

## A Randomized, Controlled, Open-Label Study of the Long-Term Effects of NGX-4010, a High-Concentration Capsaicin Patch, on Epidermal Nerve Fiber Density and Sensory Function in Healthy Volunteers

William R. Kennedy,\* Geertrui F. Vanhove,<sup>†</sup> Shiao-ping Lu,<sup>†</sup> Jeffrey Tobias,<sup>†</sup> Keith R. Bley,<sup>†</sup> David Walk,\* Gwen Wendelschafer-Crabb,\* Donald A. Simone,<sup>‡</sup> and Mona M. Selim\*<sup>†</sup>

\*Department of Neurology, University of Minnesota, Minneapolis, Minnesota.

<sup>‡</sup>Diagnostic and Biological Sciences, University of Minnesota, Minneapolis, Minnesota.

<sup>†</sup>NeurogesX, Inc., San Mateo, California.

**Abstract:** Desensitization of nociceptive sensory nerve endings is the basis for the therapeutic use of capsaicin in neuropathic pain syndromes. This study evaluated the pharmacodynamic effects of a single 60-minute application of NGX-4010, a high-concentration (8% w/w) capsaicin patch, on both thighs of healthy volunteers. Epidermal nerve fiber (ENF) density and quantitative sensory testing (QST) using thermal, tactile, and sharp mechanical-pain (pinprick) stimuli were evaluated 1, 12 and 24 weeks after capsaicin exposure. After 1 week, there was about an 80% reduction of ENF density compared to unexposed sites. In addition, there was about an 8% increase in tactile thresholds compared to baseline and the proportion of stimuli reported as sharp mechanical pain decreased by about 15 percentage points. Twelve weeks after exposure to capsaicin, ENF regeneration was evident, but not complete, and sharp mechanical-pain sensation and tactile thresholds did not differ from unexposed sites. Nearly full (93%) ENF recovery was observed at 24 weeks. No statistically significant changes in heat- or cold-detection thresholds were observed at any time point. NGX-4010 was generally well tolerated. Transient, mild warming or burning sensations at the site of application were common adverse effects.

**Perspective:** This article evaluates the effect of a single 60-minute NGX-4010 application on ENF density and QST in healthy volunteers followed for 24 weeks. The results help predict the long-term safety of NGX-4010 applications in patients.

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**Key words:** Epidermal nerve fiber density, capsaicin, NGX-4010, quantitative sensory testing (QST).

Neuropathic pain that can originate following injury to either the peripheral or central nervous systems is a challenging condition to treat. Current medications for neuropathic pain, such as antidepressants, antiepileptics, and opioids, fail to achieve satisfactory pain relief in a high proportion of patients.<sup>10</sup> In contrast to the strategy of attempting to interrupt nociceptive signaling pathways in the spinal cord or the brain, another approach is to reduce the persistent hy-

peractivity at the level of the injured peripheral nerve fibers. For instance, the relatively continuous delivery of local anesthetics such as lidocaine appears to provide pain reduction in patients with postherpetic neuralgia.<sup>12</sup>

Capsaicin is a selective agonist for the transient receptor potential vanilloid 1 receptor (TRPV1), which is preferentially expressed on small-diameter nociceptive neurons. TRPV1 is a molecular integrator of stimuli that are associated with tissue injury, such as heat, acidosis, and inflammatory mediators.<sup>5</sup> Topical application of capsaicin in humans initially activates cutaneous small-diameter sensory fibers, resulting in a burning sensation, erythema, and enhanced sensitivity to noxious and innocuous stimuli.<sup>27</sup> However, repeated topical applications of capsaicin lead to desensitization and degeneration of epidermal nerve fibers (ENFs), which are predominantly C-fiber nociceptors.<sup>2,4,6,9,14,21,24,28</sup>

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Address reprint requests to Dr. William R. Kennedy, University of Minnesota, MMC 187, 420 Delaware St. SE, Minneapolis, MN 55455. E-mail: [kenne001@umn.edu](mailto:kenne001@umn.edu)

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Desensitization and an associated decrease of ENF density is the proposed basis for the therapeutic use of capsaicin in peripheral neuropathic pain syndromes.<sup>3</sup> The effect is reversible, and regeneration of ENFs corresponds with return of the ability to detect painful sensations.<sup>21</sup> Following capsaicin exposure, sensations mediated by non-TRPV1-expressing cutaneous nerve endings are expected to remain unaltered.<sup>29</sup>

There is controversy about the efficacy of low-concentration capsaicin in providing pain relief in painful diabetic neuropathy and postherpetic neuralgia, when compared to placebo.<sup>17,32</sup> However, meta-analyses of other clinical studies with low-concentration formulations suggest efficacy against chronic pain syndromes, especially postherpetic neuralgia.<sup>11,20</sup> Still, frequent administration is required (usually 3 to 5 times daily) for periods of 2 to 6 weeks in order to provide pain relief. Compliance with low-concentration capsaicin dosing regimens is poor, due to inconvenience and burning pain associated with applications for the first weeks.<sup>22</sup> In an effort to overcome these limitations, a high-concentration (8% w/w) capsaicin dermal patch designated NGX-4010 has been developed, with the intention of providing prolonged pain relief following a single administration. A previous study in healthy volunteers showed that NGX-4010 exposure significantly reduced ENF density and was accompanied by a small (1 to 2°C), but still significant, attenuation in cutaneous warm sensitivity 1 week after treatment.<sup>19</sup>

The current study sought to evaluate further the pharmacodynamic effects of a single application of NGX-4010 on ENF density and sensory function as measured by quantitative sensory testing (QST) for touch, sharp mechanical (pinprick) pain, and temperature sensitivity in healthy volunteers at 1, 12, and 24 weeks after exposure. This study employed a longer observation period, a more sensitive ENF density quantification method, and tested more sensory modalities than previously reported.<sup>19</sup>

## Methods

### Study Participants

The study enrolled 36 healthy volunteers between 18 and 40 years of age who were in good health, had intact unscarred skin on both thighs, and had no dermatologic condition with the potential to disrupt skin integrity or alter cutaneous sensory-function assessments. Equal numbers of males and females were studied (18 males and 18 females). The mean age was 26.1 years, mean height was 171.5 cm, and mean weight was 68.8 kg. Eighty-nine percent of volunteers were white, 6% African American, 3% Asian, and 3% were classified as other. Screening included a full medical history, a complete physical exam (mental status, head and neck, dermatological, cardiovascular, respiratory, gastrointestinal, neurological and musculoskeletal assessment), laboratory assessments (complete blood profile; blood chemistry: urea nitrogen, creatinine, sodium, and potassium; liver function: SGOT, AST, SGPT, ALT; measurement of human immunodeficiency virus type I with ELISA; thyroid stim-

ulating hormone; and vitamin B12), and a urine drug screen. Detailed physical examination and laboratory assessments were repeated at the end of the study. All participants received a single 60-minute exposure to NGX-4010 and were included in the safety analyses.

Exclusion criteria were: medical history of systemic disease, painful conditions, surgery, or injury affecting the thighs, use of systemic medications that interacted with the peripheral nervous system (including beta- or alpha-adrenergic blockers, anticonvulsants, antidepressants, or opioids within 30 days prior to commencement of the study), use of prescription medications (apart from oral contraceptives), requirement for ongoing or periodic pain medication, and a history of hypersensitivity to capsaicin, local anesthetics, or adhesives.

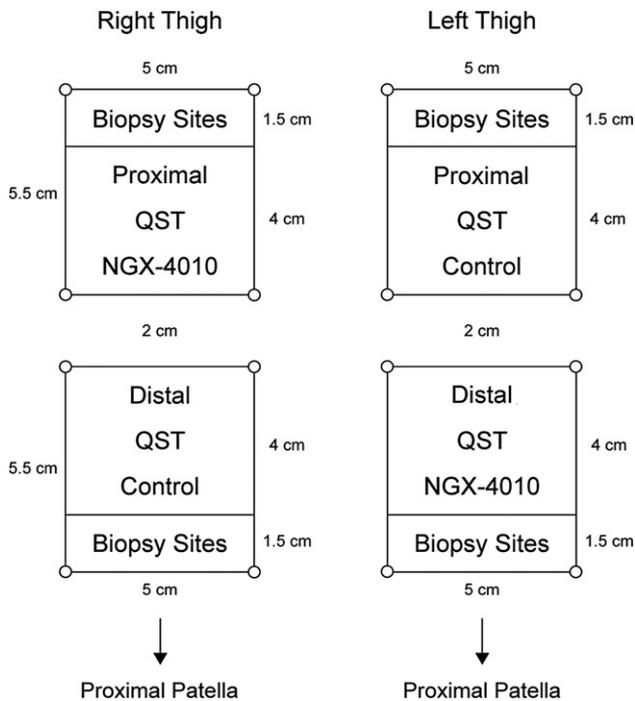
Participants agreed not to use topically applied products containing nonsteroidal anti-inflammatory drugs, menthol, methyl salicylate, local anesthetics, steroids, or capsaicin anywhere on the thighs within 30 days preceding the study patch application (day 0) and for the duration of the 24-week study. Women of childbearing age were required to have a negative pregnancy test and not be breast-feeding. All female participants were required to use an effective method of contraception and/or refrain from conception during the study and for 30 days following exposure to the study medication.

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and was consistent with Good Clinical Practice guidelines and applicable regulatory requirements. The study was approved by the Institutional Review Board at the University of Minnesota. All subjects gave written informed consent and Health Insurance Portability and Accountability Act (HIPAA) authorization prior to initiating study-related procedures.

### Study Design

This was a randomized, controlled, open-label, single-center, phase 1 study conducted over a 24-week period in healthy volunteers. The study measured ENF density and sensory function in 2 areas of skin exposed to NGX-4010 (capsaicin 640  $\mu\text{g}/\text{cm}^2$ ; 8% w/w; NeurogesX, Inc., San Mateo, CA), and in 2 adjacent control areas of skin that remained unexposed (Fig 1).

At the pre-exposure assessment visit, skin areas to be exposed to NGX-4010 and control skin areas were marked bilaterally on the participants' anteromedial thighs. All sites were 5 × 5.5 cm in size. These areas were drawn on transparencies for accurate relocation throughout the 24-week study. Skin blemishes were marked to assure accurate orientation of the transparencies. One week later (day 0), an NGX-4010 patch was applied for 60 minutes. Control skin areas remained unexposed. After patch removal, a cleansing gel was used to remove residual capsaicin remaining on the skin surface. The study participants were monitored for 1 hour after patch removal for vital signs (blood pressure, temperature, heart and respiratory rate) and then discharged. Subjects returned to the clinic at weeks 1, 12



**Figure 1.** Patch application site locations on the thighs of volunteers.

and 24 for skin biopsies, QST, and questions about potential drug-related adverse events.

## Measures and Data Analysis

### Epidermal Nerve Fiber Density

ENF density was measured by immunofluorescence analysis of skin biopsies obtained at weeks 1, 12 and 24. Study participants had a 3-mm diameter skin biopsy taken from each of the 4 designated skin areas (2 control and 2 NGX-4010-exposed areas; Fig 1), using a sterile disposable punch tool (Acu-Punch<sup>®</sup>; Acuderm Inc., Fort Lauderdale, FL). Prior to biopsy, the selected skin areas were anesthetized with subcutaneous 1% lidocaine without epinephrine. Samples were fixed and processed following a standard protocol<sup>13</sup> to localize neural and tissue antigens by indirect immunofluorescence and confocal microscopy.

For quantification of ENFs, nerve (rabbit anti-protein gene product [PGP] 9.5; AbD Serotec, Raleigh, NC) and basement membrane (mouse anti-collagen type IV; Chemicon, Temecula, CA) were localized with Cy2- and Cy3-labeled secondary antibodies (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA). ENF density was determined from confocal z-series images (16 images at 2- $\mu$ m increments) using the Neurolucida<sup>®</sup> software package (MicroBrightField Bioscience, Inc., Williston, VT), providing an equivalent thickness to a 50- $\mu$ m wet section (accounting for section compression during mounting). ENF density was expressed as number of epidermal nerve fibers per millimeter length of epidermis.

### Quantitative Sensory Testing (QST)

QST is the use of precisely measured and repeatable specific somatosensory stimuli to determine the thresh-

old and magnitude of sensations. Tactile, sharp mechanical pain and thermal (cold and heat-pain) sensations were evaluated in the present study. QST was performed at baseline (day 0) prior to application of the capsaicin patch and at weeks 1 ( $\pm 1$  day), 12 ( $\pm 3$  days), and 24 ( $\pm 7$  days) prior to skin biopsy. All sites were tested for one sensory modality before testing the next modality. The same examiners performed the same QST tests at each visit and in the order described below, consistently leaving thermal testing to the end. Random null stimuli included during the testing ensured subject reliability. QST equipment was calibrated before the start of the study.

Tactile threshold was determined with calibrated nylon von Frey-like (Semmes-Weinstein) monofilaments. Testing began with a monofilament that was clearly above threshold, and moved stepwise to a smaller-sized one until the subject's threshold was found (defined as the smallest force perceived at least 50% of the time).

Sharp mechanical-pain sensation was measured using a sharp probe (50- $\mu$ m diameter tip) attached to a calibrated nylon monofilament with a bending force of 95 mN. The probe was applied for 1 to 2 seconds at random sites within a central 9-mm<sup>2</sup> area of the sensory function assessment area (Fig 1). For each stimulus, subjects reported whether they experienced sharp mechanical pain or only touch. The number of trials perceived as sharp out of a total of 10 trials was recorded for each test area.

Sensitivity to innocuous cold and to heat-pain stimuli was determined using the CASE IV<sup>™</sup> (Computer Aided Sensory Evaluator; WR Medical Electronics Co., Stillwater, MN), a computerized thermal stimulator, with a 30-mm  $\times$  30-mm (900 mm<sup>2</sup>) thermode designed to provide a noninvasive, quantitative method of monitoring small nerve fiber function.<sup>7,8</sup>

A 4-, 2-, and 1-step testing algorithm with null stimuli for cold testing was used, and data were reported as changes in temperature of the "just noticeable difference" (JND), of which there were 25 defined levels of stimulus intensity.<sup>7</sup> Assessment of cooling detection threshold was performed prior to assessment of heat-pain detection threshold. The probe started at a baseline temperature of 30°C for cool, and started the test at level JND 13. Subsequent changes in the stimulus intensity depended on whether the stimulus was felt or not. Different intensities of cool stimuli were used and the detection threshold was estimated.<sup>7</sup>

To assess heat-pain sensitivity, nonrepeating ascending steps with null stimuli to obtain heat-pain threshold and magnitude estimates of moderate suprathreshold heat-pain were used.<sup>8</sup> For each heat stimulus, subjects were asked to rate the degree of pain, if any, on a visual analog scale ranging from 0 to 10, where 0 was no pain, 1 was minimal pain, and 10 was the worst pain imaginable. Beginning with a base temperature of 34°C, temperature increased at a rate of 4°C/second to the desired stimulus intensity. Stimulus intensities increased exponentially and were based on the JND obtained in previous studies.<sup>8</sup> Based on a subject's response to stimuli, heat-pain of .5 JND was calculated as the midpoint between

a nonpainful stimulus and the least painful stimulus defining the subject's heat-pain threshold. Heat-pain 5 JND was the stimulus intensity felt to be the intermediate pain level. Both heat-pain .5 and 5 JND were interpolated from a quadratic equation (fitted curve).

## Safety

Safety and tolerability assessments included adverse event monitoring, clinical laboratory tests, evaluation of vital signs, physical examination, and cutaneous assessment scoring, using a 7-point cutaneous irritation scoring system suggested by the US FDA.<sup>31</sup>

## Statistical Analysis

All subjects were included in the analysis. Descriptive statistics and 95% confidence intervals (CIs) were calculated for the ratio of exposed over control skin areas (averaging the sites on the left and right legs for each condition), for ENF density, thermal and tactile thresholds, and sharp mechanical-pain sensation. To test differences between exposed and control sites, a separate analysis was done for each outcome at each time, using a mixed linear model with treatment and subject sex and their interaction as fixed effects, and subject as the random effect. Least squares means (adjusted averages) and associated standard errors for the exposed and control sites, computed using the MIXED procedure of the SAS system (version 9; SAS Institute Inc, Cary, NC) are presented; these are identical to the simple treatment averages except for 24 weeks, when there was no data for 1 woman. The difference between control and exposed sites and its standard error were computed and tested as contrasts in the least squares means.

The number and percentage of subjects reporting exposure-emergent adverse effects (AEs) were summarized by AE preferred terms and AE system organ class using the Medical Dictionary for Regulatory Activities (MedDRA, version 9.0).

## Results

### Patient Disposition

Thirty-seven subjects were screened, 36 were enrolled and 1 subject relocated before completing week 24; however, data for ENF density and QST from this subject were available through week 12 and are included in all analyses (Fig 2).

### Epidermal Nerve Fiber Density

A single application of NGX-4010 resulted in an average 80% reduction in ENFs compared to unexposed sites 1 week after exposure (Figs 3 and 4; Table 1). The least square mean number of immunoreactive nerve fibers per millimeter of epidermis (ENFs/mm) was 48.7 in control areas and 10.4 in skin areas exposed to NGX-4010 ( $P < .0001$ ; Table 1). Return of ENF density with some clustering of distribution was evident by Week 12 when the mean ENF density in exposed and control skin areas was 37.7 and 47.8 ENFs/mm ( $P < .0001$ ), respectively (a 20%

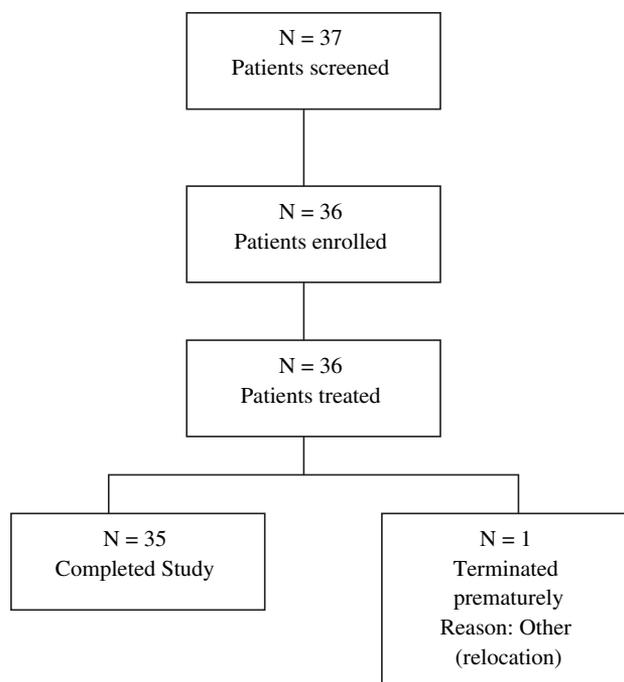


Figure 2. Patient disposition.

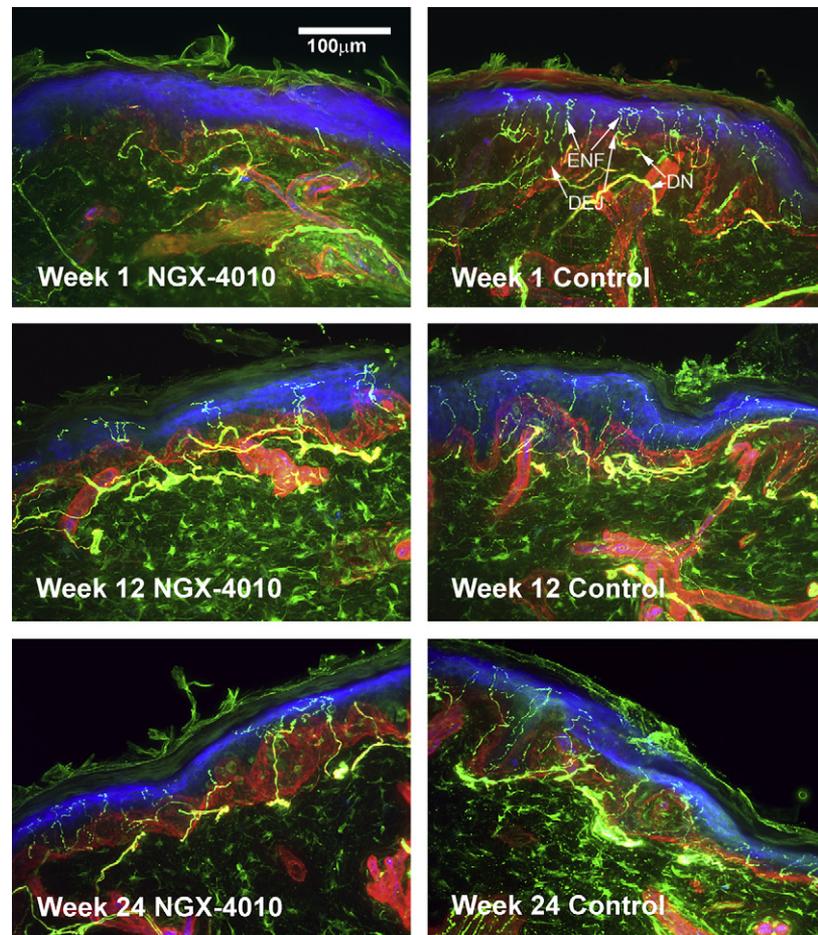
reduction of exposed sites relative to unexposed sites). ENF density in NGX-4010-exposed skin areas was restored to within a few percent of the unexposed areas (93%) by week 24, with means of 41.4 and 44.7 ENFs/mm ( $P = .02$ ) and clustering was less prevalent.

### Quantitative Sensory Testing

The results of QST are presented in Table 2. One week after exposure, the mean tactile threshold increased by approximately 8% from 4.07 mN at baseline to 4.39 mN at NGX-4010-exposed sites (Fig 5A; Table 2;  $P = .02$  compared to control). Tactile thresholds were similar in exposed and control skin areas at weeks 12 and 24.

The detection of sharp mechanical pain was lower at NGX-4010-exposed versus control sites at 1 week after patch application (Fig 5B; Table 2;  $P < .0001$ ). The proportion of stimuli reported as sharp mechanical pain dropped by about 15 percentage points at NGX-4010-exposed sites, from a mean of 87.6% at baseline to 72.5% after 1 week, but remained essentially unchanged at control sites (mean 88.9% at baseline and 88.2% at week 1). Sharp mechanical-pain detection was similar in exposed and control skin areas at weeks 12 and 24.

At weeks 1, 12 and 24, there were no significant differences between exposed and control skin areas in the mean detection threshold for cool sensation or heat-pain perception (Table 2). At baseline, the mean cool perception threshold was higher in exposed skin sites compared to control sites, with a baseline mean threshold of 8.5°C in exposed sites versus 7.7°C in control sites ( $P = .008$ ). At week 1 after application, the mean cooling threshold was 7.4°C at NGX-4010-exposed sites and 7.6°C at control sites ( $P > .05$ ). The mean heat-pain threshold



**Figure 3.** Representative images of epidermal nerve fiber (ENF) density in skin areas exposed to NGX-4010, and in unexposed control areas on the thighs of healthy volunteers. The 6 panels show immunostaining at the time points indicated in the insets. ENF and dermal nerves (DN), immunoreactive for PGP 9.5, are stained yellow or green; the basement membrane, immunoreactive for Type IV Collagen, at the dermal-epidermal junction (DEJ) and around blood vessels is red, and the epidermis is blue. ENFs are clearly lower in density 1 week following capsaicin patch application as compared with control biopsies. ENFs have increased in density 12 weeks after patch application, but still lower than control biopsies. At 24 weeks, ENF density appears similar to the control biopsy. Scale bar = 100  $\mu$ m.

was similar in exposed and control skin areas at baseline and at all subsequent time points (Table 2).

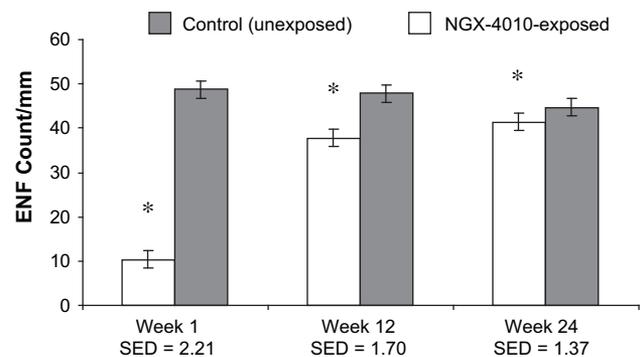
### Safety

All participants completed the full, intended 60-minute study patch application. AEs were reported by 28 of 36 study participants (78%; Table 3). The most common reactions at the sites of capsaicin application were increased sensitivity to heat (eg, warm shower, reported in 53% of subjects), warmth (14%), and burning pain (6%). All application site reactions resolved within 2 days. Cutaneous irritation<sup>31</sup> was mild (maximum score reported was 2 on a 0 to 7 scale described as: definite erythema, readily visible; minimal edema or minimal papular response). No serious AEs occurred. No subjects discontinued the study because of an AE. There were no clinically significant changes in vital signs, clinical laboratory assessments, or physical findings.

### Discussion

Application of capsaicin to the skin of healthy subjects causes a reduction in ENF density that is associated with

transient hyperesthesia followed by a sensory deficiency, called desensitization.<sup>19,21,23-25</sup> The severity of nerve loss depends upon the concentration of capsaicin, depth of penetration into the dermis, and time of exposure.<sup>21</sup> A



**Figure 4.** Mean reductions in epidermal nerve fiber (ENF) density at 1, 12 and 24 weeks after a single NGX-4010 patch application. Number of subjects is 36 for weeks 1 and 12, and 35 for week 24. Values represent least square means and error bars indicate standard errors of the means. \* indicates  $P < .05$  relative to control. SED = Standard error of difference between NGX-4010 and Control.

**Table 1. Summary of ENF Density in NGX-4010-Exposed and Control Skin Areas**

	NGX-4010	CONTROL	DIFFERENCE BETWEEN NGX-4010 AND CONTROL	P-VALUE*	RATIO†
Week 1, n	36	36	36	36	36
LSM (SE) ENFD, counts/mm	10.4 (1.95)	48.7 (1.95)	-38.3 (2.21)	<i>P</i> < .0001	.2 (.02)
95% CI of mean	(6.43, 14.34)	(44.74, 52.66)	(-42.81, -33.83)		.16, .25
Week 12, n	36	36	36	36	36
LSM (SE) ENFD, counts/mm	37.7 (2.13)	47.8 (2.13)	-10.1 (1.7)	<i>P</i> < .0001	.8 (.04)
95% CI of mean	(33.37, 42.01)	(43.47, 52.11)	(-13.55, -6.65)		.71, .87
Week 24 Termination, n	35	35	35	35	35
LSM (SE) ENFD, counts/mm	41.4 (2.08)	44.7 (2.08)	-3.3 (1.37)	<i>P</i> = .02	1 (.03)
95% CI of mean	(37.2, 45.65)	(40.49, 48.94)	(-6.08, -51)		.90, 1.02

Abbreviations: CI, confidence interval; ENFD, epidermal nerve fiber density; LSM, Least-Square-Mean; SE, Standard Error.

NOTE. LSM and SE were estimated from a mixed effects model with treatment and gender as main effects, the interaction of treatment and gender and subject as the random effect.

\**P*-values were computed testing the difference between the LSM of NGX-4010 and control using a paired Student's *t*-test.

†Ratio = average exposed/average control of raw means.

single intradermal injection of capsaicin (2 or 20- $\mu$ g) causes almost complete loss of ENFs and severe changes in the subepidermal neural plexus and superficial dermal nerves,<sup>25</sup> whereas 3 weeks of frequently applied low-concentration (0.075%) topical capsaicin results in 80% reduction in ENF density and moderate thinning of the subepidermal neural plexus.<sup>21</sup>

The high-concentration capsaicin NGX-4010 patch has been evaluated previously in healthy volunteers and was found to reduce ENF density by about 60% relative to control sites 1 week after a single 60-minute application.<sup>19</sup> The 80% reduction in ENF density following a sim-

ilar exposure in the present study is similar to that achieved with prolonged administration of low-concentration capsaicin creams.<sup>21</sup> The differences of the mean ENF density reduction reported in the 2 NGX-4010 studies may be due to differences in ENF quantification methods.<sup>13,19</sup>

The effect of NGX-4010 was reversible, such that by week 12, ENF density in exposed sites was 20% lower than at unexposed sites, and it was restored within a few percent of unexposed sites by week 24. The regeneration of ENFs 24 weeks after exposure to NGX-4010 strongly suggests that the proximal dermal origins of

**Table 2. Sensory Function in NGX-4010-Exposed and Control Skin Areas in Healthy Volunteers**

	NGX-4010	CONTROL	DIFFERENCE BETWEEN NGX-4010 AND CONTROL	P-VALUE*	RATIO†
	LSM (SE)				
Cooling Threshold, JND					
Day 0	8.5 (.4)	7.7 (.4)	.8 (.28)	<i>P</i> = .008	1.1 (.04)
Week 1	7.4 (.28)	7.6 (.28)	-.2 (.18)	<i>P</i> > .05	1 (.02)
Week 12	7.1 (.22)	7.3 (.22)	-.2 (.20)	<i>P</i> > .05	1 (.02)
Week 24	7.3 (.26)	7.4 (.26)	-.1 (.24)	<i>P</i> > .05	1 (.03)
Heat-Pain Threshold, JND 0.5					
Day 0	19. (.21)	19.2 (.21)	-.2 (.10)	<i>P</i> > .05	1 (.01)
Week 1	19.8 (.21)	19.4 (.21)	.3 (.19)	<i>P</i> > .05	1 (.01)
Week 12	19.4 (.17)	19.5 (.17)	-.1 (.11)	<i>P</i> > .05	1 (.01)
Week 24	19.3 (.22)	19.3 (.22)	-.01 (.10)	<i>P</i> > .05	1 (0)
Sharp Mechanical Pain (Pin-prick), %‡					
Day 0	87.6 (2.12)	88.9 (2.12)	-1.3 (1.26)	<i>P</i> > .05	1. (.02)
Week 1	72.5 (3.35)	88.2 (3.35)	-15.7 (3.2)	<i>P</i> < .0001	.8 (.04)
Week 12	78.1 (3.03)	80.4 (3.03)	-2.4 (1.86)	<i>P</i> > .05	1.1 (.14)
Week 24	74.8(3.68)	75. (3.68)	-.3 (2.5)	<i>P</i> > .05	1.1 (.1)
Tactile Threshold§					
Day 0	4.07 (.38)	4.03 (.38)	.04 (.2)	<i>P</i> > .05	1.12 (.1)
Week 1	4.39 (.25)	3.9 (.25)	.49 (.2)	<i>P</i> = .02	1.28 (.12)
Week 12	4.14 (.27)	3.99 (.27)	.15 (.14)	<i>P</i> > .05	1.1 (.08)
Week 24	4.52 (.34)	4.49 (.34)	.03 (.13)	<i>P</i> > .05	1.02 (.03)

Abbreviations: LSM, Least-Square-Mean; SE, Standard Error; JND, Just Noticeable Difference.

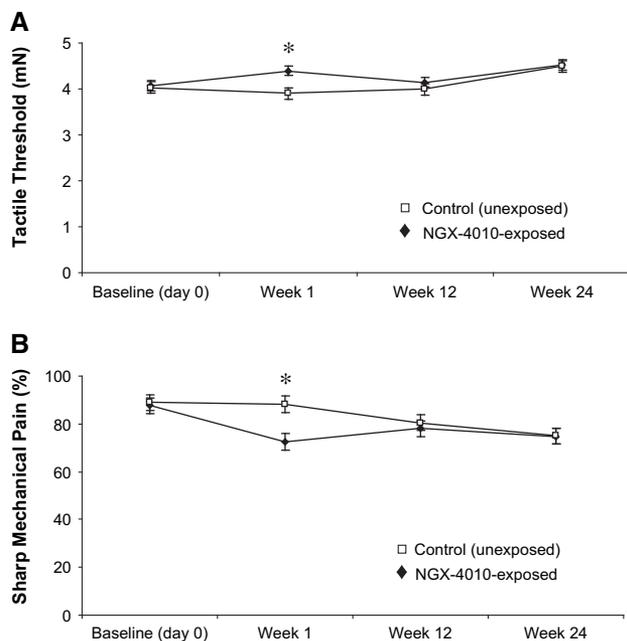
NOTE. LSM and SE were estimated from a mixed effects model with treatment, gender as main effects, the interaction of treatment and gender and subject as the random effect.

\**P*-values were computed testing the difference between the LSM of NGX-4010 and control using a paired Student's *t*-test.

†Ratio = average exposed/average control of raw means.

‡Percentage of applications (n = 10) reported as sharp pain.

§Bending force (mN).



**Figure 5.** Comparison of tactile thresholds (A) and sharp mechanical pain (pinprick) sensations (B) in skin areas exposed to a single application of NGX-4010 or unexposed control areas on the thighs of healthy volunteers. Number of subjects is 36 for baseline (day 0), weeks 1 and 12, and 35 for week 24. Values represent least square means and error bars indicate standard errors of the means. \*indicates  $P < .05$  relative to control.

ENFs remained intact and that only the distal dermal nerve segments and epidermal nerve endings are affected.

Reduction of ENF density following capsaicin exposure is usually associated with impairment of heat-pain and sharp mechanical-pain (pinprick) sensations. This is consistent with the notion that ENFs are predominantly C-fiber nociceptors, although  $A\delta$  fibers participate in mechanical nociception as well and ENFs may include unmyelinated endings of capsaicin-sensitive  $A\delta$  nociceptors that are excited by mechanical and heat stimuli.<sup>16</sup> In our analyses of sensory function (QST) following NGX-4010 exposure, we found a significant 15-percentage-point reduction in sharp mechanical pain (pinprick) and an 8% increase in tactile thresholds 1 week after patch application, and no significant difference from control sites at weeks 12 and 24. We did not detect a statistically significant effect on thermal sensations. Malmberg et al<sup>19</sup> did not detect changes in cold sensitivity following a 60- to 120-minute NGX-4010 patch application, but they did observe an increase in warmth detection thresholds of about 1.5°C. However, the effects of NGX-4010 on heat-pain sensation, tactile, or sharp mechanical-pain thresholds were not studied. Nolano et al<sup>21</sup> showed that prolonged daily exposure to low-dose capsaicin resulted in an 80% decrease in heat-pain, a 50% decrease in sharp mechanical-pain sensation, and an increase in tactile threshold. Sensation returned to near control levels during the 6 weeks after discontinuation of capsaicin. The alterations in cutaneous sensory function observed at week 1 in the presence of a significant reduction of ENF density are far less than found in other

**Table 3. Summary of Exposure-Emergent Adverse Events (AEs)**

	INCIDENCE, N (%)
Total number of subjects reporting 1 or more AEs	28 (78)
Gastrointestinal disorders	
Nausea	1 (3)
General disorders and administration site reactions	
Application site discomfort	1 (3)
Application site increased sensitivity	19 (53)
Application site irritation	1 (3)
Application site pain	2 (6)
Application site paresthesia	1 (3)
Application site warmth	5 (14)
Immune system disorders	
Seasonal allergy	2 (6)
Infections and infestations	
Herpes zoster	1 (3)
Rhinitis	1 (3)
Sinusitis	1 (3)
Upper respiratory tract infection	1 (3)
Nervous system disorders	
Dizziness	1 (3)

studies which used repeated applications of low-concentration capsaicin creams<sup>14,21</sup> or a single exposure to NGX-4010.<sup>19</sup> The high selectivity of capsaicin for the TRPV1 receptor, coupled with the selective expression of TRPV1 in nociceptive sensory nerves, suggests that even with pronounced desensitization of cutaneous nociceptors, other skin sensory nerve endings will be intact and functional.<sup>3,15</sup> Capsaicin-insensitive nerve endings would include those which arise from  $A\beta$ -fibers, which transduce tactile and proprioceptive stimuli. In addition, only a subpopulation of  $A\delta$ -fibers expresses TRPV1,<sup>15</sup> and it has been shown that secondary mechanical hyperalgesia to pinprick produced by acute capsaicin injection is signaled by  $A\delta$  nociceptors that are insensitive to capsaicin.<sup>18</sup> Non-TRPV1-expressing nerve fibers are also capable of transducing thermal stimuli, as they express TRP receptors such as TRPV2 (which is activated at 52°C) and TRPV3 (which is activated at 39°C).<sup>30</sup>

Detection of reduced heat sensitivity is dependent upon the sensitivity of the testing method. A major factor is the size of the probe that delivers thermal stimuli. The thermode excites ENFs in the skin under the probe, as well as nerve fibers in the underlying dermis, the depth of excitement depending upon the strength of stimulation. Skin of subjects exposed to capsaicin is more responsive to stimuli from a probe with a large heated surface than to a small probe because the volume of skin stimulated is many times greater and contains more ENFs that survive capsaicin. A larger probe also excites a greater number of the proximal stumps of degenerated sensory nerves in the dermis than does a smaller probe. Consequently, a small probe is better able to detect small reductions of nociceptive sensitivity such as those seen after topical capsaicin.<sup>14</sup>

Variations in the methods used to test thermal sensation, including the size of the probe, may account for differences in detection of heat-pain sensitivity after

capsaicin exposure. Most clinical studies used stimulators with fairly large probes. We therefore used a commercially available stimulator with a probe size that is identical or similar to probe sizes used in earlier studies. We could not find a decrease in heat-pain sensitivity after exposure to NGX-4010 using the CASE IV™ instrument with a thermal probe 30 × 30 mm (900-mm<sup>2</sup>). Malmberg et al<sup>19</sup> detected a mildly reduced threshold to warm stimuli using a TSA-II system (Medoc Advanced Medical Systems, Ramat Yishai, Israel) with a 16- × 16-mm thermode (256-mm<sup>2</sup>) but did not test heat pain. Much smaller probes with diameter of 3 mm (7.1 mm<sup>2</sup>)<sup>14</sup> and 2 mm (3.1 mm<sup>2</sup>)<sup>21</sup> measured markedly reduced heat-pain sensation in capsaicin exposed skin that was not detected with the large probe. Even though the temperature of the small probe (53°C) was greater than that of the larger probe (50°C) and would produce a greater discharge in individual nociceptors, sensory deficits were observed with the small probe because the critical number of nerve fibers needed to evoke a sensation were not excited in the smaller volume of heated skin. It cannot be excluded that in addition to greater spatial summation obtained with the large probe, remaining fibers after capsaicin were less responsive to heat and this contributed to the decrease in sensation observed using the small probe.

In conclusion, NGX-4010 exposure for 60 minutes in the present study reduced ENF density and resulted in modest, but statistically significant changes in tactile

NGX-4010 Pharmacodynamics in Healthy Volunteer thresholds and sharp mechanical pain detection. Tactile and sharp pain sensations returned to normal within 12 weeks and ENF density returned to within a few percent of normal by 24 weeks after exposure. No changes in thermal sensation were detected; however, the methods used lacked the sensitivity necessary to determine if heat-pain sensation was affected. Exposure to NGX-4010 was generally well tolerated. Mild cutaneous reactions that resolved within 2 days were the most common AEs. No serious AEs occurred during the 24-week study period. These results, along with the recent report of safety and efficacy against painful HIV-associated neuropathy<sup>26</sup> and postherpetic neuralgia<sup>1</sup> in large clinical studies, support further evaluation of NGX-4010 in patients with peripheral neuropathic pain syndromes.

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