Published in final edited form as:

Neuroscience. 2012 March 29; 206: 212–223. doi:10.1016/j.neuroscience.2012.01.007.

Bursting activity in myelinated sensory neurons plays a key role in pain behavior induced by localized inflammation of the rat sensory anglion

Wenrui Xie, Judith A. Strong, Daniel Kim, Sameam Shahrestani, and Jun-Ming Zhang

Pain Research Center, Department of Anesthesiology, University of Cincinnati College of Medicine, Cincinnati, OH 45267-0531, USA

Abstract

Abnormal spontaneous activity of sensory neurons is observed in many different preclinical pain models, but its basis is not well understood. In this study mechanical and cold hypersensitivity were induced in rats after inflammation of the L5 dorsal root ganglion (DRG), initiated by local application of the immune stimulator zymosan in incomplete Freund's adjuvant. Mechanical hypersensitivity was evident by day 1 and maintained for two months. The model also showed reduction of rearing behavior in a novel environment. Microelectrode recordings made in isolated whole DRG on day 3 after inflammation showed a marked increase of spontaneous activity, predominantly with a bursting pattern. The incidence was especially high (44%) in A α cells. Spontaneous activity and subthreshold membrane potential oscillations were completely blocked by tetrodotoxin (500 nM) and by riluzole (10 μ M), a blocker of persistent sodium currents. In vivo, local perfusion of the inflamed DRG for the first 7 days with riluzole gave long-lasting, dose-dependent reduction in mechanical pain behaviors. Riluzole perfusion did not affect mechanical sensitivity in normal animals. Unmyelinated C cells had a very low incidence of spontaneous activity and were much less affected by riluzole in vitro. Taken together these results suggest that high-frequency and/or bursting spontaneous bursting activity in A α sensory neurons may play important roles in initiating pain behaviors resulting from inflammatory irritation of the DRG.

Keywords

Inflammation; riluzole; persistent sodium current; spontaneous activity

Inflammatory mediators have important effects on peripheral sensory neurons in addition to their effects on immune cells. These direct, generally excitatory effects of inflammation play

© 2012 IBRO. Published by Elsevier Ltd. All rights reserved.

Correspondence to: Jun-Ming Zhang, M.D., M.Sc., Department of Anesthesiology, University of Cincinnati College of Medicine, 231 Albert Sabin Way, PO BOX 670531, Cincinnati, OH 45267-0531, Tel: 01-513-558-2427, FAX: 01-513-558-0995, Jun-Ming.Zhang@uc.edu.

Conflict of Interest: none of the authors has any to report.

Author contributions: W.X. performed behavioral, electrophysiological, and immunohistochemistry experiments, data analysis, experimental design, and helped write the manuscript. J.A.S. performed data analysis and experimental design, and drafted the manuscript. D.K. and S.S. performed behavioral experiments. J.-M. Zhang performed experimental design and helped write the manuscript.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

important roles in pain conditions that accompany inflammation of peripheral tissues. Inflammatory processes may also occur in the dorsal root ganglion (DRG), e.g. in herpes zoster (Schon et al., 1987) or chemogenic low back pain when DRG are exposed to immunogenic material released from ruptured disks (Kawakami et al., 1996, Olmarker and Myers, 1998). In addition, models of neuropathic pain with peripheral nerve injury also have inflammatory components (Beuche and Friede, 1984, Perry and Brown, 1992, Lu and Richardson, 1993, Eckert et al., 1999, Hu and McLachlan, 2002), for example due to the recruitment or activation of immune cells that remove material from damaged axons.

We previously described a model for studying the effects of inflammation in the absence of overt axotomy on DRG neurons (Xie et al., 2006). Locally inflaming the L5 DRG by depositing a small drop of an immune stimulator, zymosan, in incomplete Freund's adjuvant over the ganglion, was found to rapidly increase mechanical sensitivity in the hindpaws. This was accompanied by satellite glia activation and macrophage infiltration, as well as increases of several pro-inflammatory cytokines and decreases in anti-inflammatory cytokines. Increased spontaneous activity, especially in A α fibers, was observed using fiber recording methods. Further studies showed that one of the upregulated pro-inflammatory cytokines, GRO/KC (growth-related oncogene; Cxcl1) could modulate excitability of sensory neurons in acute primary culture (Wang et al., 2008, Yang et al., 2009a).

Abnormal spontaneous activity of sensory neurons occurs in a number of different pain models (Govrin-Lippmann and Devor, 1978, Study and Kral, 1996, Hu and Xing, 1998, Ali et al., 1999, Amir, 1999, Pan et al., 1999, Song et al., 1999, Liu et al., 2000a, Liu et al., 2000b, Xie et al., 2006, Devor, 2009, Eliav et al., 2009, Sorkin and Yaksh, 2009, Bedi et al., 2010, Lund et al., 2010, Xu and Brennan, 2010, Berger et al., 2011). It is generally observed very early and as such is a candidate for mediating abnormalities that occur at later times such as glial activation, sympathetic sprouting, and changes in gene expression. In several preclinical pain models, agents that block spontaneous activity can also block development of the chronic pain state. Hence understanding the ionic basis of spontaneous activity would contribute towards therapies aimed at preventing development of chronic pain. In the present study, we used microelectrode methods in acutely isolated whole DRG after they were locally inflamed. Compared to fiber recording methods, microelectrode recordings provide much more detail about the electrical properties of spontaneously active cells. We used this method to examine the ionic mechanisms and pharmacological sensitivity of spontaneous activity in this model. Using a method to apply drugs locally to the DRG in vivo, we also determined the behavioral effects of blocking spontaneous activity.

EXPERIMENTAL PROCEDURES

Animals

Adult Sprague Dawley rats (Harlan, Indianapolis, USA) were housed in groups of two in 40×60×30 cm plastic cages with soft bedding under a 12-h light/dark cycle, with food and water ad libitum. The experimental protocol was approved by the Institutional Animal Care and Use Committee of the University of Cincinnati.

Surgical procedure for local inflammation of the DRG (LID)

Male Sprague-Dawley rats weighing 150 – 200 g at the start of the experiment (for behavior experiments) or females weighing 100 – 120 grams at the time of sacrifice (for electrophysiological experiments) were used. Animals were anesthetized with isoflurane. An incision was made along the spine from S1 to L4 (DRG level). The L5 intervertebral foramen was visualized by exposing L5 and L4 transverse processes by separating the overlying back spine paraspinal muscles. The immune activator zymosan (2 mg/ml, 10 μ l, in

incomplete Freund's adjuvant) was slowly injected beneath the L5 intervertebral foramen, above the DRG, via a needle (30-G 1/2Prime;), which was bent into a 90 degree angle 1–2 mm from the tip. During injection, the bent part of needle was inserted into the intervertebral foramen and kept there for 1–2 minutes during and after injection to avoid leakage. This represented a simpler method than the previous model (Xie et al., 2006) which involved injecting the zymosan via a hole drilled through the transverse process. For sham operated animals used in some behavioral studies, surgery was done exactly as described above except for the final step of injecting zymosan.

Although larger, male animals were used for behavioral experiments for ease of the surgical manipulations and the lack of confounding hormonal cycles, the younger, female animals were used for electrophysiological recording because they were found to have less connective tissue, facilitating the microelectrode impalements.

In vivo local drug application to the DRG

In some experiments, riluzole (Tocris, Ellisville, MO, USA) or vehicle (artificial cerebrospinal fluid, ACSF) was applied locally to the inflamed DRG via a small glass pipette with a 40 μ m tip inserted beneath the perineurium of the L5 spinal nerve, connected via tubing to a 7-day osmotic pump with a flow rate of 1 μ l/h (Alzet, Cupertino, CA, USA). The pump was placed beneath the skin on the lower back. Construction of the pipette was based on a modification of the method for making microcapillary tubes for drug application beneath the perineurium of sciatic nerve previously described (Li et al., 2009). The insertion of the glass pipette through the perineurium was accomplished by first making a small hole in the perineurium with fine forceps. At this point, any damage to the underlying nerve (as occurred during a few of the first attempts of the procedure) was evidenced by bulging of nerve fibers out of the hole; in addition such animals ($n = 2-3$) showed evidence of motor damage (curling of the hind paw) after recovery from anesthesia. No animals with apparent motor damage were included in the study; however, these exclusions only occurred after the first attempts at the procedure. In addition, in all experiments using the osmotic pump delivery method, at the time animals were sacrificed it was confirmed that the pump had emptied and that the glass pipette was still in the proper position.

Behavior testing

Mechanical sensitivity was tested by applying a series of von Frey filaments to the heel region of the paw, using the up-and-down method (Chaplan et al., 1994). A cutoff value of 15 grams was assigned to animals that did not respond to the highest filament strength used. A wisp of cotton pulled up from, but still attached to a cotton swab was stroked mediolaterally across the plantar surface of the hindpaw to score the presence or absence of a brisk withdrawal response to a normally innocuous mechanical stimulus (light touch-evoked tactile allodynia). Measurement of rearing and exploratory behavior in a novel environment was conducted by taping animal behavior immediately after placing the animal in a 15.5" $\times$ 15.5" chamber, during the daytime but under dim red light illumination. Taping was done with web cameras attached to personal computers, with the experimenter absent from the room. Horizontal locomotion was measured by scoring the number of crossings in a 3 $\times$ 3 grid. The incidence and duration of rears was also scored during offline viewing of the videos. Gabapentin was obtained from TEVA pharmaceutical USA, Sellerville, PA; Naproxen was purchased from Sigma.

Electrophysiological recording in isolated whole DRG preparation

On postoperative day (POD) 3, or in normal DRG, intracellular microelectrode recording was performed on sensory neurons from an acutely isolated DRG preparation. This preparation also allows neurons to be recorded without enzymatic dissociation, with the

surrounding satellite glia cells and neighboring neurons intact, and allows determination of the conduction velocity. Small soluble factors (e.g. inflammatory mediators) are presumably washed away, but changes in intrinsic neuronal properties are preserved. The ganglion and attached dorsal and ventral roots were removed from the animal. The epineurium was removed and the ventral root was separated from dorsal root and teased off from the DRG. The ganglion, held in place in the recording chamber by three pieces of fine nylon thread, was mounted on the stage of an upright microscope (BX70-WI, Olympus). The DRG was continuously perfused with ACSF (in mM: NaCl 130, KCl 3.5, NaH₂PO₄ 1.25, NaHCO₃ 24, Dextrose 10, MgCl₂ 1.2, CaCl₂ 1.2, 16 mM HEPES, pH = 7.3, bubbled with 95% O₂/ 5% CO₂) at 36 – 37° C. Intracellular recordings were made from sensory neurons near the dorsal surface of the DRG using microelectrodes (40–60 MΩ) filled with 3M KCl, 10 mM HEPES, 10 mM EGTA (pH 7.5). The dorsal root was pulled into a suction electrode for extracellular stimulation to determine conduction velocity. Cells were classified by dorsal root conduction velocity as in a previous study (Stebbing et al., 1999): <1.2 m/s, C fiber; 1.2 – 7.5 m/s, Aδ; >7.5 m/s, Aαβ. For conduction velocities above ~20 m/sec the measurement was not considered accurate, due to the short conduction time overlapping with the duration of the applied stimulus; however, such cells could be clearly distinguished from C and Aδ cells and so were classified as Aαβ. Any spontaneous activity observed after impalement of the cell was recorded first, and re-confirmed at the end of the recording period. Input resistance and action potential (AP) parameters were measured with injected current pulses.

Data analysis

Comparison of values between different experimental groups was done using nonparametric methods for data that did not show a normal distribution based on the D'Agostino and Pearson omnibus normality test. The statistical test used in each case is indicated in the text or figure legend. Significance was ascribed for $p < 0.05$. Levels of significance are indicated by the number of symbols, e.g., *, $p = 0.01$ to <0.05 ; **, $p = 0.001$ to 0.01 ; ***, $p < 0.001$. Data are presented as average $\pm$ S.E.M.

RESULTS

Local DRG inflammation leads to long-lasting mechanical hypersensitivity that is partially relieved by naproxen and gabapentin

Local inflammation of the L5 DRG caused a pronounced ipsilateral mechanical hypersensitivity that could be observed as early as postoperative day 1 (POD1) and did not completely resolve even after 2 months (Fig. 1). Contralateral effects were observed on some days but were much weaker. Shaking, licking, or holding up of the ipsilateral hindpaw did not occur spontaneously and were observed only in response to von Frey stimulation; however it was noted that the rats often tended to shift their weight towards the contralateral paw at rest.

Although the data in Fig. 1 and all other figures showing behavioral data are based on experiments in male rats (150 – 200 gram), in separate experiments we confirmed that younger female animals (100 – 120 gram) such as were used for electrophysiological recordings showed a similar degree of mechanical sensitivity as the older male animals used for most behavioral experiments: females had a markedly reduced withdrawal threshold of 1.23 ± 0.28 grams ($N = 4$) 1 day after inflammation of the DRG, not statistically different from that shown for the larger males presented in Fig. 1. This reduced threshold was maintained at POD 3 (withdrawal threshold of 0.86 ± 0.11 grams), the time at which the electrophysiological recordings presented here were conducted.

In order to confirm the role of inflammatory processes in inducing the mechanical hypersensitivity, animals were tested on POD6 before and at 3 time points after administration of the anti-inflammatory drug naproxen (30 mg/kg, oral). As shown in Fig. 2, naproxen significantly reduced mechanical sensitivity at the 2 and 4 hour time points. Sensitivity was still significantly higher than the baseline value (dotted line in Fig. 2) observed before the LID surgery except for the 2 hour time point where the naproxen effect was maximal. Mechanical hypersensitivity could also be reduced by gabapentin, another drug used clinically for some chronic pain conditions (Fig. 2).

LID causes a reduction in exploratory rearing behavior

As a measure of a more complex behavior, we measured the effect of LID on the exploratory behaviors that are observed when a rat is introduced into a novel chamber. Both horizontal and vertical locomotive behaviors were elevated during the first few minutes after rats were introduced to a novel chamber, and then declined over the next 15 – 20 minutes (Fig 3). Horizontal locomotion was not significantly different between the LID and sham operated animals. However, rearing behavior was significantly reduced in LID animals; an effect which reached significance during the first 5 minute observation period when rearing is at its highest values. For these experiments LID animals were compared to sham operated animals to ensure that changes in behavior were not due to the presence of the incision and stitches along the back. In LID animals, the amount of rearing during the first 5 minute bin was shifted towards normal values by naproxen treatment.

Local DRG inflammation enhances neuronal excitability and spontaneous activity in A α β cells

Microelectrode recordings on POD3 showed that local DRG inflammation induced a striking increase in spontaneous activity in cells with A α β conduction velocities (Fig. 4). A α β cells also showed a significantly lower rheobase. The latter finding was accounted for by the large percentage of spontaneously active (SA) cells (in which rheobase was defined as zero); when considering only the non-SA A α β cells the decrease in rheobase was in the same direction but did not reach statistical significance (Normal: 1.52 ± 0.08 nA; LID: 1.27 ± 0.07 nA; $p = 0.09$, Mann-Whitney rank sum test). Spontaneous activity could be characterized as bursting, regular, or irregular as previously described (Zhang et al., 1999, Amir et al., 2002). The spontaneous activity observed after LID was predominantly of the bursting type as shown in Table 1. These SA A α β cells showed characteristic subthreshold membrane oscillations between bursts of action potentials (see example in Fig. 6)

Spontaneously active neurons have A α β conduction velocities but relatively smaller size

Of spontaneously active cells recorded on POD3, 92% were observed to have conduction velocities in the A α β range, compared to 59% of non-SA cells ($p < 0.001$, Fisher's exact test comparing A α β and non-A α β incidence in each group); Fig 5 top. For this comparison non-SA cells from normal DRG and POD3 DRG were combined, as their distributions were not significantly different. However, the spontaneously active A α β cells were clustered towards the smaller end of the size distribution for A α β cells (as estimated by membrane capacitance) – the very large A α β cells were rarely spontaneously active (Fig. 5, bottom). Due to the low incidence of SA cells in normal DRG, these cells were not included in this analysis. The average capacitance was smaller in spontaneously active A α β cells than in non-spontaneously active A α β cells (79.7 ± 3.1 pF vs. 109.6 ± 2.5 , $p < 0.001$, Mann-Whitney test), and the best fits of a Gaussian function to the capacitance distributions differed significantly between SA and non-SA cells ($p < 0.0001$); the fit to the SA cells distribution had a smaller mean (73.4 ± 2.5 vs. 103 ± 2.5) and smaller standard deviation (26.7 ± 2.5 vs. 37.0 ± 2.5) compared to the non-SA cells. Although the number of spontaneously active A δ cells from LID ganglia was small ($n = 6$; Table I), these cells also had a significantly smaller

membrane capacitance than non-spontaneously active A δ cells (52.9 ± 10.3 vs. 96.9 ± 4.2 pF, $p < 0.005$, Mann-Whitney test).

Spontaneously active A $\alpha\beta$ cells are more sensitive to riluzole

To determine if voltage-gated Na⁺ channels contributed to both the membrane oscillations and associated burst-firing after DRG inflammation, we applied tetrodotoxin (TTX; 500 nM) to bursting neurons at POD3. In all 6 A $\alpha\beta$ cells tested, spontaneous activity and underlying spontaneous oscillations were completely blocked by TTX (data not shown). In 4 out of 4 cells tested, action potentials evoked by current injection were also blocked by TTX. These experiments suggested that TTX-sensitive persistent Na currents might play an important role in generating spontaneous activity, as has been found in some other neuron types, as well as in DRG neurons in another pain model (see Discussion). We therefore next tested the effects of riluzole, a drug with anticonvulsant and neuroprotective properties that shows greater selectivity for persistent Na currents over other Na currents. For these experiments, spontaneous activity and evoked action potentials were examined in individual cells before and after riluzole application.

In all 16 spontaneously active A $\alpha\beta$ -cells tested, spontaneous activity and underlying membrane oscillations were blocked by 10 μ M riluzole (see example in Fig. 6, right panel). In this cell, the spontaneous activity was blocked 20 seconds after adding riluzole. The average time to block was 63 ± 10 seconds. This was not significantly different from the time required for TTX block (52 ± 12 seconds, $p = 0.84$) which may primarily reflect slow mixing in the recording chamber. A recovery of spontaneous activity could be observed but only following a prolonged washout (10 – 30 minutes) of the riluzole.

Although there were relatively few spontaneously active C cells even after DRG inflammation, in 2 experiments we were able to observe that the spontaneous activity in these cells was not affected by riluzole. An example is shown in Fig. 7. The spontaneous activity in this C cell was not blocked during a 15 minute exposure to riluzole.

In 12 of the 16 spontaneously active A $\alpha\beta$ -cells tested, riluzole not only blocked spontaneous activity, but also blocked the action potentials evoked by current stimulation: in these cells, either no action potential at all could be evoked ($n = 5$; see example in Figure 6, left panel), or the action potential evoked by current injection was so diminished that it would normally be considered an unhealthy evoked action potential (e.g. had a maximum voltage < -5 mV and maximum dV/dt < 80 V/s). An example of a SA cell that displayed only a diminished action potential after riluzole is given in Fig. 8 (top left); in this cell the action potential evoked by current stimulation after riluzole reached a peak voltage of -11 mV and had a maximum dV/dt value of 40 V/s. In contrast, only 1 of 15 non-SA A $\alpha\beta$ -cells in LID ganglia had diminished evoked action potentials in riluzole by these criteria ($p < 0.001$, Fisher's exact test), and 0 of 12 normal A $\alpha\beta$ -cells ($p < 0.001$). More generally, several action potential parameters were more strongly affected by riluzole in spontaneously active A $\alpha\beta$ -cells than in non-spontaneously active A $\alpha\beta$ -cells from either normal or LID ganglia. As shown in Figure 8 and Table 2, the riluzole induced changes in both maximum dV/dt and AP amplitude were greater in spontaneously active A $\alpha\beta$ cells than in non SA cells from either normal or LID ganglia. These differences were underestimated since values from the 5 SA cells that completely lacked evoked action potentials in riluzole could not be included. There were too few spontaneously active cells in normal animals, or SA C cells from LID animals, to include them in the statistical analysis for this series of experiments. Effects of riluzole on non SA C cells were generally even smaller than on non SA A $\alpha\beta$ cells, whether from normal or LID ganglia (Fig. 8; Table 2).

Although the somatic action potential evoked by intracellular current stimulation in A α β -cells was affected by riluzole to varying degrees as discussed above, it was often still possible to measure conduction velocities in the presence of riluzole (though in some instances the action potential evoked by dorsal root stimulation did not completely invade the soma). Conduction velocities could still be measured in 7 of 7 spontaneously active cells A α β -cells (including 4 in which the somatic action potential evoked by intracellular current stimulation was absent or not overshooting); in 11 of 11 non-spontaneously active A-cells from LID DRG, and in 10 of 10 non-spontaneously active A-cells from normal DRG. These results suggested that riluzole had relatively small effects on axonal sodium channels.

Riluzole applied locally to the DRG during the first week of inflammation reduces pain behaviors

The above results suggested that riluzole applied *in vivo* should preferentially reduce spontaneous activity of A α β sensory neurons in the LID model. In order to determine whether blocking spontaneous activity was anti-nociceptive, riluzole (50–200 μ M) was applied locally to the inflamed DRG *in vivo* for 7 days starting at the time of DRG inflammation (see Methods). Control animals had local perfusion of the inflamed DRG with vehicle (ACSF). As shown in Fig. 9 (top), local DRG perfusion with ACSF had no apparent anti-nociceptive effect; mechanical thresholds after inflammation of the DRG were similar to those seen with no perfusion (compare with Fig 1). However, local perfusion of the inflamed DRG with 200 μ M riluzole significantly reduced mechanical thresholds induced by LID, an effect which outlasted the duration of drug application by at least 3 weeks. The differences between LID animals perfused with ACSF vs. 200 μ M riluzole were significant ($p < 0.001$) at all days tested (except preoperative baseline). A lower dose (50 μ M) had an intermediate effect; at this dose mechanical thresholds were significantly higher than in ACSF-perfused LID animals on POD 2, 4, and 21; and significantly lower than in 200 μ M riluzole on all POD examined ($p < 0.05$ on POD 2, $p < 0.01$ on other days). On some POD (2, 4, 21, 28) the LID animals perfused with 200 μ M riluzole were not significantly different from the non-inflamed animals perfused with riluzole. Consistent with the relatively small effects of riluzole on excitability parameters in neurons from normal DRG, *in vivo* local perfusion of normal (non-inflamed) DRG had very minor effects on mechanical threshold which did not differ significantly from baseline on any of the days tested. Local DRG inflammation also increased the response to a normally innocuous stroking of the ipsilateral paw with a cotton wisp, a test for mechanical allodynia. In LID animals perfused with ACSF, the percent responding to a cotton wisp increased significantly from baseline POD 4 through 21 (Fishers exact test). Riluzole perfusion (200 μ M) also reduced responses in this test on some days tested (Fig. 9 bottom). In addition 4 younger female animals, of the size used for electrophysiological recordings, showed a response to the cotton wisp test and 2 of 4 showed a response to the acetone test, on POD 3.

DISCUSSION

We found that localized inflammation of the L5 DRG led to a robust increase in mechanical pain and a marked increase in spontaneous bursting activity in A α β cells. The data suggest an important role for spontaneous activity, especially bursting activity, in A α β cells in initiating chronic pain behaviors. Riluzole, which preferentially affected spontaneously active A α β cells, was also highly effective in suppressing pain behaviors when applied locally to the inflamed DRG. Although riluzole was not without effects on other classes of sensory neurons, its most striking effect was its ability to block activity in spontaneously active A α β cells after LID. Consistent with these electrophysiological observations, riluzole perfusion of normal DRG had no significant effect on behavioral responses to mechanical stimuli. This finding expands upon our previous study showing that early activity blockade

could reduce chronic pain behaviors in other pain models (Xie et al., 2005), as that study used local anesthetic or TTX which also blocked evoked activity as well as spontaneous activity. Some previous preclinical studies (Sung et al., 2003, Coderre et al., 2007) have demonstrated reduction of pain behaviors by systemic riluzole, but since riluzole also has effects on some CNS neurons its site of action in these studies is unknown. To our knowledge this is the first demonstration that riluzole can profoundly reduce pain behaviors through its local effects on primary sensory neurons.

It is of interest that in a different pain model, spinal nerve ligation, systemic corticosteroid administration reduced mechanical pain behaviors while having little effect on most of the measured excitability parameters including the overall incidence of spontaneous activity. However, the incidence of bursting form of spontaneous activity was significantly reduced by steroid treatment, suggesting that this particular form of spontaneous activity may be particularly important for pain behavior (Li et al., 2007).

Most nociceptors are C-cells. A number of mechanisms have been proposed by which spontaneous or evoked activity in large diameter low-threshold neurons might contribute to mechanical pain under pathological conditions, even though these neurons do not normally transmit pain signals (Price et al., 2005, Devor, 2009). In addition, some of the spontaneous activity may have occurred in myelinated nociceptors. In rat approximately 20% of A α β cells have nociceptive thresholds and response properties, and project into lamina I/II of the dorsal horn. This possibility would also be consistent with the narrower size distribution we observed for spontaneously active cells (Djouhri and Lawson, 2004, Woodbury et al., 2008). However, the incidence of spontaneous activity in A α β cells observed in our study was too high to be occurring only in myelinated nociceptors, and we did not observe evidence of ongoing spontaneous pain as might be expected from spontaneous activity in this population. Further studies are needed to characterize the origins and projections of the spontaneously active A α β cells observed in this and other pain models. However, the importance of spontaneous activity in A α β cells is highlighted by the anti-nociceptive action of riluzole, which preferentially blocks this spontaneous activity.

Both riluzole and TTX blocked spontaneous activity and underlying membrane oscillations in A α β cells, suggesting that a TTX-sensitive persistent Na current might play a key role in generation of spontaneous activity. Persistent or slowly inactivating TTX-sensitive Na currents have been demonstrated in bursting neurons in several CNS regions, where they are blocked by riluzole at lower doses than block the transient current/evoked action potential (Urbani and Belluzzi, 2000, Kononenko et al., 2004, Pena et al., 2004, Prescott and De Koninck, 2005, Sheroziya et al., 2009, Li and Bacceti, 2011). Persistent Na currents have also been previously demonstrated in DRG (Song et al., 1997, Baker and Bostock, 1998, Yang et al., 2009b). However, bursting behavior of DRG neurons differs from that observed in CNS neurons; the latter have a clearly bistable membrane potential with bursts of action potentials riding on a marked depolarizing plateau (compare to Fig. 6). Hence the details of the ionic mechanism for bursting may differ between sensory and CNS neurons. It was recently reported (Xie et al., 2011) that the chronic compression of the DRG model increased persistent Na current density in DRG neurons and that this current was blocked by riluzole (IC₅₀ 4 μ M), as were subthreshold oscillations and spontaneous activity including bursting. Unlike in our study, evoked action potentials were still observed in riluzole. This may reflect a difference between the two models, or might depend on using microelectrodes (this study) vs. patch clamp methods; spontaneous activity was only rarely observed using patch clamp methods in the chronic compression of the DRG study. In early postnatal rat DRG neurons, the rapidly inactivating Na current is 50% blocked by 3 μ M riluzole (Song et al., 1997) which would predict a greater sensitivity of the evoked action potential to riluzole, more like that observed in our study in SA neurons from adult, inflamed DRG. Given the

important roles of spontaneous activity in initiating and maintaining chronic pain, it will be important to elucidate the ionic mechanisms of spontaneous activity as possible therapeutic targets. Some of the above studies suggest that the ionic basis may differ somewhat between peripheral and central neurons, raising the possibility of developing blockers of pathological spontaneous activity in sensory neurons without untoward CNS side effects. This would be desirable given the presence of riluzole-sensitive persistent currents in normal CNS neurons.

It seems unlikely that observed behavioral effects of locally perfusing the DRG with riluzole were actually due to systemic effects of the drug acting at other sites, though we cannot eliminate the possibility. For the highest, most effective concentration of riluzole used for DRG perfusion in our study, the effective systemic dose was approximately 0.4 mg/kg/day. For comparison, in a study in which systemic riluzole given twice daily for 4 days reduced pain induced by the chronic constriction injury model, only partial effects were observed at 2mg/kg/day, which was not as effective as 8mg/kg/day (Sung et al., 2003); another study of systemic riluzole in rat pain models used doses in the range of 6 mg/kg/day (Coderre et al., 2007).

Abnormal spontaneous activity of sensory neurons occurs in a number of different pain models. The SA observed in this study using microelectrode methods confirms our previous study (Xie et al., 2006) in which local inflammation of the DRG led to spontaneous activity measured with fiber recording methods on POD3, 7, and 14. In both studies the highest incidence of spontaneous activity was observed in A α β cells, though the absolute value on POD3 was much higher in this study. This may be due to the different recording method used (fiber recording vs. microelectrode), or because the microelectrode method focuses on the neurons near the surface of the ganglion where inflammation effects may be greatest. Several agents shown to reduce or block spontaneous activity in vivo will also reduce or block the development of pain behaviors (Yoon et al., 1996, Boucher et al., 2000, Lyu et al., 2000, Chaplan et al., 2003, Sukhotinsky et al., 2004); however see (Suter et al., 2003). These studies suggest that the initial spontaneous activity plays an important role in initiating chronic pain.

Localized inflammation of the DRG also caused a reduction in rearing behavior induced by exposure to a novel chamber. Some recent studies have emphasized the importance of including more complex behavior measurements to complement the conventional reflex-based tests; it is argued that such measurements may better capture the complexities of human pain conditions involving supraspinal centers (Negus et al., 2006, Matson et al., 2010). Reduction of exploratory behavior has been proposed as one such complex behavior that is may be affected by pain conditions. Vertical (rearing) and horizontal locomotion are differentially affected in different pain models (Houghton et al., 1997, Martin et al., 2004, Matson et al., 2007) and may show different pharmacological specificity (Michael-Titus et al., 1989).

The method for implementing the LID pain model presented in this study differs only in minor detail from our previously described version (Xie et al., 2006); in both the L5 DRG was inflamed by the immune stimulators zymosan and incomplete Freund's adjuvant. The present model is easier to implement because the zymosan is deposited with a small needle into the intervertebral foramen instead of drilling a small hole into the junction of the transverse process and the lamina overlying the DRG. Both versions of the model give long-lasting mechanical hypersensitivity; in the present study we have extended the measurements to 60 days, at which time the enhanced sensitivity is beginning to resolve. Both versions of the model induced mechanical allodynia as measured by the cotton wisp test. These LID models provide a way to study the effects of inflammation on sensory neurons in the absence of overt axonal injury. Since most neuropathic pain models involve

both axon injury and some type of inflammation, a model which isolates inflammatory processes from axon injury is of general interest. In addition, these models may have direct relevance to conditions with localized inflammation of the DRGs, such as some forms of low back pain. Finally, the data suggest an important role for high-frequency and/or bursting activity which may be relevant to other types of pain models.

Acknowledgments

Supported by NIH grants NS55860 and NS45594. We thank Dr. Mark Baccei for commenting on the manuscript.

Abbreviations used

ACSF	artificial cerebrospinal fluid
DRG	dorsal root ganglion
LID	local inflammation of the DRG
POD	postoperative day
SA	spontaneously active
TTX	tetrodotoxin

References

- Ali Z, Ringkamp M, Hartke TV, Chien HF, Flavahan NA, Campbell JN, Meyer RA. Uninjured C-fiber nociceptors develop spontaneous activity and alpha-adrenergic sensitivity following L6 spinal nerve ligation in monkey. *J Neurophysiol.* 1999; 81:455–466. [PubMed: 10036297]
- Amir R. Membrane potential oscillations in dorsal root ganglion neurons: role in normal electrogenesis and neuropathic pain. *Journal of Neuroscience.* 1999; 19:8589–8596. [PubMed: 10493758]
- Amir R, Michaelis M, Devor M. Burst discharge in primary sensory neurons: triggered by subthreshold oscillations, maintained by depolarizing afterpotentials. *J Neurosci.* 2002; 22:1187–1198. [PubMed: 11826148]
- Baker MD, Bostock H. Inactivation of macroscopic late Na⁺ current and characteristics of unitary late Na⁺ currents in sensory neurons. *J Neurophysiol.* 1998; 80:2538–2549. [PubMed: 9819261]
- Bedi SS, Yang Q, Crook RJ, Du J, Wu Z, Fishman HM, Grill RJ, Carlton SM, Walters ET. Chronic spontaneous activity generated in the somata of primary nociceptors is associated with pain-related behavior after spinal cord injury. *J Neurosci.* 2010; 30:14870–14882. [PubMed: 21048146]
- Berger JV, Knaepen L, Janssen SP, Jaken RJ, Marcus MA, Joosten EA, Deumens R. Cellular and molecular insights into neuropathy-induced pain hypersensitivity for mechanism-based treatment approaches. *Brain Res Rev.* 2011; 67:282–310. [PubMed: 21440003]
- Beuche W, Friede RL. The role of non-resident cells in Wallerian degeneration. *Journal of Neurocytology.* 1984; 13:767–796. [PubMed: 6512566]
- Boucher TJ, Okuse K, Bennett DL, Munson JB, Wood JN, McMahon SB. Potent analgesic effects of GDNF in neuropathic pain states. *Science.* 2000; 290:124–127. [PubMed: 11021795]
- Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL. Quantitative assessment of tactile allodynia in the rat paw. *Journal of Neuroscience Methods.* 1994; 53:55–63. [PubMed: 7990513]
- Chaplan SR, Guo HQ, Lee DH, Luo L, Liu C, Kuei C, Velumian AA, Butler MP, Brown SM, Dubin AE. Neuronal hyperpolarization-activated pacemaker channels drive neuropathic pain. *J Neurosci.* 2003; 23:1169–1178. [PubMed: 12598605]
- Coderre TJ, Kumar N, Lefebvre CD, Yu JS. A comparison of the glutamate release inhibition and anti-allodynic effects of gabapentin, lamotrigine, and riluzole in a model of neuropathic pain. *J Neurochem.* 2007; 100:1289–1299. [PubMed: 17241130]
- Devor M. Ectopic discharge in Abeta afferents as a source of neuropathic pain. *Exp Brain Res.* 2009; 196:115–128. [PubMed: 19242687]

- Djoughri L, Lawson SN. Abeta-fiber nociceptive primary afferent neurons: a review of incidence and properties in relation to other afferent A-fiber neurons in mammals. *Brain Res Brain Res Rev.* 2004; 46:131–145. [PubMed: 15464202]
- Eckert A, Segond von Banchet G, Sopper S, Petersen M. Spatio-temporal pattern of induction of bradykinin receptors and inflammation in rat dorsal root ganglia after unilateral nerve ligation. *Pain.* 1999; 83:487–497. [PubMed: 10568857]
- Eliav E, Benoliel R, Herzberg U, Kalladka M, Tal M. The role of IL-6 and IL-1beta in painful perineural inflammatory neuritis. *Brain Behav Immun.* 2009; 23:474–484. [PubMed: 19486649]
- Govrin-Lippmann R, Devor M. Ongoing activity in severed nerves: source and variation with time. *Brain Research.* 1978; 159:406–410. [PubMed: 215270]
- Houghton AK, Kadura S, Westlund KN. Dorsal column lesions reverse the reduction of homecage activity in rats with pancreatitis. *Neuroreport.* 1997; 8:3795–3800. [PubMed: 9427373]
- Hu P, McLachlan EM. Macrophage and lymphocyte invasion of dorsal root ganglia after peripheral nerve lesions in the rat. *Neuroscience.* 2002; 112:23–38. [PubMed: 12044469]
- Hu SJ, Xing JL. An experimental model for chronic compression of dorsal root ganglion produced by intervertebral foramen stenosis in the rat. *Pain.* 1998; 77:15–23. [PubMed: 9755014]
- Kawakami M, Tamaki T, Weinstein JN, Hashizume H, Nishi H, Meller ST. Pathomechanism of pain-related behavior produced by allografts of intervertebral disc in the rat. *Spine.* 1996; 21:2101–2107. [PubMed: 8893434]
- Kononenko NI, Shao LR, Dudek FE. Riluzole-sensitive slowly inactivating sodium current in rat suprachiasmatic nucleus neurons. *J Neurophysiol.* 2004; 91:710–718. [PubMed: 14573554]
- Li H, Xie W, Strong JA, Zhang J-M. Systemic antiinflammatory corticosteroid reduces mechanical pain behavior, sympathetic sprouting, and elevation of proinflammatory cytokines in a rat model of neuropathic pain. *Anesthesiology.* 2007; 107:469–477. [PubMed: 17721250]
- Li J, Baccei ML. Pacemaker Neurons within Newborn Spinal Pain Circuits. *J Neurosci.* 2011; 31:9010–9022. [PubMed: 21677184]
- Li J, Walker SM, Fitzgerald M, Baccei ML. Activity-dependent modulation of glutamatergic signaling in the developing rat dorsal horn by early tissue injury. *J Neurophysiol.* 2009; 102:2208–2219. [PubMed: 19675290]
- Liu CN, Michaelis M, Amir R, Devor M. Spinal nerve injury enhances subthreshold membrane potential oscillations in DRG neurons: relation to neuropathic pain. *Journal of Neurophysiology.* 2000a; 84:205–215. [PubMed: 10899197]
- Liu X, Eschenfelder S, Blenk KH, Janig W, Habler H. Spontaneous activity of axotomized afferent neurons after L5 spinal nerve injury in rats. *Pain.* 2000b; 84:309–318. [PubMed: 10666536]
- Lu X, Richardson PM. Responses of macrophages in rat dorsal root ganglia following peripheral nerve injury. *Journal of Neurocytology.* 1993; 22:334–341. [PubMed: 8315414]
- Lund JP, Sadeghi S, Athanassiadis T, Caram Salas N, Auclair F, Thivierge B, Arsenaault I, Rompre P, Westberg KG, Kolta A. Assessment of the potential role of muscle spindle mechanoreceptor afferents in chronic muscle pain in the rat masseter muscle. *PLoS One.* 2010; 5:e11131. [PubMed: 20559566]
- Lyu YS, Park SK, Chung K, Chung JM. Low dose of tetrodotoxin reduces neuropathic pain behaviors in an animal model. *Brain Research.* 2000; 871:98–103. [PubMed: 10882788]
- Martin TJ, Buechler NL, Kahn W, Crews JC, Eisenach JC. Effects of laparotomy on spontaneous exploratory activity and conditioned operant responding in the rat: a model for postoperative pain. *Anesthesiology.* 2004; 101:191–203. [PubMed: 15220791]
- Matson DJ, Broom DC, Carson SR, Baldassari J, Kehne J, Cortright DN. Inflammation-induced reduction of spontaneous activity by adjuvant: A novel model to study the effect of analgesics in rats. *J Pharmacol Exp Ther.* 2007; 320:194–201. [PubMed: 17050782]
- Matson DJ, Broom DC, Cortright DN. Locomotor activity in a novel environment as a test of inflammatory pain in rats. *Methods Mol Biol.* 2010; 617:67–78. [PubMed: 20336414]
- Michael-Titus A, Dourmap N, Costentin J. MU and delta opioid receptors control differently the horizontal and vertical components of locomotor activity in mice. *Neuropeptides.* 1989; 13:235–242. [PubMed: 2546089]

- Negus SS, Vanderah TW, Brandt MR, Bilsky EJ, Becerra L, Borsook D. Preclinical assessment of candidate analgesic drugs: recent advances and future challenges. *J Pharmacol Exp Ther*. 2006; 319:507–514. [PubMed: 16751251]
- Olmarker K, Myers RR. Pathogenesis of sciatic pain: role of herniated nucleus pulposus and deformation of spinal nerve root and dorsal root ganglion. *Pain*. 1998; 78:99–105. [PubMed: 9839819]
- Pan HL, Eisenach JC, Chen SR. Gabapentin suppresses ectopic nerve discharges and reverses allodynia in neuropathic rats. *Journal of Pharmacology & Experimental Therapeutics*. 1999; 288:1026–1030. [PubMed: 10027839]
- Pena F, Parkis MA, Tryba AK, Ramirez JM. Differential contribution of pacemaker properties to the generation of respiratory rhythms during normoxia and hypoxia. *Neuron*. 2004; 43:105–117. [PubMed: 15233921]
- Perry VH, Brown MC. Role of macrophages in peripheral nerve degeneration and repair. *Bioessays*. 1992; 14:401–406. [PubMed: 1323962]
- Prescott SA, De Koninck Y. Integration time in a subset of spinal lamina I neurons is lengthened by sodium and calcium currents acting synergistically to prolong subthreshold depolarization. *J Neurosci*. 2005; 25:4743–4754. [PubMed: 15888650]
- Price TJ, Cervero F, de Koninck Y. Role of cation-chloride-cotransporters (CCC) in pain and hyperalgesia. *Curr Top Med Chem*. 2005; 5:547–555. [PubMed: 16022677]
- Schon F, Mayer ML, Kelly JS. Pathogenesis of post-herpetic neuralgia. *Lancet*. 1987; 2:366–368. [PubMed: 2886824]
- Sheroziya MG, von Bohlen Und Halbach O, Unsicker K, Egorov AV. Spontaneous bursting activity in the developing entorhinal cortex. *J Neurosci*. 2009; 29:12131–12144. [PubMed: 19793971]
- Song JH, Huang CS, Nagata K, Yeh JZ, Narahashi T. Differential action of riluzole on tetrodotoxin-sensitive and tetrodotoxin-resistant sodium channels. *J Pharmacol Exp Ther*. 1997; 282:707–714. [PubMed: 9262334]
- Song XJ, Hu SJ, Greenquist KW, Zhang J-M, LaMotte RH. Mechanical and thermal hyperalgesia and ectopic neuronal discharge after chronic compression of dorsal root ganglia. *Journal of Neurophysiology*. 1999; 82:3347–3358. [PubMed: 10601466]
- Sorkin LS, Yaksh TL. Behavioral models of pain states evoked by physical injury to the peripheral nerve. *Neurotherapeutics*. 2009; 6:609–619. [PubMed: 19789066]
- Stebbing MJ, Eschenfelder S, Habler HJ, Acosta MC, Janig W, McLachlan EM. Changes in the action potential in sensory neurones after peripheral axotomy in vivo. *Neuroreport*. 1999; 10:201–206. [PubMed: 10203309]
- Study RE, Kral MG. Spontaneous action potential activity in isolated dorsal root ganglion neurons from rats with a painful neuropathy. *Pain*. 1996; 65:235–242. [PubMed: 8826512]
- Sukhotinsky I, Ben-Dor E, Raber P, Devor M. Key role of the dorsal root ganglion in neuropathic tactile hypersensitivity. *Eur J Pain*. 2004; 8:135–143. [PubMed: 14987623]
- Sung B, Lim G, Mao J. Altered expression and uptake activity of spinal glutamate transporters after nerve injury contribute to the pathogenesis of neuropathic pain in rats. *J Neurosci*. 2003; 23:2899–2910. [PubMed: 12684477]
- Suter MR, Papaloizos M, Berde CB, Woolf CJ, Gilliard N, Spahn DR, Decosterd I. Development of neuropathic pain in the rat spared nerve injury model is not prevented by a peripheral nerve block. *Anesthesiology*. 2003; 99:1402–1408. [PubMed: 14639156]
- Urbani A, Belluzzi O. Riluzole inhibits the persistent sodium current in mammalian CNS neurons. *Eur J Neurosci*. 2000; 12:3567–3574. [PubMed: 11029626]
- Wang JG, Strong JA, Xie W, Yang RH, Coyle DE, Wick DM, Dorsey ED, Zhang JM. The chemokine CXCL1/growth related oncogene increases sodium currents and neuronal excitability in small diameter sensory neurons. *Mol Pain*. 2008; 4:38. [PubMed: 18816377]
- Woodbury CJ, Kullmann FA, McIlwrath SL, Koerber HR. Identity of myelinated cutaneous sensory neurons projecting to nociceptive laminae following nerve injury in adult mice. *J Comp Neurol*. 2008; 508:500–509. [PubMed: 18335545]
- Xie RG, Zheng DW, Xing JL, Zhang XJ, Song Y, Xie YB, Kuang F, Dong H, You SW, Xu H, Hu SJ. Blockade of persistent sodium currents contributes to the riluzole-induced inhibition of

- spontaneous activity and oscillations in injured DRG neurons. *PLoS One*. 2011; 6:e18681. [PubMed: 21541342]
- Xie W, Strong JA, Meij JT, Zhang J-M, Yu L. Neuropathic pain: Early spontaneous afferent activity is the trigger. *Pain*. 2005; 116:243–256. [PubMed: 15964687]
- Xie WR, Deng H, Li H, Bowen TL, Strong JA, Zhang J-M. Robust increase of cutaneous sensitivity, cytokine production and sympathetic sprouting in rats with localized inflammatory irritation of the spinal ganglia. *Neuroscience*. 2006; 142:809–822. [PubMed: 16887276]
- Xu J, Brennan TJ. Guarding pain and spontaneous activity of nociceptors after skin versus skin plus deep tissue incision. *Anesthesiology*. 2010; 112:153–164. [PubMed: 19996955]
- Yang RH, Strong JA, Zhang JM. NF-kappaB mediated enhancement of potassium currents by the chemokine CXCL1/growth related oncogene in small diameter rat sensory neurons. *Mol Pain*. 2009a; 5:26. [PubMed: 19476648]
- Yang RH, Wang WT, Chen JY, Xie RG, Hu SJ. Gabapentin selectively reduces persistent sodium current in injured type-A dorsal root ganglion neurons. *Pain*. 2009b; 143:48–55. [PubMed: 19269740]
- Yoon YW, Na HS, Chung JM. Contributions of injured and intact afferents to neuropathic pain in an experimental rat model. *Pain*. 1996; 64:27–36. [PubMed: 8867245]
- Zhang J-M, Song XJ, LaMotte RH. Enhanced excitability of sensory neurons in rats with cutaneous hyperalgesia produced by chronic compression of the dorsal root ganglion. *Journal of Neurophysiology*. 1999; 82:3359–3366. [PubMed: 10601467]

Highlights

- Pain behaviors are observed soon after local inflammation of the sensory ganglia.
- Inflamed DRG have very high rates of spontaneous activity with bursting pattern especially in cells with A α β fibers.
- The spontaneously active cells, but not C cells, are very sensitive to riluzole.
- Local application of riluzole to the inflamed DRG greatly reduces pain behaviors.
- Results suggest an important role of A α β cells in initiating the pain state.

Fig. 1.

Effect of localized DRG inflammation on mechanical sensitivity as assayed with von Frey filaments. Baseline measurement (plotted on POD 0) is the average of measurements made on 2 separate days prior to the surgery. Normal, unoperated animals showed a stable baseline over the same time course (open symbols). *, significant difference between normal and LID animals (repeated measures ANOVA with Bonferroni posttest). N = 6 – 8 animals per group; all ipsilateral data points for LID animals were significantly different ($p < 0.001$) from normal animals except for the baseline point.

Fig. 2.

Effect of oral naproxen and gabapentin on ipsilateral mechanical hypersensitivity induced by LID. Top: Six days after local inflammation of the DRG, rats were tested before (time = 0), 1, 2, and 4 hours after oral administration of naproxen (30 mg/kg). N = 8 (different animals from those presented in Fig. 1 or 3). Bottom: Effect of oral administration gabapentin (100 mg/kg in 0.5% carboxymethylcellulose and 0.2% Tween 80) on mechanical hypersensitivity tested with a similar protocol 7 days after LID. N = 6. Administration of vehicle had no effect (N = 8, $p = 0.82$ for comparison to pre-drug value, data not shown). *, significant difference from time 0 (before drug administration); #, significant difference from baseline value before surgery (dotted lines) (Friedman test with Dunn's posttest).

Figure 3.

Effect of LID on exploratory behaviors in a novel chamber. Three days after LID or sham surgery, rats were placed individually into a 15.5" × 15.5" chamber. The floor of the chamber was divided into a 3×3 grid. The number of crossings per five minute interval is shown on the top. The number of times the animals reared is shown in the middle panel. *, significant difference between LID and sham groups (2 way repeated measures ANOVA with Bonferroni posttest). N = 8 per group. Bottom: Treatment with naproxen (30 mg/kg, oral, starting 1 hour before exposure to the novel chamber) partially restored rearing behavior during the first 5 minute interval in LID rats. N = 8 per group. *, significant

difference between LID and LID + naproxen groups (2-way (middle) and 1-way (bottom) ANOVA with Bonferroni posttest).

Fig. 4.

Spontaneous activity is increased by LID. Top: percentage of cells in each conduction velocity class showing spontaneous activity. Value was zero in C and A δ cells from normal animals. Trend towards higher SA in A δ cells did not reach significance, $p = 0.08$). ***, significant difference, LID vs. normal A $\alpha\beta$ cells (Fishers exact test). Bottom: average rheobase in each class of cells. ***, significant difference between LID and normal A $\alpha\beta$ cells (Mann-Whitney Rank Sum test), $p < 0.001$

Figure 5.

Characteristics of spontaneously active cells. Top: distribution of conduction velocities in SA cells (from LID ganglia on POD3) and non-SA cells (combined from both LID and normal ganglia). Bin size (1.2 m/s) was manually determined so that all C fibers were represented as the first bin. Bottom: membrane capacitance distribution histogram for Aαβ cells in SA and non-SA cells. Non-SA cells from normal and LID ganglia were combined; SA cells from normal DRG (n = 7) were omitted from the analysis.

Fig 6.

Example of riluzole effects on a spontaneously active $A\alpha\beta$ cell from an LID ganglion. Left, expanded view of action potentials observed in response to injection current. In this cell, no action potential could be evoked after riluzole. Right – recordings of spontaneous activity on a slower time base. Note subthreshold oscillations between the bursts of action potentials.

Fig. 7. Example of a spontaneously active C cell that was not blocked by riluzole during 15 minutes of exposure. Spontaneous AP shown on an expanded time scale on the left; samples of the spontaneous activity are shown on the right.

Fig. 8.

Examples of action potentials before (solid trace) and after (dotted trace) application of 10 μ M riluzole. Each pair of traces is taken from a single cell. A α β cells are on the left, C cells are on the right (note different time scale that applies to all C cells). Top left: in a SA cell from an LID animal, the action potential evoked in riluzole by current stimulation (0.4 nA) was greatly diminished compared to the spontaneous action potential (rheobase defined as zero). Non-SA cells from LID animals (middle) or normal animals (bottom) showed weaker effects of riluzole on the evoked action potential. Rheobase is indicated to the right of each trace. Right: C cells were not as strongly affected by riluzole. Averaged data are shown in Table 2.

Figure 9.

Local perfusion of the DRG with riluzole reduces mechanical hyperalgesia (top) and light stroke-evoked tactile allodynia (bottom) induced by LID. The 7-day osmotic pump was implanted on day 0 at the time of L5 DRG inflammation (LID animals); normal animals had the pump placed on POD 0 but no zymosan/incomplete Freund's adjuvant injection onto the DRG. For clarity symbols showing significance of comparisons between groups (repeated measures ANOVA with Bonferroni posttest) in the top panel have been omitted; See text for significance of LID-induced responses in ACSF-perfused animals compared to baseline (bottom panel). *, significant difference between riluzole 200 μ M group and ACSF-perfused group. N = 8 – 10 animals per group.

TABLE 1

Changes in spontaneous activity after inflammation of the DRG

Conditions	C.V.	Firing patterns			Total SA	Non-SA
		Bursting	Irregular	Regular		
Normal	Aαβ	5 (4%)	1 (1%)	1 (1%)	7 (6%)	114 (94%)
LID POD 3	Aαβ	81 (35%)	22 (9%)	0 (0%)	103 (44%)	129 (56%)
Normal	Aδ	0 (0%)	0 (0%)	0 (0%)	0 (0%)	23 (100%)
LID POD 3	Aδ	2 (5%)	4 (10%)	0 (0%)	6 (15%)	34 (85%)
Normal	C	0 (0%)	0 (0%)	0 (0%)	0 (0%)	60 (100%)
LID POD 3	C	2 (3%)	1 (1%)	0 (0%)	3 (4%)	76 (96%)

Number and percentages (in parentheses) of spontaneously active (SA) cells of each firing pattern, classified by conduction velocity class, from normal DRG, or DRG isolated 3 days or 7 to 10 days ("POD 8") after local inflammation of the DRG (LID). For p values see Fig. 4 and text.

TABLE 2

Effects of riluzole on action potential parameters

	A α β cells			C cells		
	before riluzole	after riluzole	change	before riluzole	after riluzole	change
dV/dt, V/s						
SA cells, LID	286.4 $\pm$ 28.4	92.3 $\pm$ 16.3 ^{\$\$\$}	-194.1 $\pm$ 23.7	-	-	-
non SA cells, LID	408.5 $\pm$ 12.7	291.2 $\pm$ 19.9 ^{\$\$\$}	-117.3 $\pm$ 15.3 *	216.2 $\pm$ 28.1	203.0 $\pm$ 22.0	-13.2 $\pm$ 15.4
non SA cells, normal	360.5 $\pm$ 25.5	257.8 $\pm$ 27.8 ^{\$}	-102.7 $\pm$ 24.1 *	249.8 $\pm$ 9.2	193.6 $\pm$ 9.6 ^{\$\$\$}	-56.2 $\pm$ 8.9 ##
AP amplitude, mV						
SA cells, LID	67.0 $\pm$ 2.3	34.2 $\pm$ 2.9 ^{\$}	-32.8 $\pm$ 3.0	-	-	-
non SA cells, LID	78.5 $\pm$ 2.0	63.6 $\pm$ 2.6 ^{\$\$\$}	-14.9 $\pm$ 2.8 **	68.4 $\pm$ 3.7	63.9 $\pm$ 3.0	-4.5 $\pm$ 1.9
non SA cells, normal	74.5 $\pm$ 1.6	59.2 $\pm$ 3.6 ^{\$\$\$}	-15.3 $\pm$ 3.5 **	71.4 $\pm$ 2.3	61.8 $\pm$ 2.6 ^{\$\$\$}	-9.6 $\pm$ 1.7
Rheobase, nA						
SA cells, LID	0.0 $\pm$ 0.0	0.4 $\pm$ 0.0 ^{\$\$\$}	0.4 $\pm$ 0.0	-	-	-
non SA cells, LID	1.9 $\pm$ 0.2	3.1 $\pm$ 0.3 ^{\$\$\$}	1.2 $\pm$ 0.3	0.6 $\pm$ 0.1	0.7 $\pm$ 0.2	0.1 $\pm$ 0.0
non SA cells, normal	1.3 $\pm$ 0.3	1.8 $\pm$ 0.5 ^{\$}	0.5 $\pm$ 0.3	1.1 $\pm$ 0.2	1.6 $\pm$ 0.3 ^{\$}	0.4 $\pm$ 0.1

All data are from individual cells in which action potentials were measured before and after 10 μ M riluzole application.

* significantly smaller change than SA cells (Kruskal-Wallis test with Dunn's posttest). None of the values in the "change" column was significantly different comparing non SA A α β cells from LID vs. normal DRG.

significant difference between LID and normal cells (Mann-Whitney test).

\$ significant difference between "before riluzole" and "after riluzole" values (paired t-test or Wilcoxon signed rank test). There were not enough spontaneously active C cells from LID DRG, or spontaneously active cells of either conduction velocity class from normal DRG, to include in the experiments. Rheobase in SA cells was defined as zero. Spontaneously active A α β cells in which no action potential at all could be evoked in riluzole (n = 5 of 16; see text) were not included in the analysis; these would have enhanced the differences in all the parameters. N values: A α β cells, 11 SA LID cells, 24 non SA LID cells, 12 non SA normal cells. C cells: 10 cells in each group.