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Capsaicin Combined with Local Anesthetics Preferentially Prolongs Sensory/Nociceptive Block in Rat Sciatic Nerve

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Abstract

Background—Transient receptor potential vanilloid 1 channels integrate nociceptive stimuli and are predominantly expressed by unmyelinated C-fiber nociceptors, but not low-threshold mechanoreceptive sensory or motor fibers. A recent report showed that the transient receptor potential vanilloid 1 channel agonist capsaicin allows a hydrophilic quaternary ammonium derivative of lidocaine, QX-314, to selectively block C fibers without motor block. The authors tested whether a similar differential block would be produced using amphipathic *N*-methyl amitriptyline, amitriptyline, bupivacaine, or lidocaine, either alone or together with 0.05% capsaicin, in a rat sciatic nerve block model.

Methods—Rats ($n = 8$ /group) were anesthetized with sevoflurane, and 0.2 ml of drug was injected either alone or with capsaicin (simultaneously or 10 min later) next to the sciatic nerve in the sciatic notch. Motor function was assessed by the extensor postural thrust. Nociception was evaluated by the nocifensive withdrawal reflex and vocalization evoked by pinch of a skin fold over the lateral metatarsus (cutaneous pain) with a serrated forceps.

Results—*N*-Methyl amitriptyline, amitriptyline, bupivacaine, or lidocaine, followed by injection of capsaicin 10 min later, each elicited a predominantly nociceptive-specific blockade. In

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comparison, simultaneous application of each local anesthetic with capsaicin did not elicit a clinically significant differential block, with the exception of *N*-methyl amitriptyline.

Conclusions—Both tertiary amine local anesthetics and their quaternary ammonium derivatives can elicit a predominantly sensory/nociceptor selective block when followed by injection of capsaicin. The combined application of transient receptor potential vanilloid 1 channel agonists and various local anesthetics or their quaternary ammonium derivatives is an appealing strategy to achieve a long-lasting differential block in regional analgesia.

IN addition to blocking voltage-gated sodium channels in sensory nerve fibers, local anesthetics (LAs) also block sodium channels in motor and sympathetic fibers. Therefore, complete pain relief is generally only accomplished with concomitant low-threshold sensory afferent blockade, sympathetic blockade causing low blood pressure and motor blockade causing immobility. Improving the sensory selectivity of LAs will clearly extend their clinical utility beyond their current indications. (Of note, especially in the clinical anesthesia literature, the terms *sensory selective* and *differential block* are commonly used and are roughly interchangeable with *pain selective* and *nociceptor selective*).

Recently, Binshtok *et al.*¹ demonstrated a nociceptor-selective, long-lasting rat sciatic nerve blockade by injecting QX-314 followed by capsaicin. QX-314 is a permanently charged derivative of lidocaine and is therefore less able than lidocaine to acutely penetrate the membranes and block the sodium channel from the cytoplasmic side,² thereby resulting in a slow onset of blockade in some studies³ and no effect in others.¹

Capsaicin (8-methyl-*N*-vanillyl-6-nonenamide) is produced as a secondary metabolite by chili peppers, which are plants belonging to the genus *Capsicum*. Capsaicin selectively binds to the vanilloid receptor subtype 1 (VR1),⁴ now referred to as *TRPV1*, a member of the superfamily of transient receptor potential ion channels. TRPV1 is expressed peripherally in primary afferent nociceptors,⁵ most of which are unmyelinated, and is physiologically stimulated and sensitized by heat, protons, and various inflammatory mediators such as bradykinin, adenosine, adenosine triphosphate, and arachidonic metabolites such as lipoxygenase products, leukotriene B₄, and prostaglandins, which make up an “inflammatory soup.”⁶ TRPV1 permits calcium and sodium ions to pass through the membrane of the primary sensory/nociceptive neurons, causing depolarization and excitation and leading to nociceptive responses. However, initial excitation of the nociceptive neuron is followed by a long-lasting refractory state. This includes desensitization of the receptor/channel⁷⁻¹⁰ as well as changes in axon terminals, including mitochondrial swelling, release of calcitonin gene-related peptide, displacement of adenosine triphosphate by the calcium sensor calmodulin, depletion of substance P, and obvious axonal atrophy and terminal degeneration.^{7,11,12} This desensitization and the longer-lasting atrophic/degenerative changes led to clinical use of capsaicin in topical ointments to relieve neuropathic pain such as postherpetic neuralgia and minor aches and pains associated with arthritis, strains, and sprains.⁷ A single high-dose local injection of capsaicin is also currently being investigated for controlling postsurgical and osteoarthritis pain.⁷

Binshtok *et al.*¹ suggested that the mechanism underlying the observed pain-selective nerve blockade is opening of the TRPV1 receptor, allowing otherwise nonpermeant QX-314 molecules to selectively enter nociceptors while leaving motor impulse conduction intact. Of note, these investigators injected capsaicin 10 min after injection of QX-314, “with the idea that QX-314 would be present extracellularly and ready to enter TRPV1 channels as soon as they were activated.” This staggered injection (QX-314 first, followed by capsaicin) seems to be necessary for pharmacokinetic reasons, *i.e.*, neutral capsaicin penetrates membranes faster than the very hydrophilic permanently charged QX-314.

We hypothesized that activation of TRPV1 channels by capsaicin would achieve nociceptor-selective nerve block when combined with administration of (1) amphipathic quaternary ammonium sodium channel blocker (*N*-methyl amitriptyline) and (2) tertiary amine sodium channel blockers (amitriptyline, bupivacaine, and lidocaine). Although *N*-methyl amitriptyline is permanently charged, it is capable of penetrating membranes, probably because the positive charge is shielded by the additional hydrophobic arms. *N*-Methyl amitriptyline has been shown to confer some degree of nociceptor preference when applied intrathecally in sheep but not in rats.¹³ Amitriptyline is commonly used in the treatment of both clinical depression and chronic pain. This potent sodium channel blocker has not demonstrated any nociceptor selectivity when compared with bupivacaine in humans.¹⁴ Bupivacaine continues to be used more than lidocaine when the objective is relatively greater sensory-selective blockade, particularly of longer duration.

In a rat sciatic nerve block model, we investigated the duration of motor and nociceptive block using *N*-methyl amitriptyline, amitriptyline, bupivacaine, or lidocaine, either alone or with capsaicin. We demonstrate that, in addition to permanently charged LAs (QX-3141 and *N*-methyl amitriptyline), ionizable LAs (the nonclinical LA amitriptyline) and clinically used LAs (bupivacaine and lidocaine) are also capable of a much more pronounced and long-lasting nociceptor-selective nerve blockade when used with capsaicin.

Materials and Methods

Drugs

Capsaicin, amitriptyline hydrochloride, bupivacaine hydrochloride, and lidocaine hydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO). *N*-Methyl amitriptyline was custom synthesized by Sigma Chemical Co.; the purity was greater than 99% by highperformance liquid chromatography, and the molecular weight was 372.3. Capsaicin was freshly prepared with a solvent of 10% ethanol, 10% Tween 80, and 80% normal saline (pH of the final solution was 6.6). All other drugs were freshly dissolved in 0.9% NaCl (pH ranged from 5.0 to 6.0). The pH was not adjusted because it is probably buffered quickly by the pH of the tissue fluid (7.4).

Sciatic Nerve Injections

The animal experimental protocol was approved by the Standing Committee on Animals of Harvard Medical School, Boston, Massachusetts. Male Sprague-Dawley rats were purchased from Charles River Laboratories, Inc. (Wilmington, MA) and were kept in animal housing facilities with controlled relative humidity (20 -30%), at room temperature (24°C), in a 12-h (6:00 AM to 6:00 PM) light- dark cycle. Rats were handled before the procedures to familiarize them with the experimental environment and to minimize stress-induced analgesia.¹⁵ At the time of injection, animals weighed 250-300 g.

The rats were assigned to treatments *via* block randomization with a block size of 8. All rats were anesthetized by inhalation of 1-2% of sevoflurane (Abbott Laboratories, North Chicago, IL) until no withdrawal to pinch of the leg occurred (by forceps). After induction of inhalation anesthesia, the drug in a volume of 200 μ l was injected at the sciatic notch of the left hind limb with a 27-gauge needle connected to a tuberculin syringe: (1) *N*-methyl amitriptyline at 0.125%/3.4 mM, (2) amitriptyline at 0.125%/4.0 mM, (3) bupivacaine at 0.25%/7.3 mM, and (4) lidocaine at 2%/73.9 mM. Because sensory/motor separation by LAs is a partially concentration dependent, we obtained dose-response studies for (5) *N*-methyl amitriptyline and (6) amitriptyline. All drugs were given alone as well as coadministered with capsaicin at 0.05%/1.6 mM. Coadministration of capsaicin was performed either 10 min after the first drug or simultaneously (mixed). The vehicle control group received LA followed by injection of

vehicle 10 min later. Capsaicin was also injected alone, as was normal saline. The volume of drug injected was always 0.2 ml. The experimenter was blinded to the drug used (except to the administration of a second drug 10 min later, which was either capsaicin or vehicle alone).

Neurobehavioral Examination

We evaluated motor function and nociception as described previously.^{16,17} Rats were examined before injection for baseline functions and at 10, 20, 30, 60, and 90 min and 2, 3, 6, 12, 24, and 36 h after drug administration. Motor function was assayed by holding the rat upright with the control hind limb extended so that the distal metatarsus and toes of the target leg supported the animal's weight; the extensor postural thrust was recorded as the force (in grams) applied by each of the two hind limbs to a digital platform balance (Ohaus Lopro; Fisher Scientific, Florham Park, NJ). The reduction in this force, representing reduced extensor muscle contraction caused by motor block, was calculated as a percentage of the control force (preinjection control value 145-165 g). The percent reduction in force was assigned a "range" score: 0 = no block (or baseline); 1 = minimal block, force between the preinjection control value of 100% and 50%; 2 = moderate block, force between 50% of the preinjection control value and 20 g (approximately 20 g represented the approximate weight of the flaccid limb); 3 = complete block, force 20 g or less.

Nociception was evaluated by the nocifensive withdrawal reflex and vocalization to pinch of a skin fold over the lateral metatarsus (cutaneous pain) with a serrated forceps; the force and duration of this pinch was held as constant as possible. The extent of the nocifensive withdrawal reflex and vocalization were combined on a scale of 0-3 for each examination. Grading was as follows: 3 = complete block, no nocifensive reaction or vocalization; 2 = moderate block, vocalization accompanied by slow withdrawal and flexion of the leg; 1 = minimal block, brisk flexion of the leg, with some sideways movement of the body or other escape response and loud vocalization; 0 = baseline with no block and all nocifensive responses listed above.

We restricted our testing of nociception to superficial nociceptive block, *i.e.*, pinching of a skin fold at the lateral area of the dorsum of the paw, because our pilot studies using pinching of the fifth toe revealed nonreproducible results (data not shown), perhaps because the presumed preferential C-fiber block of various drug combinations does not block large motor (proprioceptive) fibers, which would allow the rat to sense the pressure of the forceps when the entire fifth toe is moved and pinched. However, firm pinch with a serrated forceps to an entire skin fold at the lateral aspect of the dorsum elicited a robust (anti)nociceptive response.

For both nociceptive and motor assessment, the examination was repeated three times at each time point and reported as an average of the three examinations.

Statistical Analysis

Because of the ordinal categorical nature of the block scores, an overall test for drug effect was obtained *via* generalized estimating equations for longitudinal ordinal data.¹⁸ A cumulative logistic ordinal model was fit with a linear and quadratic trend in time and time-by-group interaction. The group effect and group and time interaction effect were tested using contrast coefficients in generalized estimating equations analysis. The overall *P* value was calculated *via* PROC GENMOD (SAS 9.1; Cary, NC). To have an overall 5% type I error rate for each drug mode, a *P* value of less than 0.0167 was considered statistically significant ($0.05/3 = 0.0167$, because there are 3 sets of comparisons among each drug mode).

Results

After sciatic nerve block, all rats in the treatment groups ($n = 8/\text{group}$) showed a functional loss of nociceptive and motor function of different degrees and durations that were completely reversed over time.

Overall, *N*-methyl amitriptyline (fig. 1), amitriptyline (fig. 2), bupivacaine (fig. 3), and lidocaine (fig. 4), with injection of capsaicin 10 min later, produced a predominantly nociceptive-specific blockade in this rat sciatic nerve block model. In contrast, simultaneous application of the LAs with capsaicin did not produce a significant differential block, with the exception of *N*-methyl amitriptyline (tables 1 and 2).

***N*-Methyl Amitriptyline**

For the 0.125% *N*-methyl amitriptyline solution when administered alone, the duration of motor and nociceptive blockade was relatively brief (< 1 h) and incomplete. When 0.05% capsaicin was added, either 10 min after the injection of *N*-methyl amitriptyline or injected simultaneously, a prolonged sensory-selective block resulted (fig. 1).

Amitriptyline

The duration of the motor block for 0.125% amitriptyline alone was similar to the duration of the nociceptive block, but addition of capsaicin produced a large differential block and significantly decreased the motor blockade when given simultaneously (fig. 2).

Bupivacaine

Bupivacaine at a concentration of 0.25% was almost indistinguishable from 0.125% amitriptyline, when given alone or in combination with capsaicin. Injection of bupivacaine followed by capsaicin or vehicle 10 min later significantly increased the nociceptive blockade over motor blockade (fig. 3).

Lidocaine

Lidocaine at 2% showed a complete but relatively short-lasting motor and nociceptive block when given alone, and the smallest differential block among all drug combinations when the lidocaine injection was followed by capsaicin (fig. 4).

Dose-Response Studies

N-Methyl amitriptyline and amitriptyline at concentrations of 0.0625, 0.125, and 0.25% produced dose-dependent and predominantly nociceptive block when the injection was followed by capsaicin (figs. 5 and 6, respectively). With amitriptyline at a concentration of 0.25%, the motor block decreased when followed by capsaicin (fig. 6A).

Injection of capsaicin, normal saline, or vehicle only (solvent of 10% ethanol, 10% Tween 80, and 80% normal saline) caused no detectable block. In addition, injection of the vehicle 10 min after the respective LA produced an immediate and short-lived (2-3 min) intensification of both motor and nociceptive block, with overall no significant differential block (figs. 1-4 and table 1).

Intragroup comparison demonstrated significant differences among the different dosing groups (tables 1 and 2). Moreover, the complete recovery time and amount of block data summarized in tables 1 and 2, respectively, show that more hydrophobic drugs such as amitriptyline and bupivacaine (log *P* value/octanol buffer coefficient of 4.9 and 3.4, respectively) displayed significantly more differential block than the hydrophilic lidocaine (log *P* value of 2.3).

Discussion

We show that, in addition to the relatively impermeant permanently charged LA QX-314, permanently charged permeant LAs (*N*-methyl amitriptyline) also produce a pronounced differential rat sciatic nerve blockade when coinjecting with or followed by an injection of capsaicin. In addition, tertiary amine LAs also provide enhanced and longer-lasting differential block when followed by capsaicin, and more hydrophobic drugs elicit a larger differential block. Overall, this finding should add to the candidate drug pool available for further preclinical development of sensory-selective LAs.

Several potential mechanisms could explain our findings. The tissue around the sciatic nerve probably has a physiologic pH of 7.4, and therefore the LAs injected around the nerve will produce one of two forms, depending on its pK_a : protonated or neutral. The protonated form is relatively lipid insoluble and therefore cannot penetrate membranes as readily as the neutral form can. The neutral form will penetrate the membrane and, once inside the cell, convert to the protonated form, which blocks sodium channels by binding to the LA receptor located within the inner cavity.¹⁹ For some LAs, the neutral form may itself also be able to block the channel but is present at lower concentrations. The addition of capsaicin may allow the protonated form to enter the pain fibers selectively through the pore of TRPV1 channels and increase the efficacy and duration of the nociceptive block.

Furthermore, capsaicin-induced activation may lead to the opening of other large pores, such as pannexins,²⁰ providing an additional pathway for the protonated form to enter selectively into nociceptors.

Capsaicin-induced depolarization leads to activation and subsequent inactivation of sodium channels, which will “sensitize” them to the effect of LAs by virtue of their higher affinity to inactivated sodium channels.²¹ For example, *in vitro*, the affinity of amitriptyline, bupivacaine, and lidocaine is approximately 44, 19, and 20 times higher, respectively, for the inactivated state than for the resting state.^{22,23}

After activation of TRPV1 channels, the cytoplasm of C fibers becomes more acidic²⁴ and therefore would increase the charged form of LAs within the cell, which is generally more potent than its neutral counterpart²⁵ and leaves the cell more slowly.²⁶ Another possibility is that calcium entry associated with TRPV1 activation somehow induces more potent action by the intracellular LAs, perhaps because of changes in the phosphorylation state of the sodium channels.

Our results for permeant LAs demonstrate a nociceptor-predominant sciatic nerve block, but not the nociceptor-selective sciatic nerve block found for the nearly membrane-impermeable LA QX-314 when followed by capsaicin.¹ This result suggests that capsaicin facilitates the entrance of LAs into the nociceptive nerve fibers through TRPV1 channels but does not interfere substantially with traditional transmembrane crossing of LAs into motor fibers. However, we were surprised to find that simultaneous application of capsaicin decreased the absolute duration of motor block for the more hydrophobic drugs amitriptyline and bupivacaine. The injection of capsaicin could at least temporarily slightly decrease the tissue pH, causing more LA molecules to be positively charged and in turn decreasing the number of LA molecules able to enter the motor nerve fibers. Also, the pK_a of lidocaine (7.8) is lower than those of bupivacaine (8.1) and amitriptyline (9.5). Therefore, a significantly higher percentage of lidocaine will be in the uncharged form and therefore available to block motor fibers, in keeping with our results that showed the largest motor block with the drug of lowest pK_a (lidocaine).

It also seems that the vehicle itself may play a minor role in the nerve blockade. Injection of the vehicle (10% ethanol, 10% Tween 80, and 80% normal saline) 10 min after bupivacaine or lidocaine led to an intensification of both motor and nociceptive block (figs. 3 and 4). This finding is consistent with the known nerve blocking properties of ethanol, and with TRPV1 activation by ethanol.²⁷

Finally, given that sodium channels are not the only targets of LAs, the effects demonstrated here might be partly due to differential actions of the various tertiary and quaternary agents on K⁺ channels, Ca²⁺ channels, various ligand-gated channels, second messengers, and substance P neurokinin 1 receptors.^{28,29}

Clinical Implications

The finding that the addition of capsaicin to QX-314¹ produces a nociceptor-selective block has sparked renewed interest in using capsaicin (and its congener resiniferatoxin) to promote differential blockade for regional anesthesia. One concern though is that capsaicin causes a severe burning upon injection. However, in our observations, all rats seemed neurobehaviorally normal upon awakening from a short inhalational anesthesia, as indicated by normal grooming, fluid intake, and exploratory behavior, suggesting that the preceding or concomitant use of LAs eliminated this problem. The path to clinical introduction of novel LAs or LA combinations is usually hampered by toxicity. Although no formal toxicity studies have yet been performed, the overall low concentrations of drugs used by Binshtok *et al.*¹ and in the current study encourage cautious optimism, as does the full return to baseline.

Sciatic nerve block with quaternary ammonium and tertiary amine LAs followed by injection of capsaicin provides a predominantly sensory/nociceptor selective block with a duration that greatly exceeds that produced by the LA alone. Therefore, exploitation of the interaction of TRPV1 receptor agonists and several chemically distinct groups of LAs seems to be a promising path toward regional analgesia without motor block. Besides capsaicin, other TRPV1 channels activators and timings of injection in small and large animals will need to be examined to find the optimal concentrations and timing of combinations of LAs and TRPV1 agonists for clinical use.

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References

1. Binshtok AM, Bean BP, Woolf CJ. Inhibition of nociceptors by TRPV1-mediated entry of impermeant sodium channel blockers. *Nature* 2007;449:607–10. [PubMed: 17914397]
2. Strichartz GR. The inhibition of sodium currents in myelinated nerve by quaternary derivatives of lidocaine. *J Gen Physiol* 1973;62:37–57.
3. Lim TK, Macleod BA, Ries CR, Schwarz SK. The quaternary lidocaine derivative, QX-314, produces long-lasting local anesthesia in animal models *in vivo*. *ANESTHESIOLOGY* 2007;107:305–11. [PubMed: 17667576]
4. Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D. The capsaicin receptor: A heat-activated ion channel in the pain pathway. *Nature* 1997;389:816–24. [PubMed: 9349813]
5. Tominaga M, Caterina MJ, Malmberg AB, Rosen TA, Gilbert H, Skinner K, Raumann BE, Basbaum AI, Julius D. The cloned capsaicin receptor integrates multiple pain-producing stimuli. *Neuron* 1998;21:531–43. [PubMed: 9768840]
6. Woolf CJ, Ma Q. Nociceptors: Noxious stimulus detectors. *Neuron* 2007;55:353–64. [PubMed: 17678850]

7. Knotkova H, Pappagallo M, Szallasi A. Capsaicin (TRPV1 agonist) therapy for pain relief: Farewell or revival? *Clin J Pain* 2008;24:142–54. [PubMed: 18209521]
8. Immke DC, Gavva NR. The TRPV1 receptor and nociception. *Semin Cell Dev Biol* 2006;17:582–91. [PubMed: 17196854]
9. Myers BR, Julius D. TRP channel structural biology: New roles for an old fold. *Neuron* 2007;54:847–50. [PubMed: 17582323]
10. Wang H, Woolf CJ. Pain TRPs. *Neuron* 2005;46:9–12. [PubMed: 15820689]
11. Gibson SJ, McGregor G, Bloom SR, Polak JM, Wall PD. Local application of capsaicin to one sciatic nerve of the adult rat induces a marked depletion in the peptide content of the lumbar dorsal horn. *Neuroscience* 1982;7:3153–62. [PubMed: 7162630]
12. Cortright DN, Krause JE, Broom DC. TRP channels and pain. *Biochim Biophys Acta* 2007;1772:978–88. [PubMed: 17467247]
13. Gerner P, Haderer AE, Mujtaba M, Sudoh Y, Narang S, Abdi S, Srinivasa V, Pertl C, Wang GK. Assessment of differential blockade by amitriptyline and its *N*-methyl derivative in different species by different routes. *ANESTHESIOLOGY* 2003;98:1484–90. [PubMed: 12766662]
14. Fridrich P, Eappen S, Jaeger W, Schernhammer E, Zizza AM, Wang GK, Gerner P. Phase Ia and Ib study of amitriptyline for ulnar nerve block in humans: Side effects and efficacy. *ANESTHESIOLOGY* 2004;100:1511–8. [PubMed: 15166572]
15. Thalhammer JG, Vladimirova M, Bershadsky B, Strichartz GR. Neurologic evaluation of the rat during sciatic nerve block with lidocaine. *ANESTHESIOLOGY* 1995;82:1013–25. [PubMed: 7717536]
16. Hung YC, Kau YC, Zizza AM, Edrich T, Zurakowski D, Myers RR, Wang GK, Gerner P. Ephedrine blocks rat sciatic nerve *in vivo* and sodium channels *in vitro*. *ANESTHESIOLOGY* 2005;103:1246–52. [PubMed: 16306739]
17. Kau YC, Hung YC, Zizza AM, Zurakowski D, Greco WR, Wang GK, Gerner P. Efficacy of lidocaine or bupivacaine combined with ephedrine in rat sciatic nerve block. *Reg Anesth Pain Med* 2006;31:14–8. [PubMed: 16418019]
18. Lipsitz SR, Kim K, Zhao L. Analysis of repeated categorical data using generalized estimating equations. *Stat Med* 1994;13:1149–63. [PubMed: 8091041]
19. Hille B. The pH-dependent rate of action of local anesthetics on the node of Ranvier. *J Gen Physiol* 1977;69:475–96. [PubMed: 16078]
20. Pelegrin P, Surprenant A. Pannexin-1 couples to maitotoxin- and nigericin-induced interleukin-1 β release through a dye uptake-independent pathway. *J Biol Chem* 2007;282:2386–94. [PubMed: 17121814]
21. Hille B. Local anesthetics: Hydrophilic and hydrophobic pathways for the drug-receptor reaction. *J Gen Physiol* 1977;69:497–515. [PubMed: 300786]
22. Gerner P, Mujtaba M, Sinnott CJ, Wang GK. Amitriptyline *versus* bupivacaine in rat sciatic nerve blockade. *ANESTHESIOLOGY* 2001;94:661–7. [PubMed: 11379688]
23. Bean BP, Cohen CJ, Tsien RW. Lidocaine block of cardiac sodium channels. *J Gen Physiol* 1983;81:613–42. [PubMed: 6306139]
24. Hellwig N, Plant TD, Janson W, Schafer M, Schultz G, Schaefer M. TRPV1 acts as proton channel to induce acidification in nociceptive neurons. *J Biol Chem* 2004;279:34553–61. [PubMed: 15173182]
25. Nettleton J, Wang GK. pH-dependent binding of local anesthetics in single batrachotoxin-activated Na⁺ channels: Cocaine *versus* quaternary compounds. *Biophys J* 1990;58:95–106. [PubMed: 2166603]
26. Schwarz W, Palade PT, Hille B. Local anesthetics: Effect of pH on use-dependent block of sodium channels in frog muscle. *Biophys J* 1977;20:343–68. [PubMed: 21711]
27. Trevisani M, Smart D, Gunthorpe MJ, Tognetto M, Barbieri M, Campi B, Amadesi S, Gray J, Jerman JC, Brough SJ, Owen D, Smith GD, Randall AD, Harrison S, Bianchi A, Davis JB, Geppetti P. Ethanol elicits and potentiates nociceptor responses *via* the vanilloid receptor-1. *Nat Neurosci* 2002;5:546–51. [PubMed: 11992116]
28. Butterworth JF, Strichartz GR. Molecular mechanisms of local anesthesia: A review. *ANESTHESIOLOGY* 1990;72:711–34. [PubMed: 2157353]

29. Li YM, Wingrove DE, Too HP, Marnerakis M, Stimson ER, Strichartz GR, Maggio JE. Local anesthetics inhibit substance P binding and evoked increases in intracellular Ca^{2+} . ANESTHESIOLOGY 1995;82:166-73. [PubMed: 7530414]

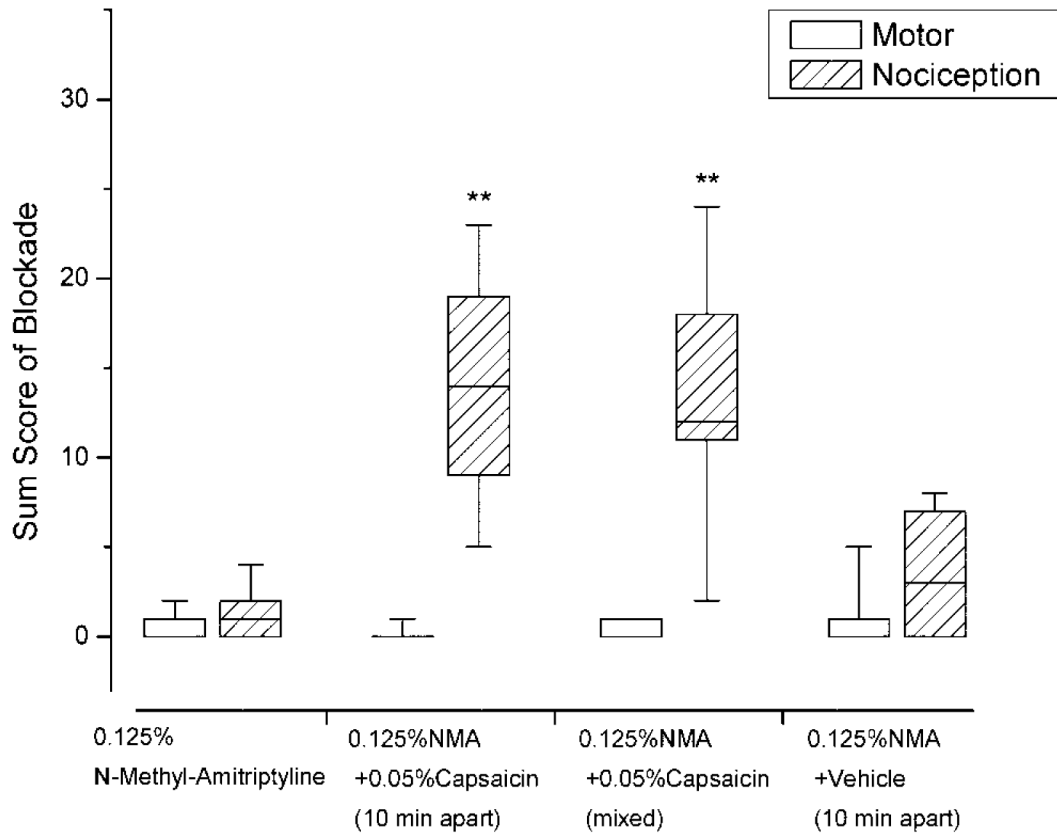


Fig. 1. Sciatic nerve block with 0.2 ml *N*-methyl amitriptyline (NMA) at 0.125%, alone or in combination with 0.05% capsaicin (simultaneously applied/mixed or 10 min later), or followed by vehicle only. $n = 8$ rats/group. Data are presented as sum score of blockade for motor or nociceptive function. The *center line* is the median, the *lower and upper boundaries* are the 25th and 75th percentiles, and the *error bars* are the 5th and 95th percentiles. ** $P < 0.01$ for nociceptive block of NMA combined with capsaicin *versus* NMA alone.

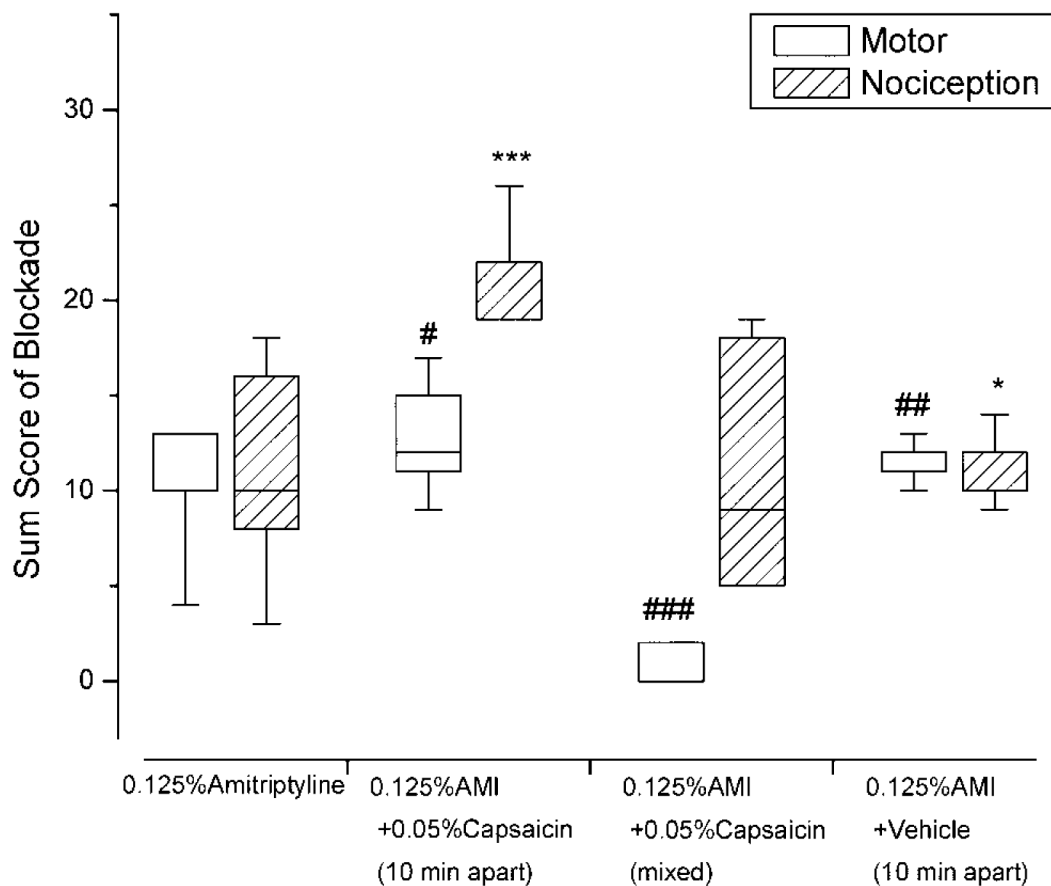


Fig. 2. Sciatic nerve block with 0.2 ml amitriptyline (AMI) at 0.125%, alone or in combination with 0.05% capsaicin (simultaneously applied/mixed or 10 min later), or followed by vehicle only. $n = 8$ rats/group. Data are presented as sum score of blockade for motor or nociceptive function. The *center line* is the median, the *lower and upper boundaries* are the 25th and 75th percentiles, and the *error bars* are the 5th and 95th percentiles. * $P < 0.05/3$ (0.0167), *** $P < 0.001$ for nociceptive block for respective amitriptyline-capsaicin or amitriptyline-capsaicin vehicle combinations *versus* amitriptyline alone. # $P < 0.05/3$ (0.0167), ## $P < 0.01$, ### $P < 0.001$ for motor block for amitriptyline-capsaicin or amitriptyline-capsaicin vehicle combinations *versus* amitriptyline alone.

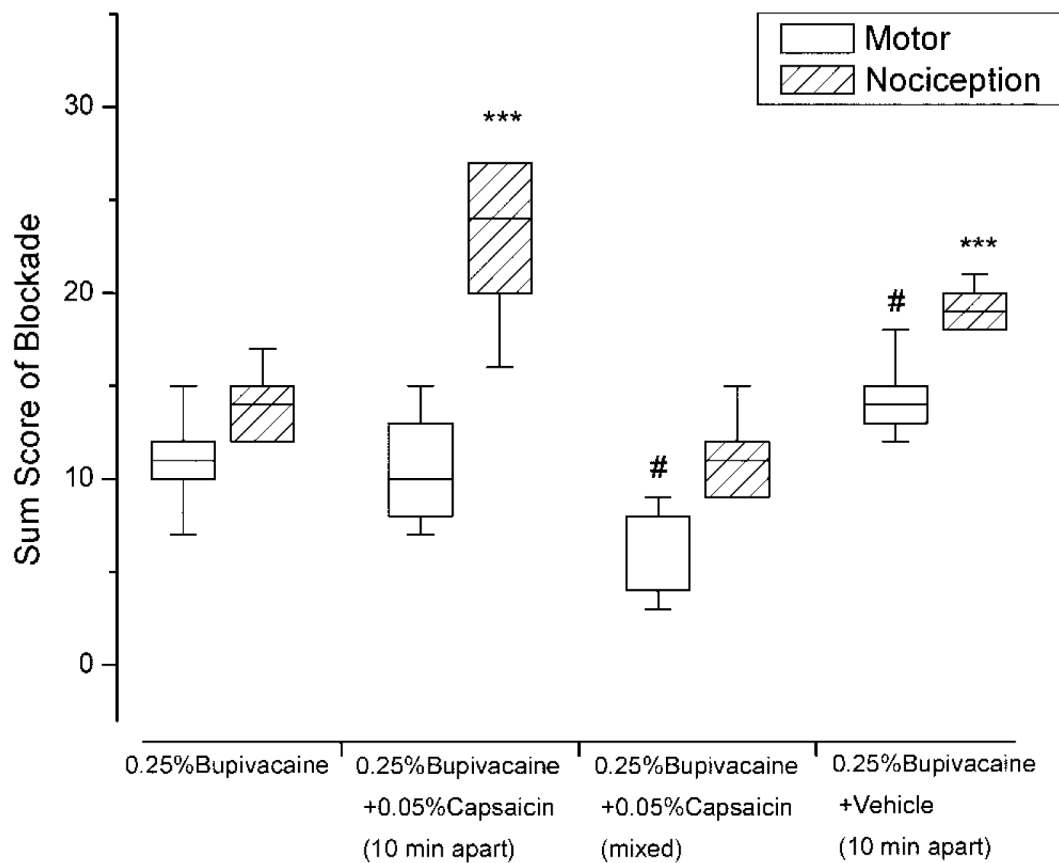


Fig. 3. Sciatic nerve block with 0.2 ml bupivacaine at 0.25%, alone or in combination with 0.05% capsaicin (simultaneously applied/mixed or 10 min later), or followed by vehicle only. $n = 8$ rats/group. Data are presented as sum score of blockade for motor or nociceptive function. The *center line* is the median, the *lower and upper boundaries* are the 25th and 75th percentiles, and the *error bars* are the 5th and 95th percentiles. *** $P < 0.001$ for nociceptive block for respective bupivacaine-capsaicin or bupivacaine-capsaicin vehicle combinations *versus* bupivacaine alone. # indicates $P < 0.05/3$ (0.0167) for motor block for bupivacaine-capsaicin or bupivacaine-capsaicin vehicle combinations *versus* bupivacaine alone.

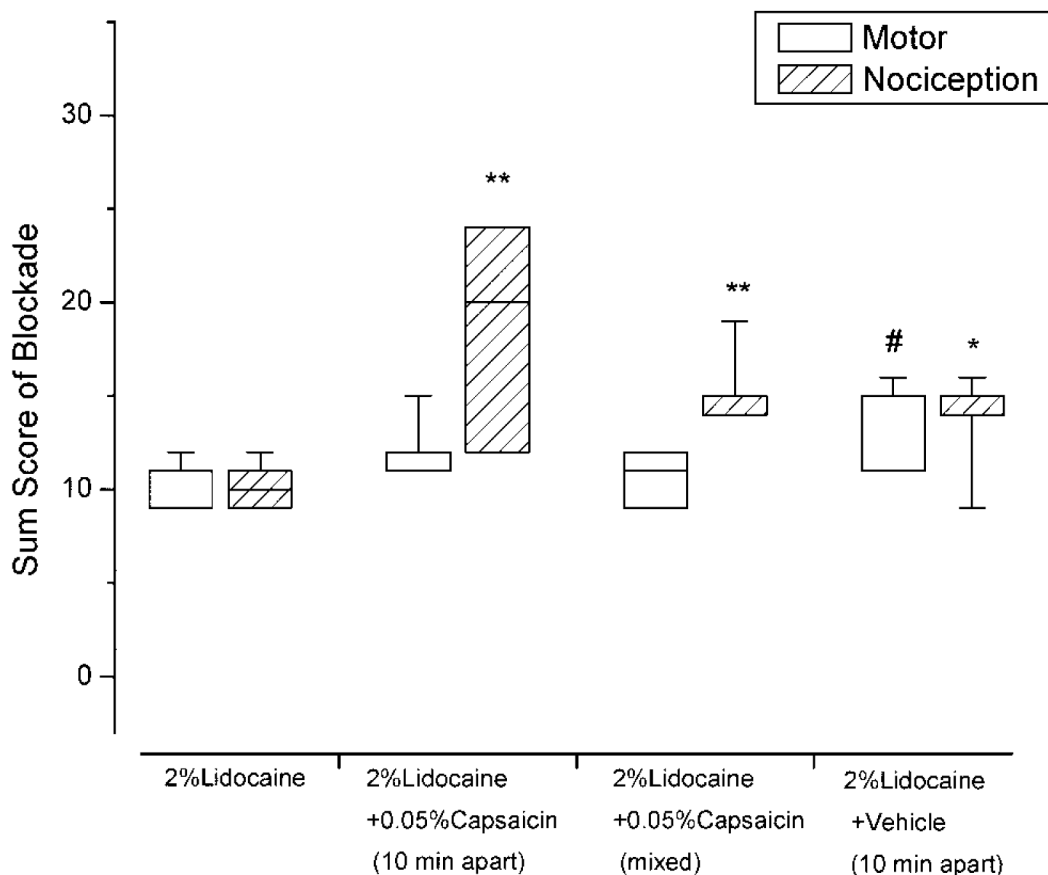


Fig. 4.

Sciatic nerve block with 0.2 ml lidocaine at 2%, alone or in combination with 0.05% capsaicin (simultaneously applied/mixed or 10 min later), or followed by vehicle only. $n = 8$ rats/group. Data are presented as sum score of blockade for motor or nociceptive function. The *center line* is the median, the *lower and upper boundaries* are the 25th and 75th percentiles, and the *error bars* are the 5th and 95th percentiles. * $P < 0.05/3$ (0.0167), ** $P < 0.01$ for nociceptive block for respective lidocaine-capsaicin or lidocaine-capsaicin vehicle combinations *versus* lidocaine alone. # $P < 0.05/3$ (0.0167) for motor block for lidocaine-capsaicin vehicle combination *versus* lidocaine alone.

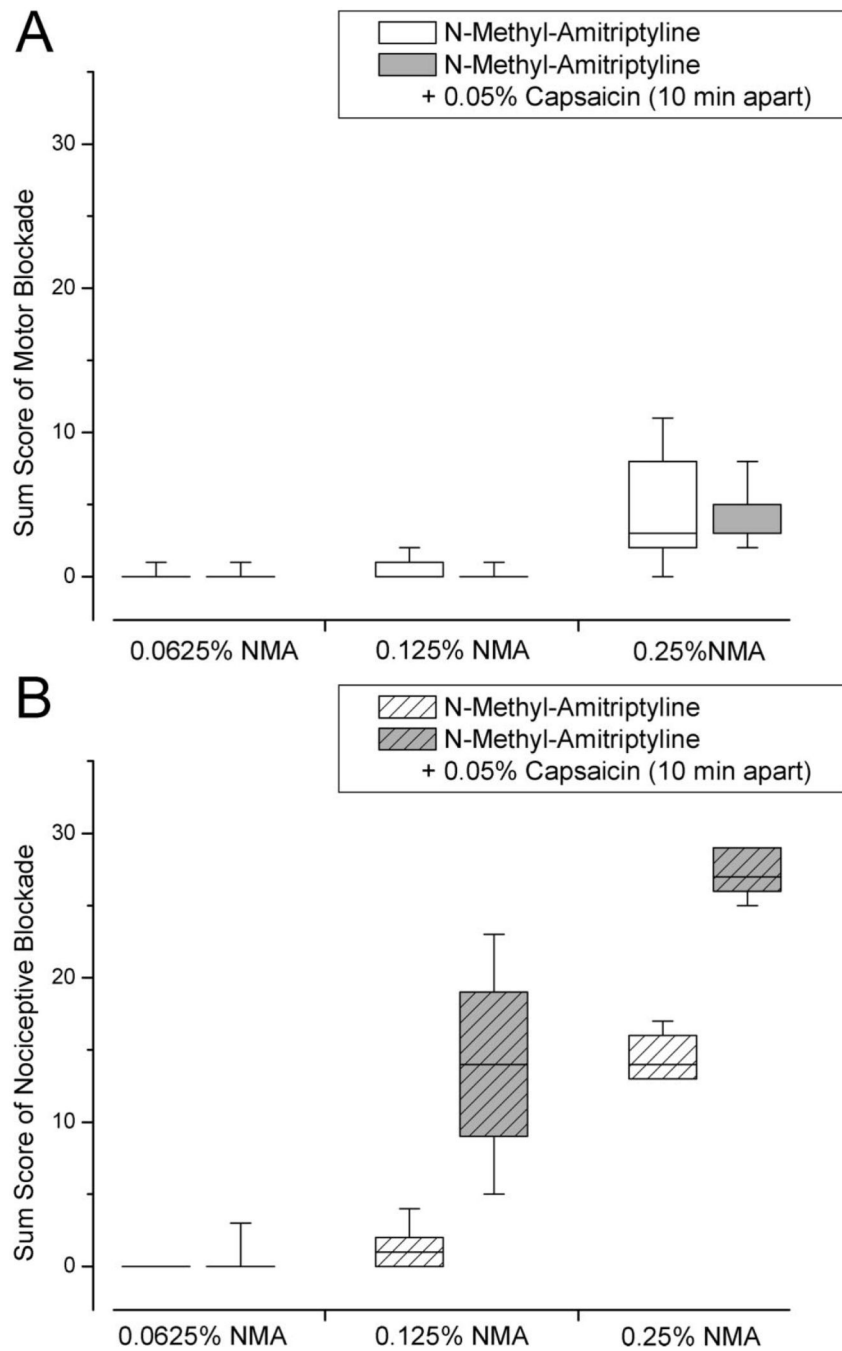


Fig. 5. Dose-response studies for *N*-methyl amitriptyline (NMA), alone or in combination with 0.05% capsaicin (applied 10 min later). $n = 8$ rats/group. Data are presented as sum score of blockade for motor (A) or nociceptive (B) function. The *center line* is the median, the *lower and upper boundaries* are the 25th and 75th percentiles, and the *error bars* are the 5th and 95th percentiles.

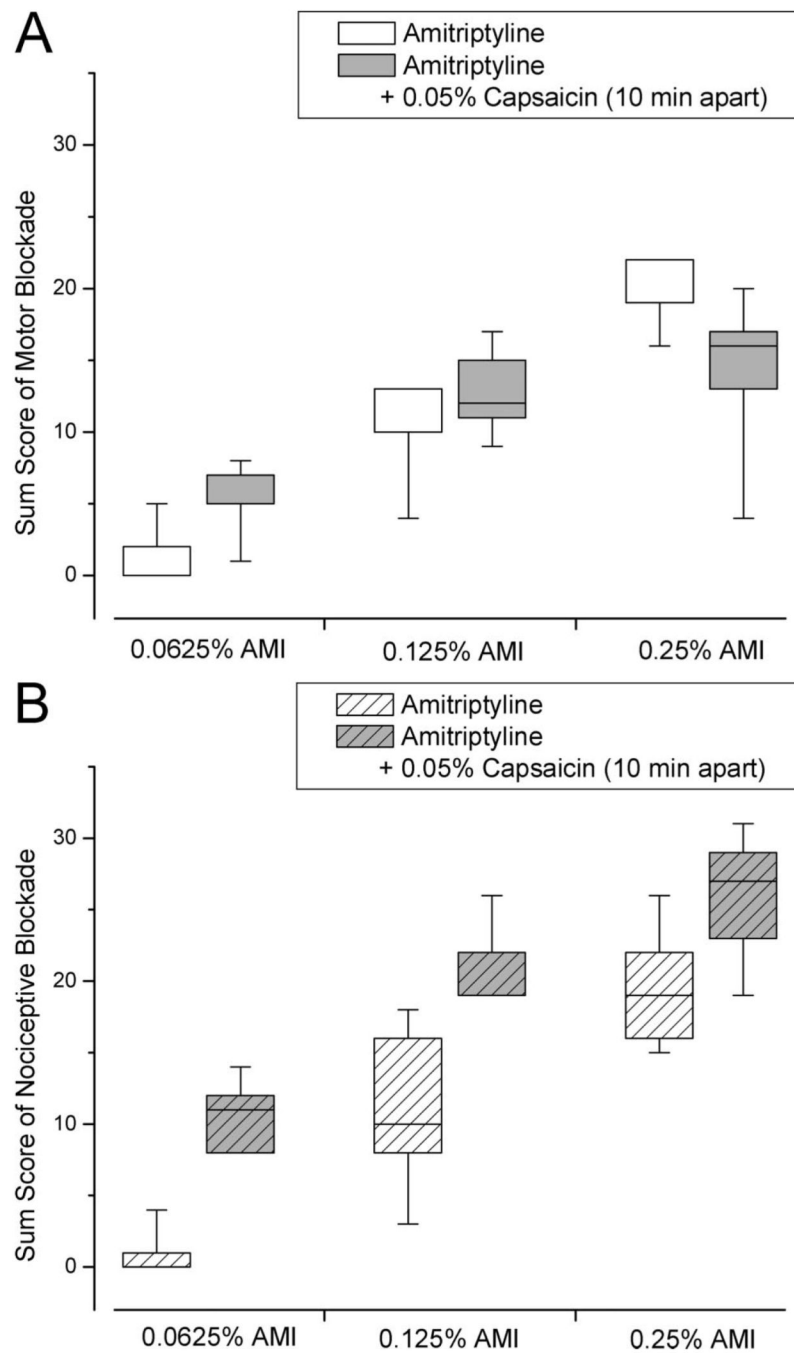


Fig. 6. Dose-response studies for amitriptyline (AMI), alone or in combination with 0.05% capsaicin (applied 10 min later). $n = 8$ rats/group. Data are presented as sum score of blockade for motor (A) or nociceptive (B) function. The *center line* is the median, the *lower and upper boundaries* are the 25th and 75th percentiles, and the *error bars* are the 5th and 95th percentiles.

Table 1
Complete Recovery Times (in Hours) for Drug Alone, Drug Combined with Capsaicin (Mixed or 10 min Apart), or Drug Followed by Capsaicin Vehicle

	Blockade	Drug Alone		Drug + 0.05% Capsaicin (10 min apart)		Drug + 0.05% Capsaicin (mixed)		Drug + Vehicle (10 min apart)	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
0.0625% <i>N</i> -Methyl amitriptyline	Motor	0.12	0.08	0.06	0.06				
	Nociception	0.00	0.00	0.13	0.08				
0.125% <i>N</i> -Methyl amitriptyline	Motor	0.31	0.16	0.04	0.04	0.12	0.06	0.38	0.18
	Nociception	0.67	0.23	11.63	1.74	10.87	2.07	0.81	0.21
0.25% <i>N</i> -Methyl amitriptyline	Motor	1.44	0.32	1.81	0.21				
	Nociception	3.63	0.18	33.00	1.13				
0.0625% Amitriptyline	Motor	0.44	0.15	1.38	0.08				
	Nociception	0.25	0.16	2.88	0.29				
0.125% Amitriptyline	Motor	3.44	0.37	3.25	0.31	0.44	0.22	2.62	0.18
	Nociception	4.56	1.04	17.25	0.75	2.69	0.69	2.13	0.21
0.25% Amitriptyline	Motor	9.13	1.33	2.31	0.63				
	Nociception	11.25	2.12	33.37	3.08				
0.25% Bupivacaine	Motor	2.31	0.21	2.31	0.21	1.56	0.11	2.87	0.23
	Nociception	2.50	1.89	23.14	3.32	1.63	0.13	4.75	0.25
2% Lidocaine	Motor	1.13	0.08	1.69	0.09	1.50	0.13	2.00	0.16
	Nociception	1.25	0.09	7.62	1.47	3.00	0.27	2.12	0.23

Table 2
 Pair-wise Analysis of Group Effect and Group and Time Effect for Each Drug Given Alone *versus* Drug with Capsaicin (10 min Apart or Simultaneous) or Capsaicin Vehicle, Showing Chi-square and P Value Obtained Using Generalized Estimating Equations

	Blockade	Drug Alone vs. Drug + 0.05% Capsaicin (10 min apart)		Drug Alone vs. Drug + 0.05% Capsaicin (mixed)		Drug Alone vs. Drug + Vehicle (10 min apart)	
		Chi-square Estimate	P Value	Chi-square Estimate	P Value	Chi-square Estimate	P Value
0.125% N-Methyl amitriptyline	Motor	x	x	x	x	x	x
	Noiception	13.22	0.0013	11.09	0.0039	2.25	0.3239
0.125% Amitriptyline	Motor	8.41	0.0149	14.37	0.0008	12.35	0.0021
	Noiception	14.58	0.0007	7.05	0.0294	8.51	0.0142
0.25% Bupivacaine	Motor	3.28	0.1941	8.73	0.0127	8.57	0.0138
	Noiception	14.15	0.0008	5.51	0.0635	17.70	0.0006
2% Lidocaine	Motor	6.79	0.0336	3.05	0.2177	8.65	0.0132
	Noiception	10.91	0.0043	9.93	0.0070	8.43	0.0148

x = Data did not fit to the model because of the very small sum score (scores consisted predominantly of 0 s, and several 1 s).