SNL performed using the same protocol by the same experimenter and with thresholds measured under blinded conditions at the same time point also revealed two populations of animals but with an almost equal distribution of animals that did, or did not, show apparent neuropathic behaviors. The mean response and standard deviation of the first and second distribution did not differ significantly between SD and HZ rats. Rather, the difference was in the relative distribution between the two strains.

A survey of multiple common “markers” associated with experimental neuropathic pain in the DRG and spinal cord was performed; the data were consistent with previously results from multiple laboratories [7; 23; 24; 34; 51; 56; 60; 61; 76]. Thus, OX42, GFAP and NPY were upregulated in the DRG and/or spinal cord after SNL while IB4, substance P and CGRP were downregulated in the DRG and/or spinal cord [7; 23; 24; 34; 51; 56; 60; 61; 76]. However, histochemical markers of neuropathy could not distinguish allodynic phenotype of SD or HZ rats.

As therapy for neuropathic pain often relies on drugs that modulate descending circuits, and because previous preclinical data have suggested a critical role for descending facilitation in maintaining neuropathic states [4; 22; 53; 62], we hypothesized that such clinical observations might be relevant preclinically to the post-SNL “pain” phenotype. As expected, RVM lidocaine did not alter response thresholds in sham-operated SD or HZ animals but reversed allodynia in SD or HZ rats with SNL. Strikingly, however, RVM lidocaine in SD or HZ rats with SNL that did not show neuropathic behaviors precipitated allodynia. In order to clarify whether these manipulations of descending pathways were associated with a tonic aversive state, allodynic or non-allodynic SD and HZ rats received RVM lidocaine and were evaluated using a conditioned place pairing paradigm. RVM lidocaine did not produce place preference or place aversion in either SD or HZ rats undergoing sham surgeries. In SD or HZ rats with allodynia, RVM lidocaine produced conditioned place preference consistent with the presence of spontaneous pain as previously reported [40; 41; 52; 64]. In HZ rats with SNL but not expressing allodynia, however, RVM lidocaine produced conditioned place aversion suggesting that blockade of descending inhibition precipitated an aversive state as would be associated with spontaneous pain. These results suggest that rats with SNL, but that do not express allodynia, may have enhanced activation of descending pain inhibitory systems from the RVM.

While it is possible that RVM lidocaine may affect fibers of passage and ascending pathways, the role of descending modulation was supported by (a) manipulations known to specifically modulate the activity of RVM cells thought to mediate facilitation and inhibition [16] and (b) blocking α -adrenergic receptors thought to mediate the actions of nor-epinephrine arising from descending pathways [35; 38; 79]. RVM ON-cells increase their firing rate after application of radiant heat and just prior to the initiation of the nociceptive reflex, whereas OFF-cells pause in their activity prior to the reflex [18; 29; 63]. These neurons have spinopetal projections, and thus may exert facilitatory and inhibitory modulation of nociceptive inputs [19; 74]. Activity of ON-cells has been linked to pain facilitation [37; 48], and may drive enhanced abnormal pain associated with nerve injury [25]. The ON-cells are directly inhibited by activation of μ -opioid receptors [30; 57], whereas OFF-cells are inhibited by activation of κ -opioid receptors [45; 57]. RVM U69,593

did not alter sensory thresholds in SD or HZ rats with allodynia or in sham-operated rats. However, RVM U69,593 precipitated allodynia in previously non-allodynic HZ rats. These data are consistent with a possible decrease of descending inhibition presumably by inhibiting OFF cell activity. The data are also consistent with the hypothesis that expression of pain after nerve injury is dependent upon the activity of an endogenous pain inhibitory system.

In order to test this possibility further, an adrenergic antagonist was administered spinally in allodynic and non-allodynic rats receiving SNL surgery. In addition to direct projections from the RVM, descending noradrenergic projections from supraspinal nuclei including the locus coeruleus, are believed to mediate a descending pain inhibition through spinal α_2 -adrenergic receptors [3; 27; 28; 59]. Spinal administration of the α_2 -adrenergic antagonist yohimbine had no effect on allodynic SD or HZ rats or in sham-operated animals. However, spinal yohimbine unmasked allodynia in previously non-allodynic HZ rats with SNL. This result is consistent with electrophysiologic studies that found that SNL is associated with a loss of α_2 -adrenoceptor mediated inhibition of deep spinal cord neurons in response to tactile stimuli [65]. As spinal yohimbine did not further alter thresholds in animals with allodynia there appears to be net facilitation in animals with allodynia. Taken, these studies suggest that descending noradrenergic inhibition blocks the expression of injury-induced pain. Although our studies emphasize noradrenergic mechanisms, RVM descending serotonergic systems may also play a role [72; 73]. Descending serotonergic projections promote neuropathic pain through spinal 5-HT₃ receptors [44; 72]. Depletion of 5-HT in RVM neurons support a role of serotonergic projections to facilitate pain [78], and depletion of spinal 5-HT abolished behavioral and electrophysiologic parameters of enhanced pain in rats with SNL [66]. Additionally, it should be noted that clinically relevant mechanisms other than descending modulation may be important in neuropathic pain.

The possibility of differences in descending modulation in SD and HZ rats was tested further by directly driving nociceptors with formalin. No differences in behavioral responses to the first phase of formalin injection were observed in SD and HZ rats. However, HZ rats had markedly reduced nocifensive behaviors compared to SD rats in the second phase of the formalin response. The behavioral data were also consistent with the responses of RVM pain modulatory cells. Thus, ON cell bursts and OFF cell pauses evoked by initial formalin injection were not different between rat strains. However, a significant reduction in OFF cell pauses as well as of ON cell bursts was observed in HZ compared to SD rats in the 60 min period following formalin. Interestingly, like the response to CFA, we did not observe two populations of second phase response to formalin in this population of HZ rats. The reasons for this are not known but may have to do with the type and nature of afferent input that is generated by a sub-chronic chemical stimulus as opposed to chronic nerve injury.

Our observations indicate that engagement of descending inhibition is a critical factor that can prevent the transition from acute to chronic neuropathic pain. While multiple peripheral mechanisms [8] may contribute to the development of chronic pain, our findings raise the interesting and unexpected concept that in many individuals, despite the presence of a mechanism capable of inducing pain, countervailing modulatory influences might prevent its behavioral manifestation. Our data demonstrate that descending modulation from the RVM reduces the impact of pain generators that would produce a chronically painful situation. Thus, failure to engage descending inhibition or failure to remove a necessary descending facilitation can result in chronic neuropathic pain. The mechanisms determining whether descending inhibition is engaged following nerve injury in the same strain of rat are not known. However, once developed, neuropathic pain is commonly treated by medications that engage descending inhibition or mimic the consequences of descending inhibition by raising levels of spinal norepinephrine [12]. Our data suggest that “chronification” of pain

may ultimately depend on descending modulation that regulates the spinal consequences of peripheral nerve injury.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Summary

A rat population with variable responses to nerve injury indicated that activation of descending inhibition with a spinal noradrenergic component prevents development of neuropathic pain.

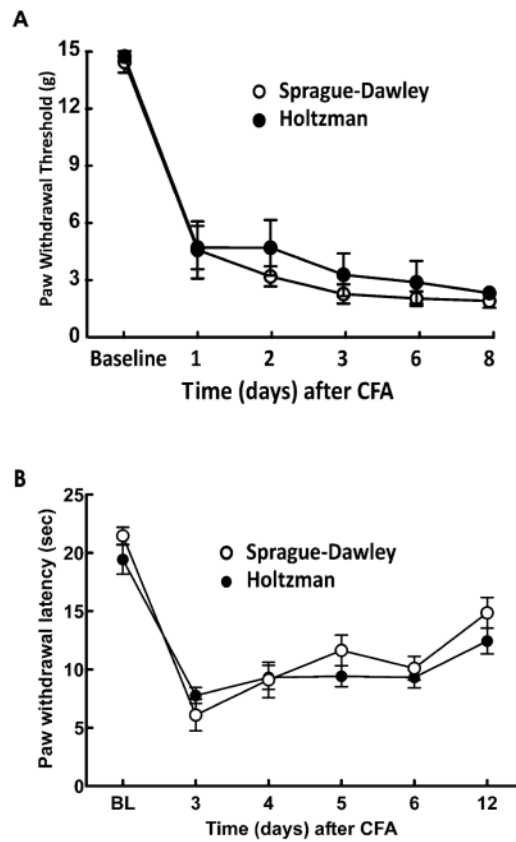


Figure 1. Baseline mechanical thresholds are not significantly different in SD and HZ rats. Following hindpaw CFA, similar magnitude and time course of decreased tactile (**A**) and noxious thermal (**B**) thresholds are observed in both strains.

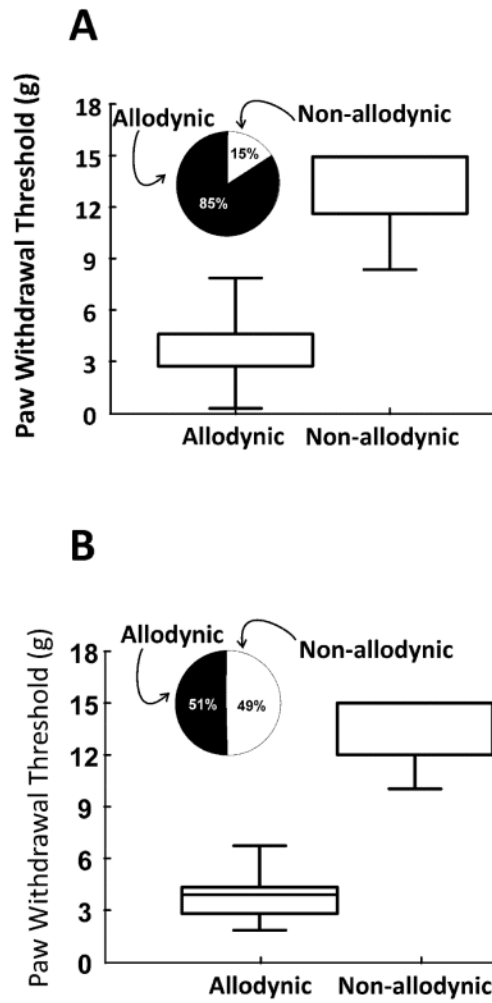


Figure 2.

A. Paw withdrawal thresholds of 783 SD rats were measured 10-14 days after SNL. Thresholds fell into a bimodal distribution, with 85% of SD rats indicating a low-threshold (allodynia) and 15% indicating a high-threshold (non-allodynic). **B.** Paw withdrawal thresholds of 185 HZ rats were measured 10-14 days after SNL. Thresholds fell into a bimodal distribution, with 51% of HZ rats indicating a low-threshold (allodynic) and 49% indicating a high-threshold (non-allodynic).

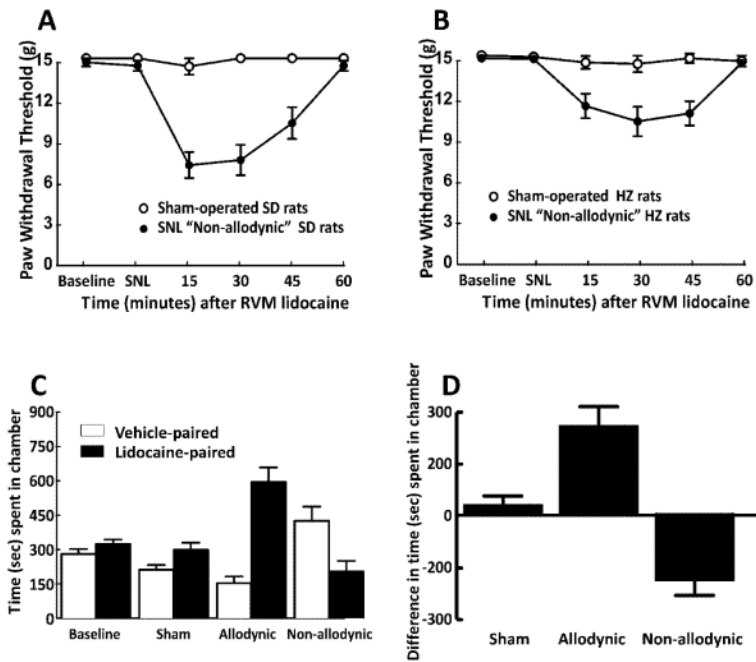


Figure 3.

SD (A) or HZ (B) rats received RVM cannula 7 days prior to SNL or sham surgery. Non-allodynic SD or HZ rats received lidocaine (4% w/v; 0.5 μ l) in the RVM producing a transient significant ($p < 0.05$) decrease in paw withdrawal thresholds indicating tactile allodynia. C. SD and HZ rats underwent conditioning to chambers that were paired to vehicle or lidocaine administered into the RVM. On day of testing, allodynic SD and HZ rats showed a preference (i.e., conditioned place preference) for the lidocaine-paired chamber, whereas the non-allodynic HZ rats showed aversion (i.e., conditioned place aversion) to the lidocaine-paired chamber. Sham-operated injected animals showed no differences from baseline. D. Data for CPP are shown as the difference in time spent in the lidocaine-paired chamber and vehicle-paired chamber.

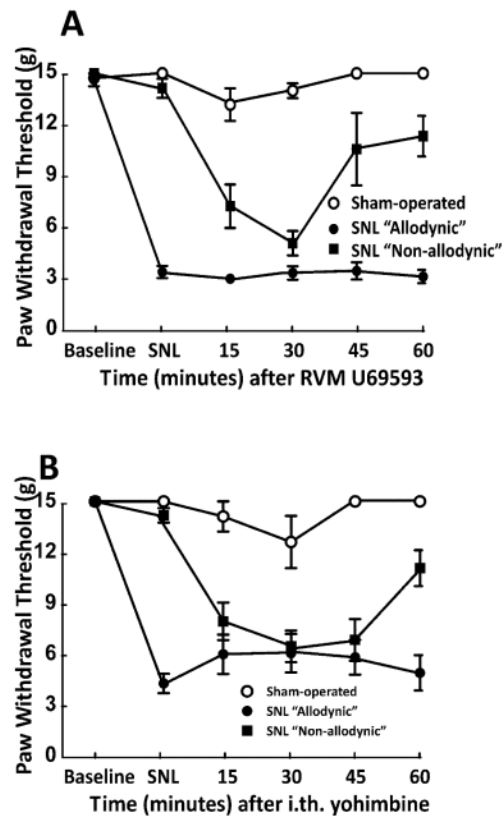


Figure 4.

A. HZ rats received sham surgery or SNL. SNL rats were separated into allodynic and non-allodynic groups and received U69593 (1 μ g) into the RVM. Non-allodynic HZ rats demonstrated reversible significant ($p < 0.05$) decreases in paw withdrawal thresholds following RVM injection, indicating tactile allodynia. Allodynic and sham-operated HZ rats showed no changes from their baseline values. **B.** HZ rats received sham surgery or SNL. SNL rats were separated into allodynic and non-allodynic groups and received yohimbine (30 μ g) intrathecally. Non-allodynic HZ rats demonstrated reversible significant ($p < 0.05$) decreases in paw withdrawal thresholds indicating tactile allodynia. Allodynic and sham-operated HZ rats showed no changes from their baseline values.

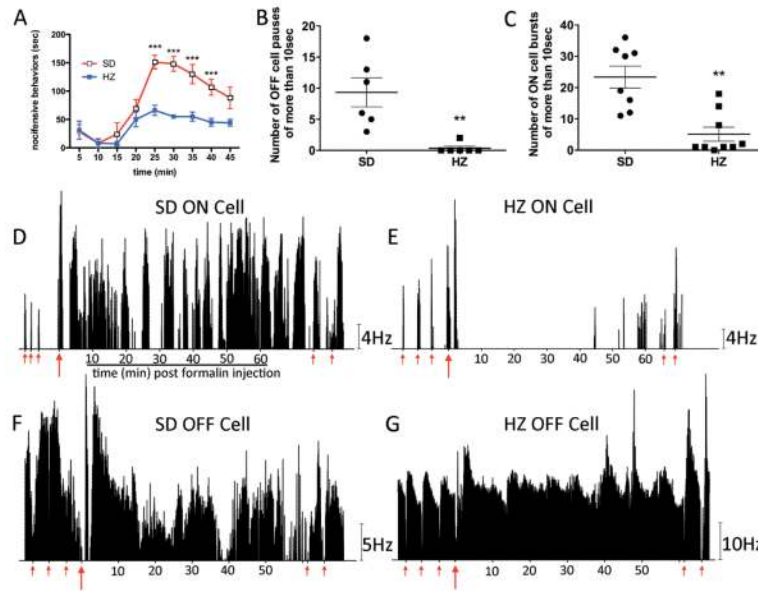


Figure 5.

C-fiber drive differentially modulates RVM ON and OFF cell activity between SD and HZ rats.

A. Behavioral responses to formalin injection into the hindpaw are shown. HZ rats showed a significant decrease in 2nd phase behaviors compared to SD rats. The total number of OFF cell pauses (**B**) and ON cell bursts (**C**) lasting for more than 10 sec following the initial burst and pause from formalin injection were significantly decreased in HZ rats compared to SD rats. Representative ratemeters for SD ON cells (**D**), HZ ON cells (**E**), SD OFF cells (**F**) and HZ OFF cells (**G**) demonstrate differences in RVM neuron responses to formalin injection. Small arrows indicate time of thermal stimulation of the tail paired to tail flick and RVM neuron responses. Large arrows indicate formalin injection. ** $p < 0.01$, *** $p < 0.001$.