The Effect of Amitriptyline on Ectopic Discharge of Primary Afferent Fibers in the L5 Dorsal Root in a Rat Model of Neuropathic Pain

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BACKGROUND: The sodium channel blocker amitriptyline has been shown to inhibit ectopic discharge in injured nerves. In the present study, we characterized ectopic discharges of afferent fibers following L5/L6 spinal nerve ligation (SNL) by their electrophysiological properties and sensitivities to inhibition by amitriptyline in the decentralized L5 dorsal root in SNL rats.

METHODS: Rats exhibiting withdrawal thresholds <4.0 g after SNL were selected for the present study. After laminectomy in pentobarbital-anesthetized rats, the L5 dorsal root was decentralized close to its entry to the spinal cord, and the spontaneous activities of single units were recorded peripherally before and after IV administration of amitriptyline. The mean frequency of afferent fiber activity and instantaneous frequency were measured.

RESULTS: The spontaneous activities of afferent fibers in naïve rats had high frequency (35.23 ± 6.63 Hz) and pattern discharge based on their instantaneous frequencies and interspike interval distributions. In rats that had received SNL, afferent fibers exhibited spontaneous discharge (mean of 11.05 ± 3.66 Hz) with an irregular discharge pattern or short bursting activity in some cases. Only 5/13 (38%) afferent fibers from naïve rats showed reduced spontaneous activities after amitriptyline (2 mg/kg, IV), whereas amitriptyline significantly inhibited ectopic discharge in 13/18 (72%) afferent fibers from SNL rats (ID_{50} = 1.66 ± 0.17 mg/kg). Furthermore, the greatest inhibitory effect of amitriptyline was consistently observed on those afferent fibers exhibiting low frequency (<20 Hz) and/or bursting discharge.

CONCLUSION: These results provide direct evidence that amitriptyline, which is used clinically for the treatment of neuropathic pain, selectively inhibits ectopic discharge of low frequency and bursting discharge in the rat neuropathic pain model.

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After peripheral nerve injury, primary afferent sensory fibers generate continuing discharges of ectopic origin (ectopic discharge), which are believed to trigger central sensitization and persistent pain.[1] Ligation of the L5 spinal nerve is a often used model of neuropathic pain in rats that results in four to sixfold increase in the prevalence of spontaneous ectopic discharges in sensory A-fibers.[2] Although the specific fiber types involved in the generation of central sensitization after neuropathy are a subject of debate,[3] studies tend to assume that inhibitory effects of test compounds on ectopic discharges recorded from afferent fibers in rat models of neuropathic pain indicate the therapeutic potential of compounds in neuropathic pain.[4,5] In the present study, we sought to characterize the properties of these afferent fibers in naïve rats and rats that had received L5/L6 spinal nerve ligation (SNL) by examining: 1) spontaneous activity frequencies, 2) discharge patterns based on instantaneous frequencies and interspike intervals, and 3) pharmacological sensitivity to amitriptyline. Although changes in mean frequency of afferent fiber discharge have been traditionally used to measure effects on fiber activity, the temporal pattern of fiber discharge has been proposed to be important in nociceptive transmission in a rat model of painful peripheral neuropathy.[6]

To quantitatively investigate this, we analyzed the instantaneous frequency of afferent fibers and classified these fibers based on their interspike interval histograms. Additionally, effects of amitriptyline on spontaneous afferent fiber activity were determined. Amitriptyline is a monoamine reuptake inhibitor and sodium channel blocker and is considered one of the first options for the management of neuropathic pain.[7,8] In rat neuropathic pain models, amitriptyline has previously been shown to inhibit ectopic discharge and behavioral hypersensitivity.[5,9–11] We specifically examined the effects of amitriptyline on the different populations of afferent fibers as defined by

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the criteria described above. The results demonstrate that amitriptyline is most effective in inhibiting activity of the low frequency, irregular bursting discharge fibers found in rats after SNL.

METHODS

The experimental protocol was approved by the Institutional Animal Care and Use Committee of Merck & Co., West Point, PA.

Neuropathic Pain Model-SNL

Male Sprague-Dawley rats weighing 350–450 g (Taconic Farms, PA) were used. All animals were tested for baseline withdrawal thresholds to mechanical stimulus. Rats were placed on a wire mesh screen, and a series of calibrated von Frey filaments with differing forces were applied to the plantar surface of the hindpaw until a withdrawal response was observed. The withdrawal threshold (g) of the paw was recorded using the "up-down" method.[12] Only animals exhibiting thresholds 15 g or more were selected for SNL surgery. Seven to 14 days after surgery, rats were tested as described above for decreased hindpaw withdrawal thresholds (allodynia), and only those rats exhibiting withdrawal thresholds not exceeding 4.0 g were selected for nerve recording. Rats which did not meet these criteria were immediately euthanized.

According to the method of Kim and Chung,[13] nerve ligation was done at L5/L6 spinal nerves, the distal process of the dorsal root ganglion. Rats were anesthetized with isoflurane, and the dorsal thoracic surface was shaved. The skin was aseptically prepped with betadine/alcohol wipes and a dorsal midline incision was made from approximately spinal nerve L3 to S2. A combination of sharp and blunt dissection was used to expose the left L6/S1 posterior interarticular process. The L6 transverse process was visualized and removed, and the left L4 and L5 spinal nerves were then exposed distal to their emergence from the intervertebral foramina. The L5 nerve was tightly ligated with 6-0 silk suture. The L6 nerve was located caudal and medial to the sacroiliac junction and tightly ligated with 6-0 suture. The muscle was closed with 4-0 absorbable suture and the skin closed with wound clips. All animals undergoing any of the above surgeries were immediately housed in cages with clean and soft bedding and monitored for any signs of possible distress.

Recording of Afferent Nerve Action Potentials

Rats were anesthetized initially with 40–45 mg/kg IP sodium pentobarbital (Nembutal®, Abbott Laboratories, Chicago, IL). Anesthesia was maintained by IV infusion of pentobarbital (5–10 $mg \cdot kg^{-1} \cdot h^{-1}$). The right femoral artery and vein were catheterized for measurement of arterial blood pressure and administration of drugs, respectively. The trachea was also cannulated. Some rats were paralyzed with IV pancuronium bromide (1 mg/kg), and their lungs subsequently ventilated with room air (55–60 strokes/min, 3–4 mL stroke volume). Mean arterial blood pressure was monitored continuously and was maintained above 80 mm Hg by IV injection of 5% dextrose in saline given in a bolus of 1–1.5 mL as required. Core body temperature was maintained at 36°C by a hot water circulating heating pad placed under the rat with feedback-controlled system (thermoprobe inserted into the thoracic esophagus). The lumbosacral spinal cord was exposed by laminectomy (T13-S2). The dura was carefully removed and the spinal cord was covered with warm (37°C) mineral oil.

The L5 dorsal root, the proximal process of the dorsal root ganglion was decentralized close to its entry to the spinal cord. Recordings were made from the distal cut end of the central processes of primary afferent fibers. The dorsal rootlet was split into thin bundles and a fine filament was isolated from the bundle to obtain a single unit. Electrical activity of the single unit was recorded by placing the teased fiber over one arm of a bipolar platinum electrode. A fine strand of connective tissue was placed across the other pole of the electrode as a balance. Action potentials were monitored continuously by analog delay and displayed on a storage oscilloscope after initial amplification through a low-noise AC differential amplifier. The action potentials were processed through a window discriminator and counted using the spike2/CED 1401 data acquisition program (CED, Cambridge, England). Peristimulus time histograms (1-s binwidth), raw action potentials, and arterial blood pressure were displayed on-line continuously. In some experiments, two or three afferent fibers were recorded, and their wave forms were discriminated off-line by Spike2 program.

Experimental Protocol

The spontaneous activities were recorded from L5 dorsal root in naive and SNL rats. The stable firing was recorded continuously for at least 10 min. Once stable firing was obtained, cumulative doses of IV amitriptyline were tested. Each dose of drug was given 2 min after the previous testing.

In some additional experiments in naïve rats, we purposely searched for the mechanosensitive afferent nerve fibers innervating the left hindpaw from the L5 dorsal root. The mechanosensitive afferents were identified by electrical stimulation of the left hindpaw (single pulse 0.5 ms square-wave at 3–8 mA) and responses to mechanical stimulation using von Frey filaments. The fibers with conduction velocities (conduction velocities) <2.5 m/s were considered unmyelinated C-fibers, and those with conduction velocities >2.5 m/s were considered thinly myelinated Aδ-fibers.[14] We did not use noxious stimuli to search for afferents to avoid sensitization of afferent terminals. However, light stroking across the skin was used to identify low threshold, mechanoreceptive afferents.

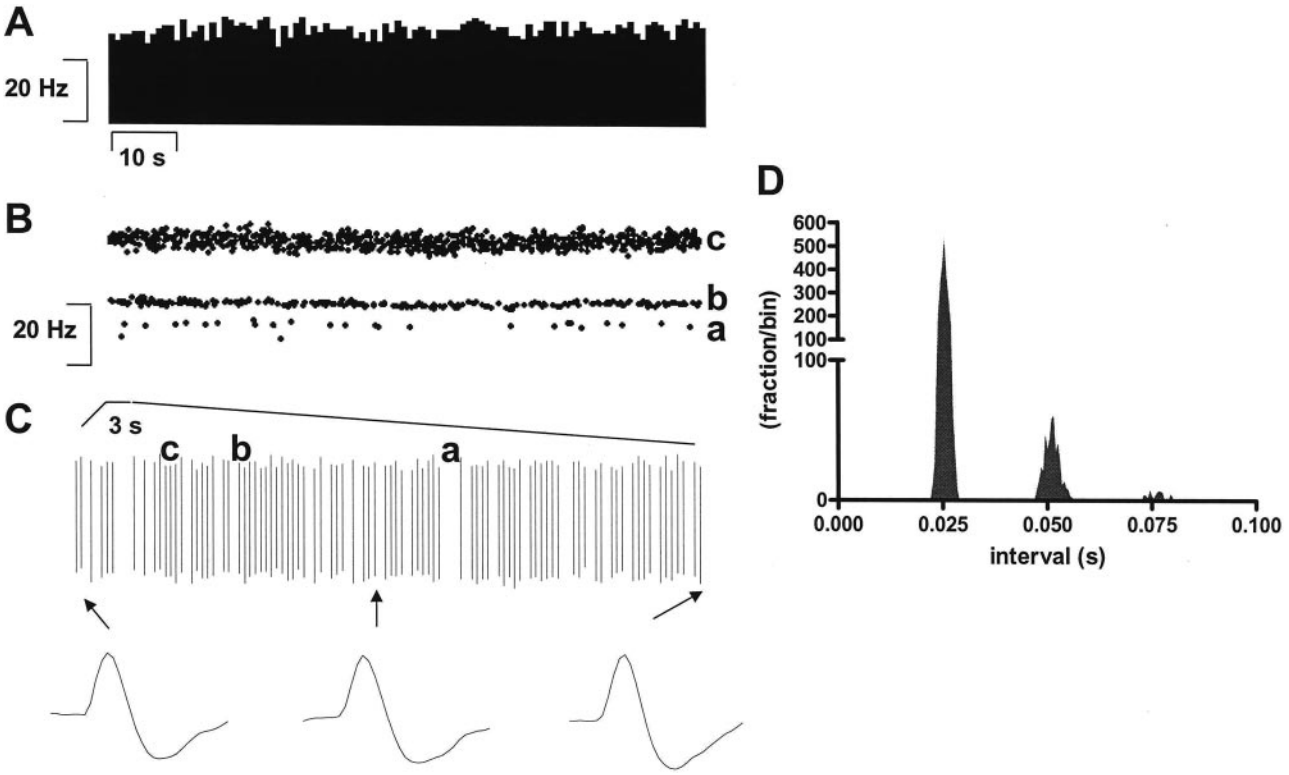

Figure 1. Example of spontaneous activity of an afferent fiber recorded from a naïve rat. (A) The frequency discharge of the fiber is illustrated as a peristimulus time histogram with a 1-s binwidth. (B) The instantaneous frequency of fiber discharge is represented by each dot. Note that this fiber contains instantaneous frequency patterns with three events marked as "a," "b," and "c." (C) The discharge wave form of the afferent fiber during the first 3 s recording period. The interspike intervals "a," "b," and "c" correspond to instantaneous frequencies of "a," "b," and "c" in (B). The action potential wave forms illustrated below have the same shapes providing evidence that the spontaneous activities are from the same single fiber. (D) The interspike interval histograms (5 ms bins) on the time axis. The fiber activity has interspike interval patterns with three events at intervals of 0.025, 0.05, and 0.075 s.

Fibers that did not respond to light touch/stroking were tested with a pinch stimulus by pinching the hindpaw using forceps and fingers. Receptive fields of the afferent nerves were located using noxious mechanical stimulation by von Frey filaments. The loose property of skin was exploited to carefully discriminate cutaneous versus deep receptive field. Stimulus-response functions to 10 s force of 0.6, 1, 1.4, 2, 4, 6, 8, 10, 15, 26, 60, 100, 180, and 300 g, at 2-min intervals, were determined in all fibers. Responses of mechanosensitive afferents to repeated noxious force of 200 g every 3 min were then evaluated in studies involving IV administration of 2 mg/kg amitriptyline, given 60 s before force testing.

Data Analysis

The mean frequency of afferent fiber activity in 1 s bins, instantaneous frequency and interspike interval histograms were analyzed by the Spike2 program. For mechanosensitive afferent fibers, the resting activity of a fiber was counted for 60 s before stimulation and the response to a stimulus was determined as the increase in discharge during stimulation above its resting activity. The response threshold was determined by the first response to the lowest force. Mechanosensory afferent fibers having response thresholds more than 15 g were considered high threshold, and all recorded fibers from naïve rats had this characteristic.

All data are expressed as mean ± SEM. Results were analyzed with Student's *t*-test or an analysis of variance for repeated measures by Sigmastat (Systat Software). The inhibitory dose 50 (ID50; dose to produce 50% inhibition of nerve activity) and 95% confidence intervals were calculated from the 20% to 80% component of the cumulative dose-response curve by Prism (GraphPad Software). A value of $P < 0.05$ was considered statistically significant.

RESULTS

Thirty-one afferent fibers on the left side of the L5 dorsal root were studied for spontaneous nerve activities. Thirteen fibers were recorded from naïve rats, and 18 fibers were recorded from SNL rats in which the hindpaw withdrawal threshold had decreased from 15 g to 3.62 ± 0.20 g ($n = 12$, $P < 0.05$), indicating allodynia.

Spontaneously Active Primary Afferent Fibers in Naïve Rats had High-Frequency Discharges with Distinct Firing Patterns

The mean frequency of discharge was stable in all spontaneously active afferent fibers in naïve rats with a mean of 35.23 ± 6.63 Hz (0.75–75.84 Hz). Twelve of 13 afferent fibers displayed a distinct pattern discharge in

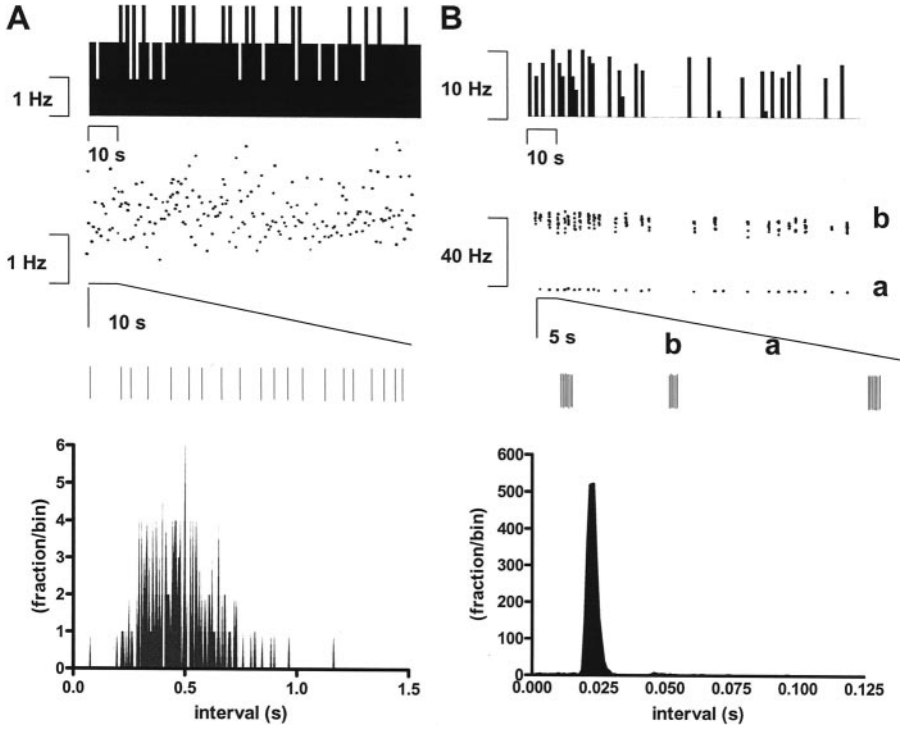

Figure 2. Representative examples of discharge patterns of afferent fibers recorded from spinal nerve ligation (SNL) rats. (A) Example of irregular, low-frequency discharge of an afferent fiber. The discharge frequency of the fiber is illustrated in the top panel as the peristimulus time histogram with a 1-s binwidth. Each dot represents an action potential plotted as instantaneous frequency, and this fiber had irregular pattern discharge. The action potential wave forms illustrated underneath. The bottom histogram shows the unimodal distribution of interspike intervals with 5 ms intervals. (B) An example of short bursting discharge of an afferent fiber. The discharge frequency of the fiber is illustrated in the top panel as the peristimulus time histogram with a 1-s binwidth. Each dot represents an action potential plotted as instantaneous frequencies. Interburst and intraburst interspike interval marked with "a" and "b," respectively.

which the interspike intervals or instantaneous frequencies were even and regularly interrupted by one or two relatively long but fixed interspike intervals or low instantaneous frequency discharges. Thus, spike generation appeared to be precise and time-locked or patterned. Figure 1A shows an example of a stable fiber discharge from a naïve rat with a mean frequency of 32.47 Hz. The instantaneous frequency trace of this fiber in Figure 1B shows that fixed instantaneous frequencies (approximately 40 Hz) (marked "c") were interrupted by relatively low instantaneous frequencies marked "a" (approximately 13 Hz) and "b" (approximately 20 Hz). The low instantaneous frequencies at "a" and "b" also had fixed values similar to instantaneous frequencies at "c." Interspike interval histograms constructed from the spontaneous activity of this fiber are shown in Figure 1D, in which interspike intervals were collected in equal bins of 5 ms width. Interspike interval histograms of this fiber revealed three distinct populations of firing events: more events having interspike intervals of 0.025 s and fewer events having interspike intervals of 0.075 s. The interspike intervals of 0.025, 0.050, and 0.075 s corresponded to instantaneous frequencies of 40, 20, and 13 Hz, respectively.

IV injection of pancuronium bromide (1 mg/kg) did not affect the firing pattern or mean frequency discharge (prepancuronium = 20.32 ± 4.31 Hz; postpancuronium = 20.61 ± 4.58 Hz; $n = 6$, $P > 0.05$).

Spontaneously Active Primary Afferent Fibers in SNL Rats had Low-Frequency Irregular or Burst Discharge

In SNL rats, the mean frequency of spontaneous fiber discharge was 11.05 ± 3.66 Hz (0.77–60.14 imp/s). Based on the instantaneous frequency or the interspike interval, discharges of 11/18 fibers were irregular, whereas seven displayed regular interval patterns. Among the seven fibers displaying patterned discharges, three of them had high-basal frequencies of discharge (22, 40, and 60 Hz). The remaining four fibers had lower discharge frequencies (3, 4, 5, and 15 Hz) and displayed "on-off" short bursting discharge. The bursting discharges were interrupted by long silent periods. As an example, the afferent fiber in Figure 2A had a mean frequency of 2.52 Hz and the instantaneous frequency depicted in the middle panel illustrates the typical irregular discharge. Interspike interval histograms shown on the lower panel for this fiber revealed one distinct population of instantaneous frequency discharge. In another example (Fig. 2B), this fiber had a mean spontaneous frequency of 3 Hz. The instantaneous frequency depicted in the middle panel shows a relatively long interruption "a" (interspike interval of approximately 2 s, instantaneous frequency of approximately 0.5 Hz) of bursting discharges "b" (interspike interval of approximately 0.025 s, instantaneous frequency of approximately 40 Hz) for each cycle. Interspike interval histograms constructed from interspike intervals of this fiber in bins of 5 ms width are shown in the lower panel, revealing at least two distinct populations of discharge events.

Amitriptyline Selectively Attenuated Ectopic Discharges in SNL Rats

Only 5/13 (38%) afferent fibers from naïve rats showed reduced spontaneous activities after IV amitriptyline (2 mg/kg), whereas amitriptyline significantly inhibited ectopic discharge in 13/18 (72%) afferent fibers from SNL rats. The mean ID_{50} values for amitriptyline in naïve rats and SNL rats were 3.98 ± 0.61 mg/kg

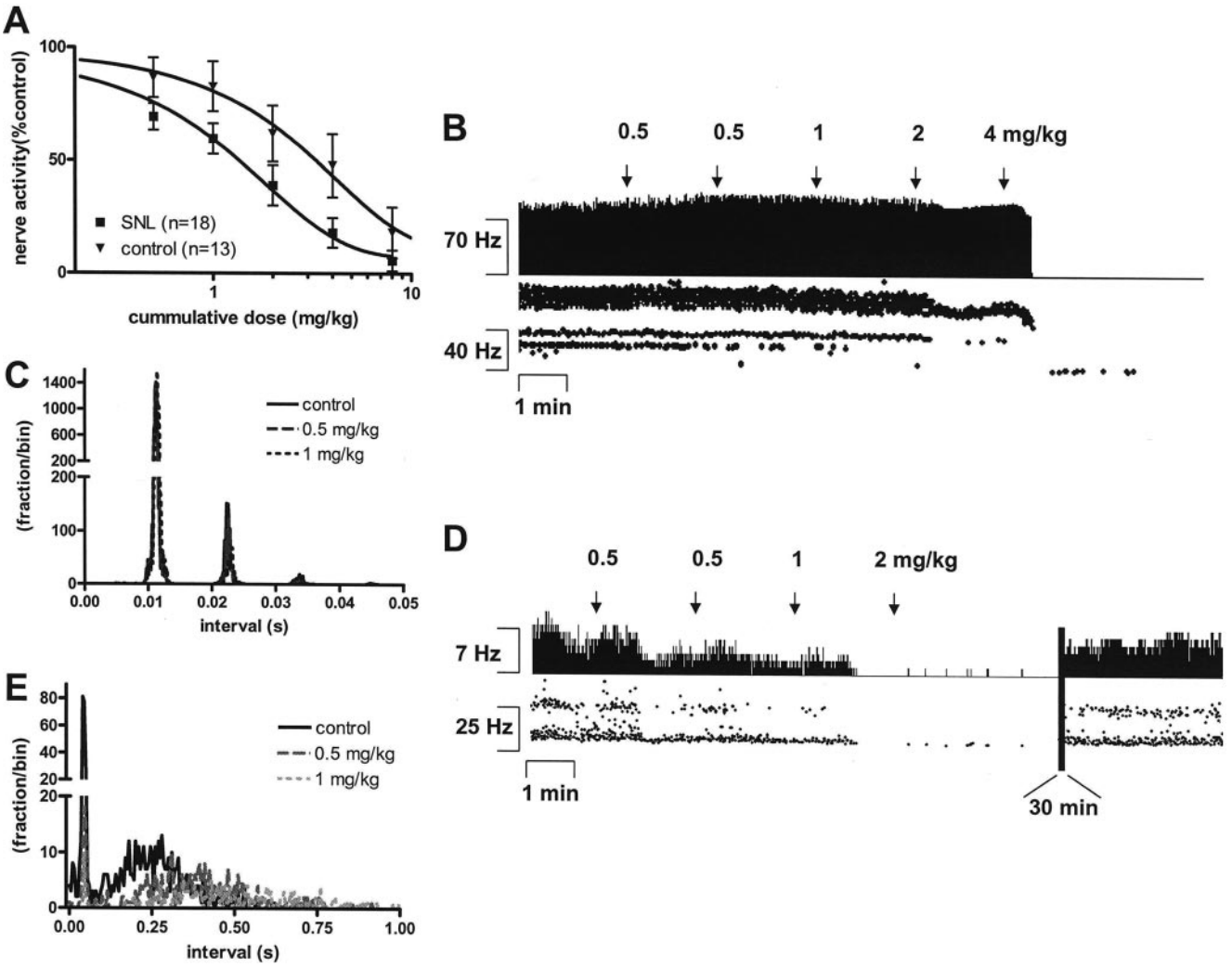

Figure 3. Inhibition of spontaneously active afferent fibers in naïve rats and spinal nerve ligation (SNL) rats by amitriptyline. (A) Dose-response curve after IV administration of amitriptyline. The activity is represented as % of control ± SEM. (B) Example of amitriptyline effect on a patterned spontaneously active afferent fiber in a naïve rat. The arrows indicate the cumulative dose of IV amitriptyline. Instantaneous frequency was not affected before complete block occurred. (C) Histogram of the distribution of interspike intervals with 5 ms bins before and after amitriptyline. The interspike interval histogram illustrates that the interspike interval was not affected by IV administration of amitriptyline. (D) Example of pronounced inhibition of short bursting discharge in an afferent fiber by amitriptyline in a SNL rat. The arrows indicate the cumulative dose of IV amitriptyline. The fast instantaneous frequency events were reduced dose dependently leading to a complete block of activity which recovered after 30 min. (E) Histogram of the distribution of interspike interval histogram with 5 ms bins before and after amitriptyline. The interspike interval histogram illustrates that the counts/bin of the fast event was reduced, but the peak of the fast event was not shifted. However, the peak of the slow event was reduced and prolonged after IV administration of amitriptyline.

(2.77–5.19, slope: −0.21) and 1.66 ± 0.17 mg/kg (1.31–2.00, slope: −0.42), respectively (Fig. 3A). The example in Figure 3B from a naïve rat demonstrates that amitriptyline did not inhibit spontaneous fiber discharge until a relatively high dose of 4 mg/kg was administered. The interspike interval histogram in Figure 3C shows that amitriptyline also did not change the firing pattern of this fiber at ineffective doses. In SNL rats, amitriptyline dose-dependently inhibited spontaneous fiber discharges, an example is shown in Figure 3D. We examined the effects of amitriptyline on on-off/burst discharge, concentrating on changes both in interspike interval during burst, and to the on-off cycle. Before complete inhibition of discharge, burst events were reduced by cumulative administration of 0.5 and 1 mg/kg amitriptyline, evidenced by decreasing but not shifting the peak (fraction/bin) of short interspike intervals. The peak of slow events was reduced and lengthened by amitriptyline (Fig. 3E). This same phenomenon was found in other bursting discharge fibers.

The efficacy of amitriptyline was found to vary between afferent fibers in SNL rats, especially at cumulative doses <2 mg/kg (Fig. 4A). The inhibition of spontaneous discharge by amitriptyline was found to be correlated to mean frequencies of afferent discharge (slope of 1.53 ± 0.5; $P < 0.05$; Fig. 4B), such that amitriptyline strongly suppressed activity in fibers displaying low-frequency discharges.

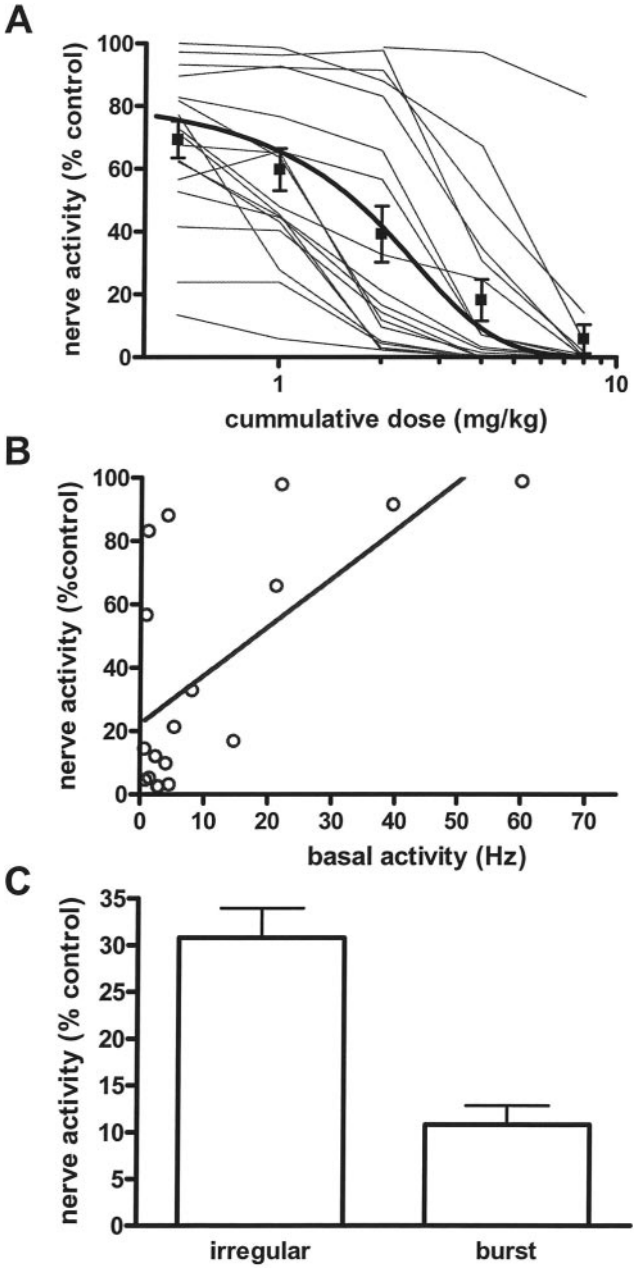

Figure 4. Summary of the effects of amitriptyline in spinal nerve ligation (SNL) rats. (A) Dose-response curve after IV administration of amitriptyline. The activity is represented as % of control ± SEM. Light-weighted lines show the activities of individual fibers. (B) Inverse regression comparing inhibition of activity by amitriptyline (2 mg/kg) to basal mean frequency afferent activity in naïve and SNL rats ($P < 0.05$). (C) Inhibition of irregular and short bursting discharges by 2 mg/kg amitriptyline in SNL rats, expressed as % of control.

Figure 4C summarizes the inhibition of afferent fibers with irregular and burst discharges in SNL rats by 2 mg/kg amitriptyline. In those afferents displaying discharge frequencies <20 Hz, amitriptyline inhibited activity to 30.79% ± 4.13% and 10.8% ± 9.96% of control for fibers displaying irregular and bursting discharges, respectively. The inhibition of discharge in bursting fibers was significantly greater compared with that in irregular fibers ($P < 0.05$).

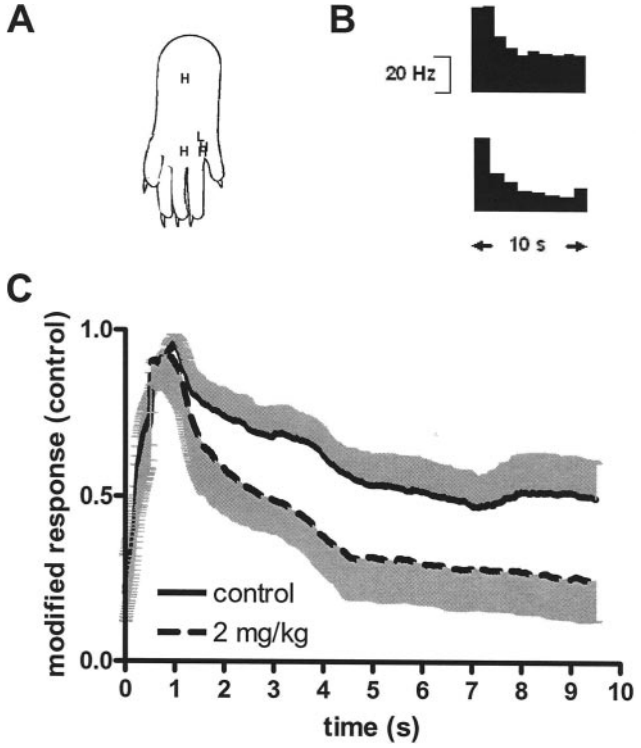

Figure 5. Inhibition of responses of afferent fibers to acute mechanical stimulation by amitriptyline. (A) Receptive fields of all five fibers (four high-threshold afferents marked "H" and one low-threshold afferent marked "L"). (B) Example of a response of a mechanosensory afferent fiber to noxious mechanical stimulation (200 g, 10 s every 3 min) before (top) and after (bottom) IV administration of 2 mg/kg amitriptyline. (C) Mean fiber responses during 10 s mechanical stimulation before and after 2 mg/kg amitriptyline expressed relative to the maximal control response. The shaded area represents the SEM of each data point.

Amitriptyline Inhibits Responses of Cutaneous Afferent Nerve to Noxious Mechanical Stimulation in Naïve Rats

Five mechanosensitive afferents (one C-fiber and four Aδ-fibers) were identified in naïve rats, which responded to mechanical stimulation of the hindpaw with von Frey filaments. The receptive fields of these afferent fibers were small (approximately 1 mm diameter, Fig. 5A). One Aδ-fiber had low thresholds for mechanical response and exhibited spontaneous discharge of 12 Hz and four fibers had high thresholds for response, of which three had spontaneous activity of 0.77 ± 0.27 Hz and one had no spontaneous activity. Amitriptyline (IV 2 mg/kg) inhibited responses of all five mechanosensitive afferent fibers to noxious mechanical stimulation to 33.15% ± 16.4% control ($P < 0.05$). Figure 5B illustrates a typical inhibition of response to phasic mechanical stimulation before and after administration of amitriptyline. We also noticed that the inhibition by amitriptyline (2 mg/kg) was not seen at the initial high frequency response to mechanical stimulation but rather started 1–2 s after the onset of stimulation and was enhanced during continuous stimulation (Fig. 5C).

DISCUSSION

In the present study, we found that afferent fibers from rats that had received SNL displayed ectopic discharge with irregular discharge patterns or short bursting activity. The ectopic activity was dose-dependently inhibited by amitriptyline. Moreover, these irregular fibers with low-frequency discharges or bursting fibers showed enhanced sensitivity to inhibition by amitriptyline. The inhibitive effect of amitriptyline on ectopic discharges is consistent with amitriptyline's efficacy in treating neuropathic pain both in the clinic and in rat models.[7,11,15]

Ectopic Discharge Frequency and Pattern from SNL Rats

The mean frequency discharge of fibers after SNL in the present study (11 ± 3.6 Hz) is consistent with that found by others.[2,5,16] Sun et al.[17] found that the mean frequency of fiber discharge after SNL was time-dependent as higher mean frequency discharge was observed 24 h post-SNL (25 Hz), which diminished by 7–14 days post-SNL (3–7 Hz).

Afferent fibers after SNL were found to exhibit irregular discharge or short bursting activity. Instantaneous frequencies of activity are proportional to the instantaneous membrane potential, and the exact timing of spikes has been proposed to be essentially determined by membrane potential fluctuations.[18,19] Thus, changes in the expression and location of sodium and potassium channels along fiber axons following neuropathy may cause alterations in the instantaneous membrane potential resulting in aberrant oscillation frequencies and irregular fiber discharges.[20–24] The short bursting activity observed in approximately 1/3 of fibers in SNL rats has also been reported by others.[17,24–26] Action potential bursting activity has been shown to modulate cell secretion and neurotransmitter release,[27] and thus this burst firing pattern found in fibers from SNL rats may contribute to the development of central sensitization.

In the present study, many spontaneously active afferent fibers were recorded from naïve rats that displayed relatively high mean discharge frequencies, which were patterned based on their instantaneous frequency and interspike interval distributions. High frequency spontaneous activity has been observed in uninjured spinal nerve.[28] Possible sources of this high-frequency spontaneous activity in naïve rats may include cutaneous thermoreceptors and/or slowly adapting Type II mechanoreceptors innervating muscle spindles or joints.[29,30]

Attenuation of Ectopic Discharge by Amitriptyline

In addition to its role as a monoamine reuptake inhibitor, amitriptyline has been reported to block sodium channel activity.[10,31,32] The present study clearly demonstrates the amitriptyline inhibition on ectopic discharge in SNL rats. Moreover, potent effects are observed on those fibers exhibiting low frequency or short bursting activity. This observation is consistent with the notion that sodium channels have an important role in the electrogenic rhythm associated with ectopic discharge.[24,33] The high degree of frequency-dependent and state-dependent inhibition by sodium channel blockers observed in *in vitro* electrophysiological studies may be pharmacologically relevant for the treatment of neuropathic pain.[32] In the present study, we observed the frequency-dependent inhibition of afferent fiber activity by amitriptyline. However, amitriptyline is most effective in inhibiting activity of low-frequency discharges. Similarly, inhibition of ectopic discharge by sodium channel blockers lidocaine and lamotrigine at fixed concentrations demonstrated a negative correlation between frequency of ectopic discharge and efficacy of tested drugs *in vivo*.[33] Thus, both studies suggest that frequency dependence is not the primary mechanism of sodium channel blockers on neuropathic pain.

Only 38% of afferent fibers from naïve rats showed weak reduction in spontaneous activities after IV amitriptyline administration (2 mg/kg). The effective inhibition of ectopic discharge by the sodium channel blocker amitriptyline can be interpreted by its selective inhibition on different sodium channel subtypes. After injury of peripheral nerves, the composition of sodium channel isoforms expressed in sensory neurons undergoes significant changes,[34] with an increased expression of tetrodotoxin-sensitive NaV1.3 in cell bodies of sensory neurons.[21] Amitriptyline more potently blocks tetrodotoxin-sensitive sodium channels than tetrodotoxin-resistant sodium channels. The potency shifted from 8- to 1.4-fold at a holding potential of −80 and −140 mV, respectively.[35] The shifted potency at different levels of resting potentials could be attributed to the difference in affinities of resting and inactivated channels by amitriptyline. It has been reported that there is an accumulation of inactivated sodium channels at less negative resting membrane potentials after peripheral neuropathy.[36–38] Amitriptyline blocks voltage-gated sodium channels in a state-dependent manner, with a preference for open and inactivated rather than for resting channel states.[39,40] It is important to note that at the less negative resting membrane potentials in injured nerves, the fraction of inactivated channels is higher for tetrodotoxin-sensitive sodium channels compared with tetrodotoxin-resistant sodium channels based on their different inactivation properties. Thus, it is conceivable that variability of inhibition on ectopic discharge by amitriptyline might be dependent on the fraction of available inactivated tetrodotoxin-sensitive sodium channels. Taken together, both altered expression of sodium channel subtypes and reduced resting membrane potentials in injured nerves may account for the differences in discharge characteristics and pharmacological sensitivities of ectopic discharge in injured nerves by amitriptyline. Changes in sodium channels in patients with neuropathic pain are more

complex; owing to the heterogeneity of pathological conditions. Whether altered expression of sodium channels contributes to the inconsistent efficacy in pain treatment by sodium channel blockers, e.g., amitriptyline requires further investigation.

Use-dependent block arises from amitriptyline binding to inactivated channels recruited during repetitive firing and from amitriptyline dissociation from inactivated states with a time constant slower than the frequency of the discharges. We simulated this process by analyzing the time course of mechanical responses of afferent fibers after amitriptyline administration in naïve rats. We found that amitriptyline did not block the initial high-frequency response but produced a pronounced inhibition of mechanosensitive afferent responses to maintained phasic mechanical stimulation. This sustained mechanical stimulation could result in the accumulation of inactivated sodium channels, and thus, use-dependent inhibition of afferent fiber responses by amitriptyline may contribute to its mechanism of action.

Finally, rats were pretreated by systemic administration of sodium pentobarbital in this study. Anesthetics may target at sites of synaptic transmission across the entire central nervous system. In addition, by competing with the common pathway, sodium channel blockers have been reported to antagonize the effect of anesthetics.[41] The potential effects of the anesthetic on the peripheral nerves are not understood. However, pentobarbital might produce a similar interaction on ectopic discharges and their pharmacological responses.

In summary, the results from the present study demonstrate afferent fibers from SNL rats exhibited lower mean frequency discharge that was irregular or consisted of short bursting activity in some cases. An effect of amitriptyline in inhibiting fiber activity was observed in SNL rats, and amitriptyline appeared most effective in inhibiting those fibers displaying short bursting activity. These results provide direct functional evidence that amitriptyline inhibits ectopic discharge, demonstrating a peripheral site of action associated with the antinociceptive effect of amitriptyline in the SNL model.

REFERENCES

1. 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
2. Liu C-N, Wall PD, Ben-Dor E, Michaelis M, Amir R, Devor M. Tactile allodynia in the absence of C-fiber activation: altered firing properties of DRG neurons following spinal nerve injury. Pain 2000;85:503–21
3. Gold M. Spinal nerve ligation: what to blame for the pain and why. Pain 2000;84:117–20
4. Pan HL, Eisenach JC, Chen S R. Gabapentin suppresses ectopic nerve discharges and reverses allodynia in neuropathic rats. J Pharmacol Exp Ther 1999;288:1026–30
5. Abdi S, Lee DH, Chung JM. The anti-allodynic effects of amitriptyline, gabapentin, and lidocaine in a rat model of neuropathic pain. Anesth Analg 1998;87:1360–6
6. Tanner KD, Reichling DB, Gear RW, Paul SM, Levine JD. Altered temporal pattern of evoked afferent activity in a rat model of vincristine-induced painful peripheral neuropathy. Neuronscience 2003;118:809–17
7. Teng J, Mekhail N. Neuropathic pain: mechanisms and treatment options. Pain Pract 2003;3:8–21
8. Dick IE, Brochu RM, Purohit Y, Kaczorowski GJ, Martin WJ, Priest BT. Sodium channel blockade may contribute to the analgesic efficacy of antidepressants. J Pain 2007;8:315–24
9. Esser MJ, Sawynok J. Acute amitriptyline in a rat model of neuropathic pain: differential symptom and route effects. Pain 1999;80:643–53
10. Gerner P, Mujtaba M, Khan M, Sudoh Y, Vlassakov K, Anthony D. Wang GK. *N*-phenylethyl amitriptyline in rat sciatic nerve blockade. Anesthesiol 2002;96:1435–42
11. Lynch ME, Clark AJ, Sawynok J, Sullivan MJ. Topical amitriptyline and ketamine in neuropathic pain syndromes: an open-label study. J Pain 2005;6:644–9
12. Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL. Quantitative assessment of tactile allodynia in the rat paw. J Neurosci Methods 1994;53:55–63
13. Kim SH, Chung JM. An experimental model of peripheral neuropathy produced by segmental spinal nerve ligation. Pain 1992;50:355–63
14. Su X, Urban MO. Effect of metabotropic glutamate receptor subtype 5 antagonist, 2-methyl-6-(phenylethynyl)-pyridine on mechanosensory afferents innervating rat hind paw following inflammation. Neurosci Lett 2005;390:123–8
15. Carter GT, Galer BS. Advances in the management of neuropathic pain. Phys Med Rehabil Clin N Am 2001;12:447–59
16. Gorodetskaya N, Constantin C, Janig W. Ectopic activity in cutaneous regenerating afferent nerve fibers following nerve lesion in the rat. Eur J Neurosci 2003;18:2487–97
17. Sun Q, Tu HY, Xing GG, Han JS, Wan Y. Ectopic discharges from injured nerve fibers are highly correlated with tactile allodynia only in early, but not late stage in rats with spinal nerve ligation. Exp Neurol 2005;191:128–36
18. Amir R, Martin M, Devor M. Membrane potential oscillations in dorsal root ganglion neurons: role in normal electrogenesis and neuropathic pain. J Neurosci 1999;19:8589–96
19. Kretzberg J, Egelhaaf M, Warzecha A. Membrane potential fluctuations determine the precision of spike timing and synchronous activity: a model study. J Comput Neurosci 2001; 10:79–97
20. Everill B, Kocsis JF. Reduction in potassium currents in identified cutaneous afferent DRG neurons after axotomy. J Neurophysiol 1999;82:700–8
21. Cummins TR, Waxman SG. Downregulation of tetrodotoxin-resistant sodium currents and upregulation of a rapidly repriming tetrodotoxin-sensitive sodium current in small spinal sensory neurons following nerve injury. J Neurosci 1997;17:3503–14
22. Kim CH, Oh Y, Chung JM, Chung K. The changes in expression of three subtypes of TTX-sensitive sodium channels in sensory neurons after spinal nerve ligation. Mol Brain Res 2001; 95:153–61
23. Liu C-N, Devor M, Waxman SG, Kocsis JD. Subthreshold oscillations induced by spinal nerve injury in dissociated muscle and cutaneous afferents of mouse DRG. J Neurophysiol 2002;87:2009–17
24. Matzner O, Devor M. Hyperexcitability at sites of nerve injury depends on voltage-sensitive Na^+ channels. J Neurophysiol 1994;72:349–59
25. Tal M, Wall PD, Devor M. Myelinated afferent fiber types that become spontaneously active and mechanosensitive following nerve transaction in the rat. Brain Res 1999;824:218–23
26. Michaelis M, Liu X, Janig W. Axotomized and intact muscle afferents but no skin afferents develop ongoing discharges of dorsal root ganglion origin after peripheral nerve lesion. J Neurosci 2000;20:2742–8
27. Duan K, Yu X, Zhang C, Zhou Z. Control of secretion by temporal patterns of action potentials in adrenal chromaffin cells. J Neurosci 2003;23:11235–43
28. Wu G, Ringkamp M, Hartke TV, Murinson BB, Campbell JN, Griffin JW, Meyer RA. Early onset of spontaneous activity in uninjured C-fiber nociceptors after injury to neighboring nerve fibers. J Neurosci 2001;21:RC140

29. De-Doncker L, Picquet F, Petit J, Falempin M. Characterization of spindle afferents in rat soleus muscle using ramp-and-hold sinusoidal stretches. J Neurophysiol 2003;89:442–9
30. Leem JW, Willis WD, Chung JM. Cutaneous sensory receptors in the rat foot. J Neurophysiol 1993;69:1684–99
31. Perry PJ, Zeilmann C, Arndt S. Tricyclic antidepressant concentrations in plasma: an estimate of their sensitivity and specificity as a predictor of response. J Clin Psychopharmacol 1994; 14:230–40
32. Bräu ME, Dreimann M, Olschewski A, Vogel W, Hempelmann G. Effect of drugs used for neuropathic pain management on tetrodotoxin-resistant Na^+ currents in rat sensory neurons. Anesthesiology 2001;94:137–44
33. Ritter AM, Ritchie C, Martin WJ. Relationship between the firing frequency of injured peripheral neurons and inhibition of firing by sodium channel blockers. J Pain 2007;8:287–95
34. Lai J, Hunter JC, Porreca F. The role of voltage-gated sodium channels in neuropathic pain. Curr Opin Neurobiol 2003; 13:291–7
35. Leffler A, Reiprich A, Mohapatra DP, Nau C. Use-dependent block by lidocaine but not amitriptyline is more pronounced in tetrodotoxin (TTX)-Resistant Nav1.8 than in TTX-sensitive Na^+ channels. J Pharmacol Exp Ther 2007;320:354–64
36. Matzner O, Devor M. Method for distinguishing between drug action on impulse propagation versus impulse generation. J Neurosci Methods 1993;49:23–31
37. Novakovic SD, Tzoumaka E, McGivern JG, Haraguchi M, Sangameswaran L, Gogas KR, Eglen RM, Hunter JC. Tetrodotoxin-resistant sodium channel PN3 in rat sensory neurons in normal and neuropathic conditions. J Neurosci 1998;18:2174–87
38. Elliott AA, Elliott JR. Characterization of TTX-sensitive and TTX-resistant sodium currents in small cells from adult rat dorsal root ganglia. J Physiol 1993;463:39–56
39. Nau C, Wang GK. Interactions of local anesthetics with voltage-gated Na^+ channels. J Membr Biol 2004;201:1–8
40. Ragsdale DS, McPhee JC, Scheuer T, Catterall WA. Molecular determinants of state-dependent block of Na^+ channels by local anesthetics. Science 1994;265:1724–8
41. Angel A. Central neuronal pathways and the process of anaesthesia. Bri J Anaesthesia 1993;71:148–63

