LETTERS

# Inhibition of nociceptors by TRPV1-mediated entry of impermeant sodium channel blockers

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**Most local anaesthetics used clinically are relatively hydrophobic molecules that gain access to their blocking site on the sodium channel by diffusing into or through the cell membrane[1]. These anaesthetics block sodium channels and thereby the excitability of all neurons, not just sensory neurons. We tested the possibility of selectively blocking the excitability of primary sensory nociceptor (pain-sensing) neurons by introducing the charged, membrane-impermeant lidocaine derivative QX-314 through the pore of the noxious-heat-sensitive TRPV1 channel. Here we show that charged sodium-channel blockers can be targeted into nociceptors by the application of TRPV1 agonists to produce a pain-specific local anaesthesia. QX-314 applied externally had no effect on the activity of sodium channels in small sensory neurons when applied alone, but when applied in the presence of the TRPV1 agonist capsaicin, QX-314 blocked sodium channels and inhibited excitability. Inhibition by co-applied QX-314 and capsaicin was restricted to neurons expressing TRPV1. Injection of QX-314 together with capsaicin into rat hindpaws produced a long-lasting (more than 2 h) increase in mechanical and thermal nociceptive thresholds. Long-lasting decreases in pain sensitivity were also seen with regional injection of QX-314 and capsaicin near the sciatic nerve; however, in contrast to the effect of lidocaine, the application of QX-314 and capsaicin together was not accompanied by motor or tactile deficits.**

Although the goal of topical or regional anaesthesia is to block the transmission of signals in nociceptors to prevent pain, the administration of local anaesthetics also produces numbness from block of low-threshold pressure and touch receptors, paralysis from block of motor axons, and block of autonomic fibres. Nociceptor neurons are unique in expressing high-threshold transducer channels, including, in most, the TRPV1 receptor, which forms ion channels activated by both noxious heat and capsaicin, the pungent ingredient in chilli peppers[2]. The pore of TRPV1 channels is suggested to be large enough to pass compounds at least as large as FM1-43, a dye of molecular mass 452 Da (ref. 3). QX-314, a positively charged derivative of lidocaine with a molecular mass of 263 Da, has no effect on neuronal sodium channels when applied extracellularly but does block sodium channels when applied intracellularly[4–8]. This indicated to us that it might be possible to use the TRPV1 channel to deliver QX-314 or other charged channel blockers selectively into nociceptor neurons by applying such blockers together with TRPV1 agonists.

We recorded current through voltage-dependent sodium channels by using whole-cell voltage-clamp recordings from adult rat dorsal root ganglion (DRG) neurons and identified nociceptors by responsiveness to a short (1-s) application of 1 μM capsaicin. In all small (24 ± 5 μm (mean ± s.e.m.); $n = 25$) neurons tested, capsaicin produced a prolonged (10 ± 3 s) inward current (Fig. 1a, upper panel), verifying the expression of TRPV1 receptors. Bath application of

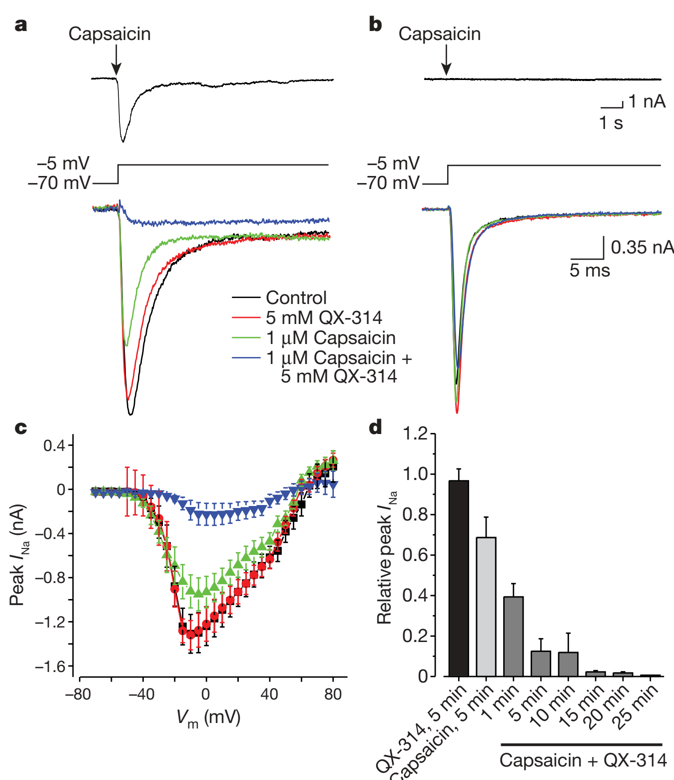


**Figure 1 | Application of extracellular QX-314 (5 mM) and capsaicin (1 μM) together selectively blocks sodium currents in capsaicin-responsive DRG neurons. a**, Lower panel: effect on sodium current (elicited by a step from −70 to −5 mV) of a 10-min wash-in of 5 mM QX-314 alone (red trace), 1 μM capsaicin alone (green trace), and 5mM QX-314 and 1 μM capsaicin applied together (blue trace) in a small (24 μm) capsaicin-sensitive cultured adult DRG neuron. Black trace, control. Upper panel: capsaicin induced a prolonged inward current (holding voltage −70 mV) in this neuron. **b**, Lack of effect on sodium current of the same drug applications on a large (52 μm) capsaicin-insensitive neuron. **c**, Peak inward current as a function of test pulse recorded in control (black), in the presence of 5 mM QX-314 alone (red; 5 min application), 1 μM capsaicin alone (green; 5 min application), and 5 mM QX-314 and 1 μM capsaicin applied together (blue; 5 min application). Results are means ± s.e.m. for 25 small capsaicin-sensitive neurons. Currents were elicited by 150-ms depolarizing steps from a holding potential of −70 mV to a range of test potentials in 5-mV increments. **d**, Time course of the combined effect of capsaicin and QX-314 on peak sodium current. Results are means and s.e.m. of peak sodium current relative to control. ($n = 25$).

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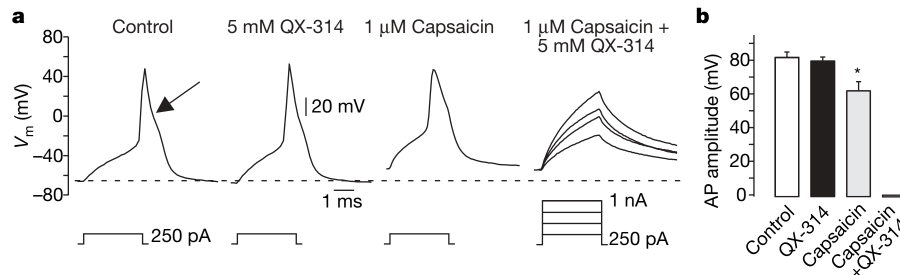


**Figure 2 | Application of QX-314 and capsaicin together blocks excitability in nociceptor neurons. a**, First panel: a depolarizing current step (250 pA, 4 ms) applied to a small (23 μm) DRG neuron evoked a nociceptor-like broad action potential with a prominent deflection on the falling phase (arrow). Second panel: application of QX-314 (5 mM, applied for 2 min) had no effect. Third panel: capsaicin (1 μM, 5 min) decreased the action potential amplitude, probably as a result of a modest decrease in sodium current and inactivation of sodium current secondary to capsaicin-induced depolarization. Fourth panel: QX-314 and capsaicin (2 min) applied together completely abolished action potential generation even with larger current injections. **b**, Mean action potential amplitudes. Results are means and s.e.m. ($n = 25$ for QX-314; $n = 15$ for capsaicin and capsaicin plus QX-314). Asterisk, $P < 0.05$ (Student's *t*-test).

5 mM QX-314 alone had little effect on sodium current elicited by a depolarizing step (decrease by 3% ± 0.5% after application for 5 min; $n = 25$) (Fig. 1a, c). Application of capsaicin alone (1 μM for 5 min) decreased sodium current moderately (31% ± 9% inhibition ($n = 25$), which is consistent with earlier data from nociceptors[9]. However, when QX-314 was applied together with capsaicin, the sodium current was almost totally blocked (inhibition by 98% ± 0.4%; $n = 25$) (Fig. 1a, c). As expected if the block of sodium current resulted from gradual entry of QX-314 through TRPV1 receptors, inhibition developed over several minutes and was nearly complete after 15 min (Fig. 1d).

In large DRG neurons (with a soma diameter of more than 40 μm) (Fig. 1b), capsaicin neither elicited an inward current (10 of 10) nor altered sodium current (3% ± 2% increase after application for 10 min; $n = 10$), as reported[9]. Application of QX-314 and capsaicin together had little or no effect on sodium current in large-diameter neurons (decrease by 9% ± 5% after application for 10 min; $n = 10$). The lack of effect reflects the failure of QX-314 to enter the large neurons, because a fivefold lower concentration of QX-314 (1 mM) applied intracellularly by inclusion in the recording pipette produced a complete block of sodium current within 30–60 s (10 of 10 cells; 48 ± 7 μm diameter; data not shown). Thus, the ability of QX-314 and capsaicin applied together to inhibit sodium current is restricted to neurons expressing TRPV1 receptors, as expected if QX-314 enters neurons through TRPV1 receptors. In current-clamp experiments using physiological internal and external solutions, QX-314 and capsaicin applied together blocked action potential generation completely in small-diameter neurons (Fig. 2; 15 of 15 neurons).

We next examined whether the combination of capsaicin and QX-314 can reduce pain behaviour *in vivo*. Injection of QX-314 alone (10 μl of 2% solution) into the hindpaw of adult rats had no significant effect on the mechanical threshold for eliciting a withdrawal response, as determined by von Frey hairs ($P = 0.33$; Fig. 3a). Capsaicin alone (1 μg μl$^{-1}$) elicited spontaneous flinching (40 ± 6 flinches in 5 min), reflecting the direct irritant action of the capsaicin on nociceptors, and after 15 and 30 min it significantly decreased the mechanical threshold ($P < 0.05$; Fig. 3a), as reported previously[10]. Injection of capsaicin and QX-314 together did not significantly change the number of flinches during the first 5 min after the injection (30 ± 7, $P = 0.24$). However, the combination completely abolished the later decrease in mechanical threshold normally produced by capsaicin alone ($P = 0.14$, measured at 15 min). Moreover, 60 min after the combined injection of capsaicin and QX-314 the mechanical threshold actually increased, reaching twice the baseline value 2 h after injection (46 ± 5 g versus 24 ± 3 g, $P < 0.05$). In three animals the paw was insensitive to even the highest-value von Frey filament (57 g). The elevated mechanical threshold lasted for about 3 h and then gradually returned to basal levels by 4 h (Fig. 3a).

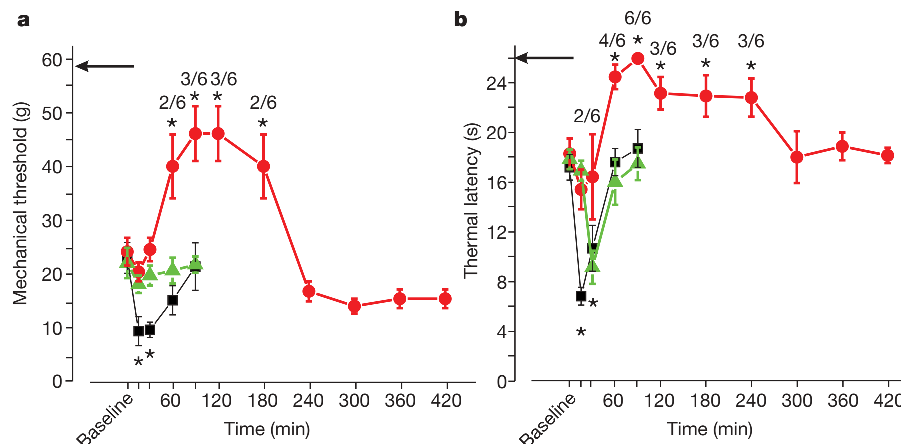


**Figure 3 | Intraplantar injection of capsaicin and QX-314 together produces prolonged local anaesthesia to mechanical (von Frey) and thermal noxious stimuli. a**, Mechanical threshold for paw withdrawal after intraplantar injection of QX-314 alone (2%, 10 μl; green), capsaicin alone (1 μg μl$^{-1}$; black), or QX-314 and capsaicin applied together (red). Results are means ± s.e.m. ($n = 6$ for each group; asterisk, $P < 0.05$ (ANOVA followed by Dunnett's test)). The numbers of animals that did not respond to the highest-value von Frey filament (57 g, arrow) are indicated. **b**, Latency for paw withdrawal from radiant heat (controlled radiant heat stimulus focused on an 8 mm × 8 mm spot on the plantar surface). Results are means ± s.e.m. ($n = 6$ for each group; asterisk, $P < 0.05$ (ANOVA followed by Dunnett's test)). The arrow indicates a cutoff of 26 s; the numbers of animals not responding within this time are indicated.



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Similar effects were found for a controlled radiant noxious heat stimulus. QX-314 alone transiently reduced the thermal response latency 30 min after the injection ($P < 0.01$ at 30 min; $P > 0.05$ for all other time points; Fig. 3b). Capsaicin (1 μg μl$^{-1}$) alone also, as expected, decreased the thermal response latency ($P < 0.01$ at 15 and 30 min; Fig. 3b). However, whereas both QX-314 and capsaicin administered alone increased sensitivity to heat, the application of QX-314 and capsaicin together progressively anaesthetized the animals to noxious heat such that 2 h after the injection no animal reacted to the radiant noxious heat stimulus applied for 25 s. This effect remained for 4 h after injection (Fig. 3b).

We next tested whether the administration of capsaicin and QX-314 together could be used to produce a regional nerve block without the motor deficits produced by lidocaine. Injection of standard 2% lidocaine solution in close proximity to the sciatic nerve caused complete paralysis of the lower limb at 15 min (six of six animals) and complete or partial paralysis was still present at 30 min (motor score 1.67 ± 0.2, $P < 0.01$; Fig. 4c). There was a complete loss of the tactile-stimulus-evoked placing reflex for at least 30 min in all animals, with full recovery of the sensory and motor deficits by 45 min (Fig. 4). In pilot experiments it became clear that although QX-314 is a simple derivative of lidocaine with a similar if not higher 50% inhibitory concentration ($IC_{50}$) for sodium channels, much lower concentrations of QX-314 could be used to produce effective local anaesthesia when applied with capsaicin. Injection of QX-314 (0.2%, 100 μl) alone had no effect on motor function (six of six animals; Fig. 4c) or on mechanical threshold ($P = 0.7$) and noxious thermal response latency ($P = 0.66$; Fig. 4 a, b). Capsaicin alone (0.5 μg μl$^{-1}$, 100 μl) injected near the nerve decreased both the mechanical threshold ($P < 0.05$) and the thermal latency ($P < 0.05$) for 30 min after injection (Fig. 4a, b). During this period four of the six animals showed a sustained flexion of the injected limb leading to a slight impairment of locomotion (mean motor score 0.7 ± 0.2, $P < 0.01$), but movement of the knee and hip as well as the placing reflex were unchanged. We interpret the sensitivity and motor changes as reflecting the activation of nociceptor axons producing a sustained flexion reflex. For the application of QX-314 and capsaicin together into the parasciatic nerve region, we injected QX-314 first, followed 10 min later by capsaicin, with the idea that QX-314 would be present extracellularly and ready to enter TRPV1 channels as soon as they were activated. Indeed, there was little or no behavioural response to the capsaicin injection when preceded by QX-314 injection, and an effective anaesthesia to noxious stimuli developed with a very marked increase in mechanical threshold (all animals showed no response to the stiffest von Frey hair (57 g) compared with a pre-injection withdrawal threshold of 15.2 ± 3.4 g; $P < 0.01$, $n = 6$), and in thermal response latency (22.3 ± 2.3 s versus 14.9 ± 0.4 s; $P < 0.05$, $n = 6$). The changes were evident 15 min after the capsaicin injection for mechanical stimuli and at 30 min for thermal stimuli, and lasted for 90 min (Fig. 4a, b). Five of six animals had no motor deficit (mean motor score 0. 17 ± 0.17, $P = 0.34$; Fig. 4c) or a change in the placing reflex. One animal showed sustained flexion similar to, but more transient than, that observed when capsaicin was injected alone.

These results show that excitability of nociceptors can be selectively inhibited by a membrane-impermeant local anaesthetic derivative when it is applied together with an agonist of TRPV1 receptors, and that this effect is restricted to neurons expressing TRPV1 receptors. Because QX-314 has no effect on neuronal sodium channels when present externally but blocks from the inside of the membrane[4–8], it seems clear that the application of QX-314 together with capsaicin results in a rapid entry of QX-314 molecules into the neurons. The simplest possibility is that QX-314 permeates directly through TRPV1 channels opened by capsaicin. This seems plausible because the pore of TRPV1 channels is unusually wide compared

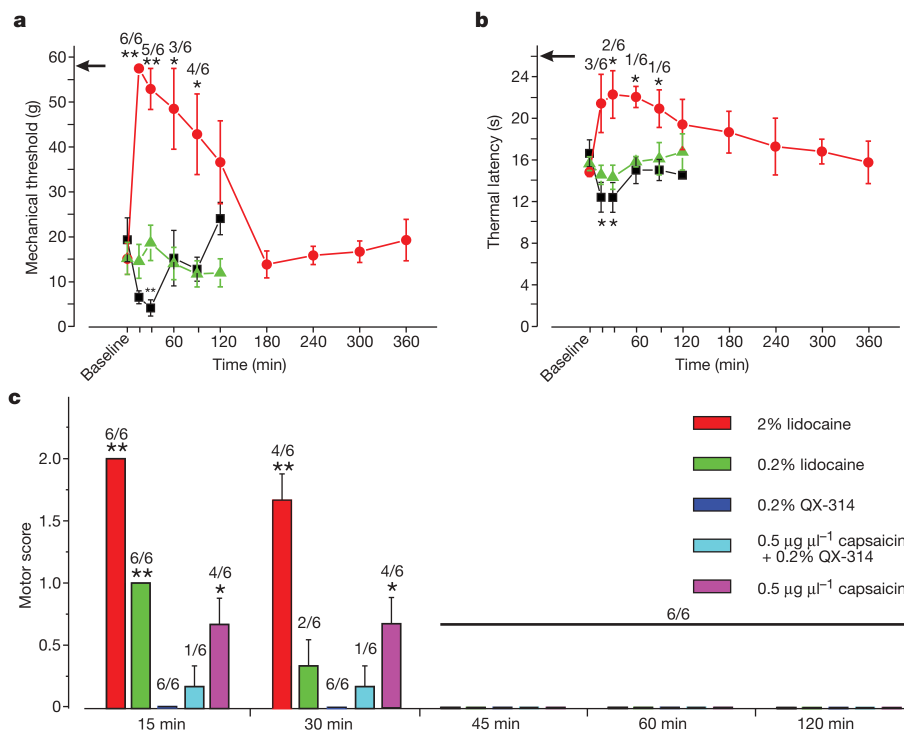


**Figure 4 | Injection of QX-314 followed by capsaicin adjacent to the sciatic nerve anaesthetized the animals to noxious mechanical and thermal stimuli of the hindlimbs without producing any motor deficit. a,** Mechanical threshold for paw withdrawal after sciatic injection of QX-314 alone (0.2%, 100 μl; green), capsaicin alone (0.5 μg μl$^{-1}$, 100 μl; black) or QX-314 injected 10 min before capsaicin (red). Results are means ± s.e.m. ($n = 6$ for each group; asterisk, $P < 0.05$; two asterisks, $P < 0.01$ (ANOVA followed by Dunnett's test)). The numbers of animals that did not respond to the highest force (57 g, arrow) are indicated. **b,** Latency for paw withdrawal from radiant heat. Symbols are as in **a. c,** Change in motor function (score: 2, full paralysis; 1, partial paralysis; 0, no impairment) evaluated after sciatic injection of lidocaine (2% or 0.2%), QX-314 (0.2%), capsaicin (0. 5 μg μl$^{-1}$), or QX-314 followed by capsaicin. Results are means and s.e.m. ($n = 6$ for each group; asterisk, $P < 0.05$; two asterisks, $P < 0.01$ (ANOVA followed by Dunnett's test, referenced to animals before injection; that is, motor score of 0)). Numbers of animals affected are indicated above columns.



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with other cation-selective channels (including other members of the TRP family)[11] and is large enough to allow permeation of the large cations tetraethylammonium and *N*-methyl-D-glucamine[12]. QX-314 can permeate slowly through the pore of the cardiac ($Na_v1.5$) sodium channel[8,13], which probably has a smaller pore than TRPV1 channels. Direct entry of QX-314 would also be consistent with the proposal that that the dye FM1-43, with a larger molecular mass than QX-314, permeates through the pore of TRPV1 channels[3].

Our results show that the application of QX-314 and capsaicin together produces long-lasting decreases in the response to painful mechanical and thermal stimuli, and the regional anaesthesia produced by this mechanism is without the motor deficits that accompany conventional local anaesthesia with lidocaine and other lipid-soluble local anaesthetics. This strategy for blocking pain should be advantageous for generating pain-restricted local anaesthesia when preserving motor and autonomic responses and non-painful sensations is desirable, such as in childbirth and some dental procedures, as well as in treating nociceptor-driven chronic pain such as postherpetic neuralgia.

## METHODS SUMMARY

**Electrophysiology.** Whole-cell voltage-clamp or current-clamp recordings were made at room temperature (21–23 °C) from primary cultures of neurons from DRGs from 6–8-week-old Sprague–Dawley rats. Voltage-clamp recordings used solutions designed to isolate sodium currents by blocking potassium and calcium currents and with decreased external sodium concentrations to improve voltage clamp. Current-clamp recordings were made by using solutions with physiological ionic composition. Solution changes were made in less than 1 s with a multibarrel drug delivery system.

**Behaviour.** For intraplantar injections, rats were habituated to handling and tests were performed with the experimenter blind to the treatment. Intraplantar injections of vehicle (20% ethanol, 5% Tween 20 in saline; 10 μl), capsaicin (1 $\mu g \mu l^{-1}$), QX-314 (2%) or a mixture of capsaicin and QX-314 were made into the left hindpaw. The mechanical threshold was determined with von Frey filaments[14] and thermal sensitivity was tested by latency to paw withdrawal from a controlled radiant heat source[15] focused on an 8 mm × 8 mm spot on the plantar surface.

For sciatic nerve injections, animals were habituated to handling for 10 days, and lidocaine (0.2% or 2%; 100 μl), QX-314 (0.2%, 100 μl) alone, capsaicin (50 μg in 100 μl) alone, or QX-314 followed by capsaicin (10-min interval) were injected into the area of sciatic nerve below the hip joint. Mechanical threshold was determined with von Frey filaments, and thermal sensitivity was tested by latency to paw withdrawal from a radiant heat source. Motor function of the injected leg was assessed every 15 min with a grading score of 0 (no effect; normal gait and limb placement), 1 (limb movement but with abnormal placement and movement) or 2 (complete loss of limb movement)[16]. Walking, climbing and the placing reflex were examined.

**Statistical analysis.** Data are presented as means ± s.e.m.. Statistics were analysed with Student's *t*-test or, when appropriate, one-way analysis of variance followed by Dunnett's test.

**Full Methods** and any associated references are available in the online version of the paper at www.nature.com/nature.

**Received 21 June; accepted 28 August 2007.**


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**Acknowledgements** We thank T. Herbert and S. Lin for technical assistance.

**Author Contributions** B.P.B. conceived of introducing charged ion channel blockers through TRPV1 channels; A.M.B., C.J.W. and B.P.B. designed the experiments; A.M.B. did all experiments; and A.M.B., B.P.B. and C.J.W. wrote the paper.

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doi:10.1038/nature06191 

## METHODS

**Electrophysiology.** DRGs from 6–8-week-old Sprague–Dawley rats were removed and placed in DMEM containing 1% penicillin–streptomycin (Sigma), treated for 90 min with 5 mg $ml^{-1}$ collagenase, 1 mg $ml^{-1}$ Dispase II (Roche), then with 0.25% trypsin for 7 min, followed by 2.5% trypsin inhibitor. Cells were triturated in the presence of DNAase I inhibitor (50 U), centrifuged through 15% BSA (Sigma), resuspended in 1 ml of Neurobasal medium (Sigma), 10 μM AraC, 50 ng $ml^{-1}$ nerve growth factor and 2 ng $ml^{-1}$ GDNF (glial cell line-derived neurotrophic factor) and plated onto 35-mm tissue culture dishes (Becton Dickinson) coated with 500 μg $ml^{-1}$ polylysine and 5 mg $ml^{-1}$ laminin, at 8,000–9,000 cells per dish. Cultures were incubated at 37 °C under 5% $CO_2$. Recordings were made at room temperature within 48 h of plating.

Whole-cell voltage-clamp or current-clamp recordings were made with an Axopatch 200A amplifier (Molecular Devices) and patch pipettes with resistances of 1–2 MΩ. For voltage-clamp recordings the pipette capacitance was decreased by wrapping the shank with Parafilm or coating the shank with Sylgard (Dow Corning). Cell capacitance was compensated for with the use of the amplifier circuitry, and linear leakage currents were subtracted with a *P*/4 procedure. Series resistance (usually 3–7 MΩ and always less than 10 MΩ) was compensated for by about 80%. Voltage-clamp recordings used solutions designed to isolate sodium currents by blocking potassium and calcium currents and with a decreased external sodium concentration, to improve voltage clamp. Pipette solution was 110 mM CsCl, 2 mM $MgCl_2$, 1 mM $CaCl_2$, 11 mM EGTA, 10 mM HEPES, pH adjusted to 7.4 with about 25 mM CsOH. The external solution was 60 mM NaCl, 60 mM choline chloride, 4 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 0.1 mM $CdCl_2$, 15 mM tetraethylammonium chloride, 5 mM 4-aminopyridine, 10 mM glucose, 10 mM HEPES, pH adjusted to 7.4 with NaOH. No correction was made for liquid junction potential (−2.2 mV).

Current-clamp recordings were made with the fast current-clamp mode of the Axopatch 200A amplifier by using a pipette solution of 135 mM potassium gluconate, 2 mM $MgCl_2$, 6 mM KCl, 10 mM HEPES, 5 mM MgATP, 0.5 mM $Li_2GTP$, pH adjusted to 7.4 with KOH, and an external solution of 145 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 10 mM HEPES, 10 mM glucose, pH adjusted to 7.4 with NaOH. Membrane potential was corrected for the liquid junction potential (−15 mV).

Command protocols were generated and data were digitized with a Digidata 1200 A/D interface with pCLAMP 8.2 software (Molecular Devices). Voltage-clamp current records were low-pass filtered at 2 kHz, and current-clamp recordings were low-pass filtered at 10 kHz (−3 dB, four-pole Bessel filter).

Drugs were applied with a multibarrel drug delivery system placed 200–250 μm from the neuron. Solution exchange was complete in less than 1 s.

**Behaviour.** For intraplantar injections, thermal sensitivity was determined with the Hargreaves test method[15] (Ugo Basile Biological Research Apparatus).

For sciatic nerve injections, it was not possible to assay sensory sensitivity during the period of paralysis produced by lidocaine.

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