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ARTICLE

Effects of Mexiletine and Lacosamide on Nerve Excitability in Healthy Subjects: A Randomized, Double-Blind, Placebo-Controlled, Crossover Study

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Selective voltage-gated sodium channel blockers are of growing interest as treatment for pain. For drug development of such compounds, it would be critical to have a biomarker that can be used for proof-of-mechanism. We aimed to evaluate whether drug-induced changes in sodium conductance can be detected in the peripheral nerve excitability profile in 18 healthy subjects. In a randomized, double-blind, 3-way crossover study, effects of single oral doses of 333 mg mexiletine and 300 mg lacosamide were compared with placebo. On each study visit, motor and sensory nerve excitability measurements of the median nerve were performed (predose; and 3 and 6 hours postdose) using Qtrac. Treatment effects were calculated using an analysis of covariance (ANCOVA) with baseline as covariate. Mexiletine and lacosamide had significant effects on multiple motor and sensory nerve excitability variables. Depolarizing threshold electrotonus (TEd_{40} (40–60 ms)) decreased by mexiletine (estimated difference (ED) −1.37% (95% confidence interval (CI): −2.20, −0.547; $P = 0.002$) and lacosamide (ED −1.27%, 95% CI: −2.10, −0.443; $P = 0.004$) in motor nerves. Moreover, mexiletine and lacosamide decreased superexcitability (less negative) in motor nerves (ED 1.74%, 95% CI: 0.615, 2.87; $P = 0.004$, and ED 1.47%, 95% CI: 0.341, 2.60; $P = 0.013$, respectively). Strength-duration time constant decreased after lacosamide in motor- (ED −0.0342 ms, 95% CI: −0.0571, −0.0112; $P = 0.005$) and sensory nerves (ED −0.0778 ms, 95% CI: −0.116, −0.0399; $P < 0.001$). Mexiletine and lacosamide significantly decrease excitability of motor and sensory nerves, in line with their suggested mechanism of action. Results of this study indicate that nerve excitability threshold tracking can be an effective pharmacodynamic biomarker. The method could be a valuable tool in clinical drug development.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
☑ For clinical drug development of novel sodium channel blockers, a pharmacodynamic biomarker is critical. Based on their mechanism of action, sodium channel blockers are expected to decrease neuronal excitability. Excitability threshold tracking is a peripheral nerve stimulation technique used to estimate motor and sensory nerve excitability.

WHAT QUESTION DID THIS STUDY ADDRESS?
☑ Can drug-induced changes in sodium conductance be detected in the peripheral nerve excitability profile of healthy subjects?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
☑ Orally administered sodium channel blockers (mexiletine and lacosamide) significantly decrease excitability of motor and sensory nerves in healthy subjects.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?
☑ This study shows that excitability threshold tracking can be an effective biomarker in pharmacological studies. It could therefore be a useful tool in early phase drug development of novel sodium channel blockers, as a translational tool, for proof-of-mechanism, to help identify target engagement in healthy subjects and to assist in dose finding for patient studies.

Selective voltage-gated sodium channel (Na$_V$) blockers are subject to growing interest as treatment for pain.[1] It is of importance that pharmacodynamic (PD) effects of such treatments are detected in the early phase of clinical development, preferably in healthy subjects. Detection of PD effects early in the development program is useful as proof-of-mechanism, to show target engagement, to aid in dose escalation study designs, and to assist dose finding for the translation to patient studies. A reliable clinical biomarker for effects of drugs that target Na$_V$-channels is lacking, so development of such a PD biomarker would be highly valuable.

Nerve excitability threshold tracking (NETT), also called nerve excitability testing, is a noninvasive peripheral nerve stimulation technique, which can be used to estimate axonal excitability of motor and sensory nerves.[2,3] Excitability of the axonal membrane is largely dependent on Na$_V$ and potassium channel conductance,[4] and pharmacological modulation of these channels influences axonal excitability. Therefore, we performed a study aimed to evaluate whether pharmacologically induced changes in Na$_V$-conductance can be detected using NETT in healthy subjects. As a proof-of-concept, effects of a single dose of mexiletine and lacosamide, two Na$_V$-blockers that are expected to decrease axonal excitability based on their mechanism of action, were compared with placebo in double-blind fashion. To our knowledge, this is the first placebo-controlled study in which effects of Na$_V$-blockers were investigated on NETT in healthy human subjects and our results encourage the use of NETT as a biomarker in early phase clinical drug development.

METHODS
The study (Netherlands Trial Registry: NL7327) was conducted at Centre for Human Drug Research, Leiden, The Netherlands, in accordance with the Declaration of Helsinki after approval by Ethics Committee Stichting "Beoordeling Ethiek Biomedisch Onderzoek", The Netherlands.

Subjects
Subjects gave signed informed consent before commencement of study activities. Medical screening was performed to determine eligibility. Healthy, male subjects, 18–45 years old, with body mass index between 18 and 30 kg/m^2, were included. Health status was confirmed by evaluation of medical history, physical examination, and laboratory tests. Nicotine users and subjects with a history of drug or alcohol abuse, or a positive test for these substances, were excluded from this study. Subjects with conditions considered to influence electrophysiological measurements were excluded. Use of medication, dietary supplements, CYP450 iso-enzyme modulating products, alcohol, caffeine, and nicotine were prohibited. Strenuous physical activity was prohibited from 48 hours before each study day.

Study design
This was a randomized, double-blind, double-dummy, placebo-controlled, three-way crossover study. On three separate study visits, subjects received a single dose of mexiletine, lacosamide, or placebo in randomized order. Between each visit was a wash-out period of 7 days. On each visit, 3 motor and sensory NETT measurements were performed: predose, and 3 and 6 hours postdose. Blood samples for pharmacokinetic (PK) analysis were drawn predose and before and after each postdose NETT measurement. Evoked pain tests, and intraepidermal electrical stimulation, were performed before and after dosing, these results will be reported separately. Measurements and meals were at approximately the same clock-time, to prevent influence of diurnal variation or food.

Primary objectives were to evaluate the sensitivity of NETT to detect effects of mexiletine and lacosamide, and to evaluate the test–retest reliability of NETT. These outcomes were evaluated with motor and sensory NETT end points, and variability was expressed in coefficient of variation (CV%), respectively. The exploratory objective was to determine concentration-effect relations between the drug concentrations and NETT variables.

No important changes to study methods or trial outcomes were made after the first subject's first dose.

Study drugs
Mexiletine (Namuscla, 167 mg; Lupin Europe GmbH, Frankfurt am Main, Germany) capsules and lacosamide (Vimpat, 100 mg; UCB Pharma S.A., Brussels, Belgium) filmcoated tablets were over-encapsulated. For both treatments, matching placebo was produced, enabling double-blind and double-dummy drug administration.

A dose of 300 mg of mexiletine hydrochloride for a duration of 3 months has been reported to exhibit significant effects on nerve excitability in patients with neuropathic pain.[5] Therefore, a similar dose of 333 mg mexiletine was selected for this study, to reach therapeutic plasma concentrations with a single dose. Moreover, 333 mg mexiletine was deemed to have an acceptable safety profile, as single doses up to 600 mg mexiletine have been administered to healthy subjects.[6]

A single dose of 300 mg lacosamide was chosen, because it would lead to therapeutic concentrations for the treatment of epilepsy and was considered safe for healthy subjects. The suggested reference range based on effect and tolerability is 10–40 μmol/L, or 2.5–10 mg/L.[7,8] Previously reported mean maximum plasma concentration (C_{max}) after a single dose of 300 mg lacosamide was 7.366 mg/L.[9]

Study staff and subjects remained blinded until database lock. The block-randomization was produced using SAS (version 9.4, SAS Institute Inc., Cary, NC) by a statistician uninvolved in the clinical study conduct. Subjects were randomly assigned to one of six treatment sequences in a balanced study design. Randomization numbers were assigned to participants sequentially after medical screening by blinded study staff.

PK analysis
Plasma concentrations of the study drugs were analyzed using a validated liquid-chromatography tandem mass spectrometry method. Mexiletine concentrations were evaluated by Leiden University Medical Centre (Leiden, The Netherlands) laboratory; lacosamide concentrations by the laboratory of Apotheek Haagse Ziekenhuizen (The Hague, The Netherlands). Lower limit of quantification was 0.06 mg/L for mexiletine and 0.75 mg/L for lacosamide. Reproducibility of the assays was in line with the European Medicines Agency (EMA) bioanalytical method development guideline, with CV% < 15%.

Nerve excitability threshold tracking
Motor and sensory nerve excitability of the median nerve was measured using NETT. The nerve was stimulated using surface electrodes (Red Dot; 3M, St. Paul, MN), with the active electrode located at the wrist and the reference 10 cm proximal to the active electrode on the radial side. Electrical stimulation was induced using an isolated bipolar constant current stimulator (DS5; Digitimer, Hertfordshire, UK). Compound muscle action potentials (CMAPs) were recorded from the abductor pollicis brevis, using a belly-tendon montage (Disposable Tab Electrodes; Natus Medical, Pleasanton, CA). Sensory nerve action potentials (SNAPs) were recorded antidromically using ring electrodes (Disposable Wide Ring Electrode; Natus Medical) on digit three. When no SNAP could be recorded from digit three, digit two was used. CMAP and SNAP signals were amplified using an electromyography amplifier (D440-2; DigiTimer), gain 10,000 for sensory measurements and 300 for motor measurements, bandpass filter 3 to 3,000 Hz. Signals were digitized

using an analog-digital convertor (NI-USB-6341; National Instruments, Austin, TX). Hum Bug (Quest Scientific Instruments, North Vancouver, BC, Canada) was used to minimize 50 Hz noise. To maintain stable temperature conditions, the hand and forearm were warmed using a heat blanket (Norm-O-Temp with Maxi-Therm Lite infant hyper-hypothermia blanket; Maxi-Therm, Cincinnati, OH) programmed at 35°C, from 30 minutes prior stimulation until the end of the measurement. Skin temperature was registered before and after the measurement using a temperature probe (BioSignals Plux, Arruda dos Vinhos, Portugal).

Stimulation was guided by QTRAC-S software (version 28-5-2018; Institute of Neurology, London, UK) with the TRONDNF stimulation paradigm (Institute of Neurology). This paradigm and corresponding variables were described previously.[2,3] Each NETT measurement consists of four protocols[10]: (i) stimulus response curve (relationship between stimulus current and amplitude of the muscle/sensory action potential); (ii) strength-duration relationship (relationship between stimulus duration and stimulus charge); (iii) threshold electrotonus (threshold changes during a depolarizing or hyperpolarizing conditioning currents of 10–300 ms, the current set to 20% or 40% of the current needed for the unconditioned target response); current–voltage (I/V) relationship (threshold changes due to conditioning currents, currents are between + 50% depolarizing and − 100% hyperpolarizing); and (iv) recovery cycle (threshold changes due to supramaximal conditioning pulses at inter-stimulus intervals (ISI) of 200 to 2 ms between the conditioning and test pulse). For this study, the following changes were made to TRONDNF. First, for motor and sensory measurements, the maximal delay in threshold electrotonus was increased from 200 to 300 ms, to evaluate the full accommodation to hyperpolarization. Additionally, changes were made to allow for direct comparison between the motor and sensory nerve end points. Test-stimulus duration of sensory measurements was increased from 0.5 to 1 ms (with exception of the strength-duration paradigm) and for sensory recovery cycles measurements the conditioning width was changed from 0.5 to 1 ms. Stimulus duration in the sensory strength-duration measurements was programmed to decrease with steps of 0.2 ms instead of 0.1 ms. Finally, fraction of the peak (window fraction) was set from 40% to 10%.

QTRAC-P (version 26-10-2018; Institute of Neurology) was used to process data and generate the following end points (the description is based on previous publications[10,11]): threshold for 50% CMAP/ SNAP (current required for 50% of maximal CMAP/SNAP), rheobase (slope of strength-duration relation), strength-duration time constant (SDTC; negative x-intercept of the strength-duration relation), TEd_{40} peak and TEd_{20} peak (peak threshold decrease due to depolarizing currents set to 40% and 20% of the resting threshold), TEd_{40} (X–X ms) and TEd_{20} (X–X ms) (mean threshold decrease due to 40% and 20% depolarizing currents, with conditioning stimulus latency between brackets (X–X ms)), S2-accommodation (difference between TEd_{40} peak and TEd_{40} (90–100 ms)), accommodation half-time (time when TEd_{40} is halfway between TEd_{40} peak and TEd_{40} (90–100 ms)), TEh_{40} (X–X ms; mean threshold decrease due to 40% and 20% hyperpolarizing currents, with conditioning stimulus latencies between brackets (X–X ms)), fanning (sum of values of TEd_{40} (190–200 ms) and TEh_{40} (190–200 ms)), hyperpolarizing I/V-slope (slope between 100% and 80% hyperpolarizing currents), minimum I/V slope (smallest slope in the I/V curve), resting I/V slope (slope between −10% and +10% conditioning stimuli), relative refractory period (ISI at which threshold returns to baseline), refractoriness at 2 ms (threshold change due to conditioning stimulus with ISI 2 ms), subexcitability (peak threshold change (highest value) after superexcitability), and superexcitability (peak threshold change (lowest value) after refractory period).

A blind data review was performed before statistical analysis, to exclude measurements with technical errors.

Statistical analysis

Treatment effects (placebo vs. mexiletine; placebo vs. lacosamide) on NETT outcomes were calculated using a mixed model analysis of variance (ANCOVA), with baseline as covariate. Time, period, treatment, and treatment by time were used as fixed factors. Subject, subject by treatment, and subject by time were implemented as random factors. Normal distribution of the residuals was checked graphically, and, in case of log-normal distribution, variables were log transformed before analysis. The between-day intrasubject variability and intersubject variability of NETT, expressed in CV%, were calculated from the baseline values of each visit, and were derived from the model covariate variables (the random factors subject, subject by time and subject by treatment). For statistical significance, 5% level was used. Sample size was based on a previous NETT study,[10] which showed significant PD effects of retigabine in patients with amyotrophic lateral sclerosis in a similar cross-over design.

Concentration-effect relationships

For analysis of concentration-effect relationships, PK data were linked to PD measurements, based on closest available clock-time. Each variable was modeled with an intercept only, a linear concentration-effect relationship and nonlinear (maximum effect (E_{max})) concentration-effect relationship in a mixed effects model with random effects by subject and subject by treatment on baseline to evaluate the potential concentration-effect relationships. Linear and nonlinear relationships were compared with the intercept-only model with an analysis of variance, fits of linear and nonlinear relationship were compared based on the Akaike information criterion (AIC), in which the model with the lowest AIC or a P value of < 0.05 was selected. Concentration-effect models were estimated in R (version 3.6.1, The R Foundation, Vienna, Austria).

RESULTS

The clinical phase of the study ran from September 2019 to February 2020. Eighteen subjects were enrolled, demographics are listed in **Table S1**.

Figure S1 shows individual—and mean ± SD—plasma concentrations of mexiletine and lacosamide. No observations in the absorption phase are available. Mean concentrations ranged between 5.88 and 4.83 mg/L for lacosamide, and 0.903 and 0.639 mg/L for mexiletine. The summary plasma concentrations by protocol time are listed in **Table S2**.[12,13] All adverse events in this study were mild and transient.

Excitability measurements

A total of 162 motor and 162 sensory NETT measurements were performed. As a result of the blinded data review, subexcitability was excluded from 19 measurements; superexcitability, accommodation half-time and minimum and hyperpolarizing I/V slope from one measurement; refractoriness from 3 measurements; and all threshold electrotonus variables from 5 measurements.

Raw baseline excitability variables before administration of the study drugs, and postdose estimated means, are shown in **Table S3**. Test–retest reliability (CV%) is listed in **Table S4**.

Pharmacodynamic effects on motor nerve excitability

Effects of mexiletine and lacosamide on motor nerve excitability are listed in **Table 1**. A representative selection of significant variables from each NETT paradigm is shown in **Figure 1**, depicted as the estimated mean change from baseline. Furthermore, to visualize effects on NETT recordings, average recordings of 3 and 6 hours postdose (treatment vs. placebo, without baseline correction) are shown in **Figures 2** and **3**, for mexiletine and lacosamide, respectively.

Table 1 Treatment effects of mexiletine vs. placebo, and lacosamide vs. placebo, on motor and sensory nerve excitability threshold tracking end points (estimated mean difference with placebo, 95% CI, *P* value)

		Motor nerve excitability			Sensory nerve excitability		
		Estimated mean treatment period	Estimated difference treatment vs. placebo (95% CI)	*P* value	Estimated mean treatment period	Estimated difference treatment vs. placebo (95% CI)	*P* value
CMAP (mV)/SNAP (µV)	Placebo	13.4			44.2		
	Mexiletine	13.1	−0.351 (−1.05, 0.346)	0.312	39.2	−4.95 (−8.62, −1.29)	**0.010**
	Lacosamide	13.7	0.249 (−0.447, 0.946)	0.469	44.1	−0.0664 (−3.74, 3.61)	0.971
Threshold for 50% CMAP/SNAP (mA)	Placebo	4.18			2.64		
	Mexiletine	4.33	0.147 (−0.128, 0.423)	0.285	2.74	3.7% (−5.6%, 14.0%)	0.434
	Lacosamide	4.14	−0.0406 (−0.312, 0.231)	0.763	2.77	4.8% (−4.6%, 15.2%)	0.314
Rheobase (mA)	Placebo	2.57			1.54		
	Mexiletine	2.63	0.164 (−0.0422, 0.370)	0.115	1.67	8.0% (−4.0%, 21.5%)	0.190
	Lacosamide	2.54	0.0609 (−0.143, 0.265)	0.547	1.72	11.6% (−0.8%, 25.6%)	0.065
Strength-duration time constant (ms)	Placebo	0.394			0.537		
	Mexiletine	0.378	−0.0167 (−0.0397, 0.0062)	0.147	0.516	−0.0218 (−0.0597, 0.0161)	0.251
	Lacosamide	0.360	−0.0342 (−0.0571, −0.0112)	**0.005**	0.460	−0.0778 (−0.116, −0.0399)	**<0.001**
TEd_{40} (10–20 ms) (%)	Placebo	66.0			58.1		
	Mexiletine	64.9	−1.11 (−2.33, 0.0997)	0.070	56.9	−1.15 (−3.05, 0.747)	0.225
	Lacosamide	63.8	−2.21 (−3.41, −1.00)	**0.001**	55.9	−2.17 (−4.09, −0.247)	**0.028**
TEd_{40} (40–60 ms) (%)	Placebo	49.4			45.8		
	Mexiletine	48.0	−1.37 (−2.20, −0.547)	**0.002**	45.0	−0.816 (−2.70, 1.07)	0.382
	Lacosamide	48.1	−1.27 (−2.10, −0.443)	**0.004**	45.5	−0.285 (−2.19, 1.62)	0.761
TEd_{40} (90–100 ms) (%)	Placebo	45.5			41.5		
	Mexiletine	44.4	−1.06 (−1.95, −0.179)	**0.020**	40.7	−0.784 (−2.74, 1.17)	0.419
	Lacosamide	44.2	−1.28 (−2.16, −0.395)	**0.006**	41.4	−0.0998 (−2.08, 1.88)	0.919
TEd_{40} (190–200 ms) (%)	Placebo	45.8			40.7		
	Mexiletine	44.4	−1.35 (−2.25, −0.452)	**0.005**	39.9	−0.782 (−2.73, 1.17)	0.418
	Lacosamide	43.7	−2.04 (−2.94, −1.14)	**<0.001**	39.7	−0.968 (−2.94, 1.00)	0.322
TEd_{40} peak (%)	Placebo	65.1			58.1		
	Mexiletine	63.9	−1.19 (−2.18, −0.195)	**0.023**	56.6	−1.46 (−3.36, 0.446)	0.128
	Lacosamide	62.8	−2.35 (−3.34, −1.36)	**<0.001**	55.9	−2.16 (−4.10, −0.222)	**0.030**
TEd_{40} accommodation half-time (ms)	Placebo	19.9			16.7		
	Mexiletine	19.6	−0.27 (−1.19, 0.64)	0.547	16.0	−0.75 (−1.78, 0.27)	0.144
	Lacosamide	18.7	−1.25 (−2.15, −0.34)	**0.009**	14.5	−2.21 (−3.26, −1.16)	**<0.001**

(Continued)

Table 1 (Continued)

		Motor nerve excitability			Sensory nerve excitability		
		Estimated mean treatment period	Estimated difference treatment vs. placebo (95% CI)	P value	Estimated mean treatment period	Estimated difference treatment vs. placebo (95% CI)	P value
S2 accommodation (%)	Placebo	19.6			16.5		
	Mexiletine	19.5	−0.182 (−1.14, 0.778)	0.702	15.9	−0.682 (−1.77, 0.402)	0.209
	Lacosamide	18.6	−1.04 (−2.00, −0.0893)	0.033	14.4	−2.12 (−3.22, −1.01)	0.001
TEd_{20} (10–20 ms) (%)	Placebo	34.0			−32.0		
	Mexiletine	33.8	−0.13 (−0.81, 0.55)	0.700	−31.4	−0.61 (−2.11, 0.88)	0.410
	Lacosamide	33.0	−0.95 (−1.62, −0.28)	0.008	−31.6	−1.13 (−2.65, 0.38)	0.136
TEd_{20} peak (%)	Placebo	36.4			31.7		
	Mexiletine	35.9	−0.521 (−1.23, 0.186)	0.1406	30.7	−1.02 (−2.62, 0.569)	0.198
	Lacosamide	34.9	−1.57 (−2.26, −0.868)	<0.001	30.2	−1.55 (−3.17, 0.0739)	0.061
TEh_{40} (10–20 ms) (%)	Placebo	−73.7			−66.0		
	Mexiletine	−74.0	−0.323 (−2.03, 1.39)	0.702	−65.9	0.0958 (−2.04, 2.24)	0.928
	Lacosamide	−72.3	1.34 (−0.368, 3.05)	0.120	−64.8	1.25 (−0.910, 3.41)	0.247
TEh_{40} (90–100 ms) (%)	Placebo	−124			−85.2		
	Mexiletine	−123	0.386 (−4.23, 5.00)	0.865	−87.1	−1.84 (−4.92, 1.24)	0.233
	Lacosamide	−122	1.98 (−2.61, 6.57)	0.384	−85.6	−0.349 (−3.48, 2.78)	0.821
TEh_{40} (190–200 ms) (%)	Placebo	−123			−78.7		
	Mexiletine	−124	−0.299 (−4.88, 4.29)	0.894	−78.9	−0.164 (−2.98, 2.65)	0.906
	Lacosamide	−121	2.64 (−1.92, 7.20)	0.244	−77.9	0.813 (−2.04, 3.67)	0.565
Fanning, sum of TEd_{40}- and TEh_{40} (190–200 ms)	Placebo	169			119		
	Mexiletine	168	−1.14 (−6.07, 3.80)	0.638	119	−0.443 (−4.64, 3.76)	0.831
	Lacosamide	164	−4.65 (−9.56, 0.258)	0.062	118	−1.86 (−6.12, 2.40)	0.380
Hyperpolarizing I/V-slope	Placebo	0.345			0.322		
	Mexiletine	0.330	−4.2% (−9.6%, 1.5%)	0.138	0.345	0.0230 (0.0033, 0.0427)	0.024
	Lacosamide	0.347	0.5% (−5.2%, 6.6%)	0.851	0.358	0.0358 (0.0158, 0.0558)	0.001
Minimum I/V-slope	Placebo	0.240			0.309		
	Mexiletine	0.234	−0.0063 (−0.0152, 0.0025)	0.153	0.318	0.0084 (−0.0038, 0.0206)	0.171
	Lacosamide	0.248	0.0072 (−0.0017, 0.0161)	0.107	0.328	0.0182 (0.0056, 0.0307)	0.006
Resting I/V-slope	Placebo	0.580			0.768		
	Mexiletine	0.586	0.0051 (−0.0164, 0.0265)	0.630	0.778	1.3% (−5.1%, 8.1%)	0.688
	Lacosamide	0.606	0.0258 (0.0043, 0.0474)	0.021	0.760	−0.9% (−7.3%, 5.8%)	0.771

(Continued)

Table 1 (Continued)

		Motor nerve excitability			Sensory nerve excitability		
		Estimated mean treatment period	Estimated difference treatment vs. placebo (95% CI)	P value	Estimated mean treatment period	Estimated difference treatment vs. placebo (95% CI)	P value
Relative refractory period (ms)	Placebo	2.57			3.33		
	Mexiletine	2.63	0.0532 (0.0013, 0.105)	**0.045**	3.35	0.0188 (−0.142, 0.180)	0.812
	Lacosamide	2.54	−0.0323 (−0.0840, 0.0193)	0.211	3.18	−0.152 (−0.313, 0.0089)	0.063
Refractoriness at ISI 2 ms (%)	Placebo	35.0			64.6		
	Mexiletine	38.1	3.06 (−0.568, 6.69)	0.095	62.9	−1.70 (−8.77, 5.37)	0.626
	Lacosamide	31.0	−4.02 (−7.64, −0.395)	**0.031**	50.9	−13.71 (−20.75, −6.66)	**0.001**
Subexcitability (%)	Placebo	11.6			10.4		
	Mexiletine	12.1	0.480 (−1.05, 2.01)	0.520	10.6	0.280 (−1.30, 1.86)	0.718
	Lacosamide	11.1	−0.483 (−2.06, 1.10)	0.533	7.74	−2.62 (−4.21, −1.03)	**0.002**
Superexcitability (%)	Placebo	−24.3			−18.5		
	Mexiletine	−22.6	1.74 (0.615, 2.87)	**0.004**	−16.9	1.58 (0.609, 2.56)	**0.002**
	Lacosamide	−22.8	1.47 (0.341, 2.60)	**0.013**	−17.8	0.714 (−0.260, 1.69)	0.145

The figures in bold indicated statistical significance. CI, confidence interval; CMAP, compound muscle action potential; ISI, interstimulus interval; SNAP, sensory nerve action potential.

Mexiletine. Significant effects of mexiletine were observed on threshold electrotonus with depolarizing conditioning currents 40% of threshold (TEd_{40}). Mexiletine decreased the peak in threshold reduction due to the depolarizing currents (TEd_{40} peak). Furthermore, it lowered the threshold reduction induced by depolarizing conditioning pulses of 40–200 ms (TEd_{40} (40–60 ms) (**Figure 1b**); TEd_{40} (90–100 ms); TEd_{40} (190–200 ms)). Thus, there was a shift to lower values for the TEd_{40} curve without S2-accommodation.

In the recovery cycles, different phases of excitability after an action potential are measured, namely the relative refractory period (RRP), followed by a period of superexcitability (increased excitability, characterized by a threshold reduction) and subexcitability (decreased excitability, characterized by a threshold increase). Superexcitability significantly decreased (less negative) after mexiletine administration (**Figure 1d**). Moreover, a small, but significant increase in RRP duration was observed when comparing mexiletine to placebo. Additionally, mexiletine significantly increased refractoriness at ISI 2 ms.

Lacosamide. Strength-duration time constant was significantly shortened by lacosamide compared with placebo (**Figure 1a**). Additionally, similar to mexiletine, lacosamide induced a shift to lower values for TEd_{40}: it lowered TEd_{40} peak and decreased TEd_{40} with conditioning stimulus durations 10–200 ms (TEd_{40} (10–20 ms); TEd_{40} (40–60 ms; **Figure 2b**); TEd_{40} (90–100 ms); and TEd_{40} (190–200 ms)). Accommodation half-time and S2-accommodation were significantly reduced by lacosamide. Furthermore, lacosamide had significant effects on threshold electrotonus with 20% depolarizing currents (TEd_{20}): TEd_{20} peak and TEd_{20} (10–20 ms) were lowered compared with placebo.

Lacosamide induced a significant increase in resting I/V-slope (**Figure 1c**) and, last, we found a significantly reduced superexcitability (less negative; **Figure 1d**) and refractoriness at ISI 2 ms by lacosamide.

Pharmacodynamic effects on sensory nerve excitability

Effects of mexiletine and lacosamide on sensory nerve excitability are shown in **Table 1**. Estimated mean change from baseline of one representative variable from each stimulation paradigm is shown in **Figure 4**. Moreover, average postdose NETT recordings (treatment vs. placebo, without baseline correction), are shown in **Figures 5** and **6**, for mexiletine and lacosamide, respectively.

Mexiletine. Mexiletine significantly reduced SNAP amplitudes. Consistent with motor nerves, mexiletine decreased superexcitability (less negative; **Figure 4d**). Moreover, hyperpolarizing I/V slope was significantly increased by mexiletine (**Figure 4c**).

Lacosamide. Lacosamide significantly shortened SDTC (**Figure 4a**). Additionally, lacosamide significantly reduced TEd_{40} peak, TEd_{40} (10–20 ms; **Figure 4b**), accommodation half-time and S2-accommodation. These results are in line with our findings in motor nerves. Hyperpolarizing I/V-slope (**Figure 4c**) and minimum I/V-slope were significantly increased by lacosamide.
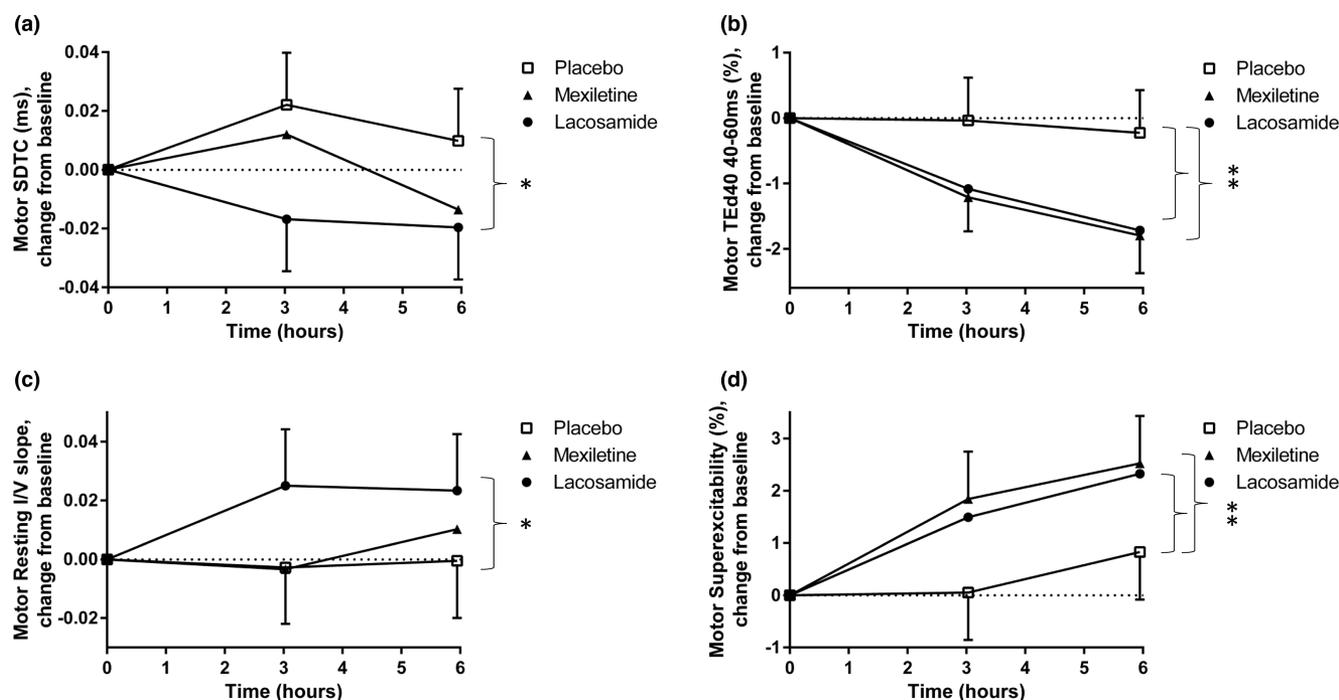
Figure 1 Estimated mean change from baseline of motor nerve excitability threshold tracking variables. Every graph shows one selected variable with significant treatment effects from each threshold tracking paradigm: (**a**) strength duration time constant (SDTC), (**b**) TEd$_{40}$ (40–60 ms), (**c**) resting I/V slope, and (**d**) superexcitability. Error bars indicate the 95% confidence interval. The time after dosing (hours) is indicated on the x-axis. Significant effects of mexiletine and/or lacosamide vs. placebo in the treatment period are highlighted with an asterisk. $N = 18$.

Furthermore, lacosamide decreased refractoriness at ISI 2 ms and subexcitability.

Concentration-effect relationships

As displayed in **Table S5**, significant treatment effects were supported by significant concentration-effect relationships in 27 out of 30 significant variables. The only significant treatment effects in which no concentration-effect relation could be detected were the effect of lacosamide on SNAP, effect of mexiletine on hyperpolarizing I/V slope, and the effect of lacosamide on refractoriness.

DISCUSSION

This study was performed to evaluate whether NETT is a useful tool to determine PD effects of Na$_V$-blockers in early phase clinical drug development. As a proof-of-concept, we evaluated effects of mexiletine and lacosamide on motor and sensory NETT. We found a significant reduction of nerve excitability by both study drugs, indicating that NETT is sensitive to detect drug-induced changes in Na$_V$-conductance.

Effects of Na$_V$-blockers on NETT

To our knowledge, this is the first study to demonstrate effects of oral Na$_V$-blockers on NETT in healthy subjects. However, proposed effects of reduced Na$_V$-conductance by tetrodotoxin (TTX) on NETT have been evaluated previously using theoretical nerve modeling.[14] Kiernan et al. concluded that TTX-effects are mainly caused by a threshold increase and flattening

of the threshold/potential relationship. This in turn results in a decrease in SDTC and an increase in rheobase. SDTC is a membrane-time constant derived from the rate of decline of current strength required at increasing stimulus durations, thought to be dependent on persistent Na$_V$-channel properties.[4] Our study, with Na$_V$-blockers with different modes of action than TTX, also showed a decrease of SDTC by lacosamide, but interestingly not by mexiletine. Rheobase was unaffected. Threshold electrotonus examines the threshold reduction due to depolarizing and hyperpolarizing conditioning currents, to demonstrate internodal membrane properties.[4] The model by Kiernan et al. also predicts a clear decrease in depolarizing threshold electrotonus and an increase in hyperpolarizing threshold electrotonus. Our results are in line with the TTX-effect on depolarizing threshold electrotonus, but not with the TTX-effect on hyperpolarizing threshold electrotonus. Furthermore, the nerve model by Kiernan et al. shows a reduction of all phases of the recovery cycles by Na$_V$-blockade, resulting in a flattening of the recovery cycles curve, corroborating our findings. Last, the model predicts an increased hyperpolarizing I/V-slope, which is explained by Kiernan et al. as activation of hyperpolarization mediated I$_H$ currents, corresponding to our findings for both mexiletine and lacosamide.

Based on the resemblance between the theoretical nerve model with TTX[14] and our findings, we conclude that the significant effects of mexiletine and lacosamide on nerve excitability are in line with expected effects of Na$_V$-blockade. The above-described differences between the TTX-model and mexiletine and lacosamide
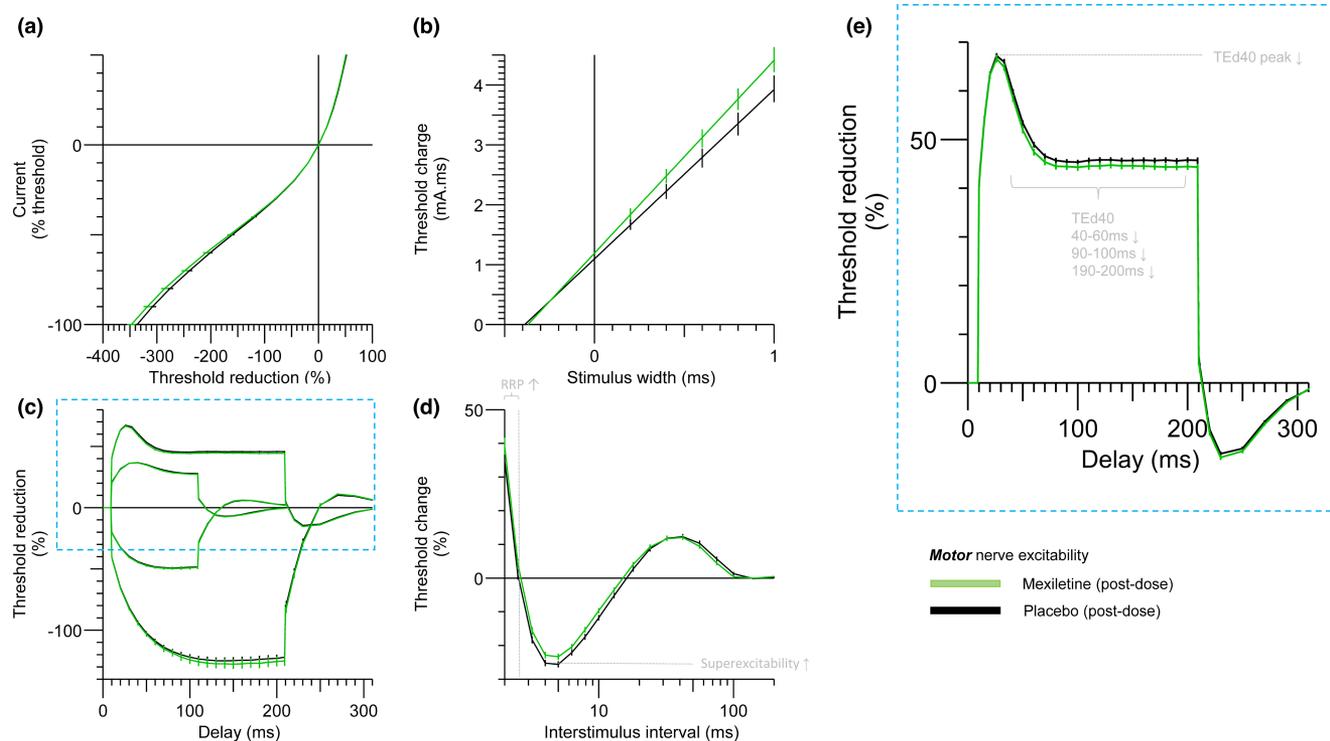
Figure 2 The average postdose (3 and 6 hours) motor nerve excitability threshold tracking recordings of placebo (black) vs. mexiletine (green). Variables that were significantly affected by mexiletine are highlighted with ↑ (for increase) and ↓ (for decrease). Subgraphs of excitability recordings are as follows: (**a**) I/V relationship; (**b**) strength-duration relationship; (**c**) threshold electrotonus; and (**d**) recovery cycles. Graph (**e**) is zoomed in on the depolarizing threshold electrotonus with 40% depolarizing currents. Indication of variables is reproduced from Kiernan *et al.*[3] Note that these graphs show mean combined postdose measurements for placebo vs. active treatment and baseline measurements are not considered, therefore these do not exactly match the statistical analysis. Moreover, these figures include all measurements including the minimal amount of data excluded in the blinded data review.

(rheobase, hyperpolarizing threshold electrotonus), may be explained by the difference in mechanism of action. TTX binds to Na$_V$ extracellularly at the outer pore, preventing access of cations,[14] whereas mexiletine binds to the inner pore and exhibits a state-dependent Na$_V$-block.[15] The binding site and action mechanism of lacosamide is much less clear. Lacosamide was originally suggested to selectively enhance slow Na$_V$-inactivation without affecting fast inactivation, through an unknown binding site.[16,17] More recent findings suggest that lacosamide does bind to fast-inactivated state of sodium channels, but with slow binding and unbinding kinetics.[18] Another possible explanation for the lack of effects of mexiletine and lacosamide on rheobase and hyperpolarizing threshold electrotonus, may be a larger reduction of Na$_V$-conductance by TTX. Overall, this data supports the hypothesis that the observed effects are a result of direct Na$_V$-blockade, however, it should be noted that additional (indirect) effects, for example, on membrane potential or other ion channels could also contribute to the observed pattern of NETT effects, as was described for lidocaine.[19] To better understand the exact mechanisms for the observed NETT effects, in future work, it would be of interest to perform nerve modeling with our data to clarify this further, as described above for TTX.[14]

When comparing effects between the Na$_V$-blockers—mexiletine and lacosamide—within our study, many observed effects are similar, such as effects on depolarizing threshold electrotonus and

superexcitability. However, lacosamide affected a more extensive set of variables than mexiletine, often with larger effect sizes. Difference in target site concentration and/or potency at the relevant involved ion-channels are potential causes for these discrepancies. A difference in mechanism of action or binding kinetics of the drugs is another possible explanation.

Apart from theoretical model simulations, there is a limited amount of prior clinical data available, investigating Na$_V$-blocking effects on NETT in humans, to place our findings into context. Effects of a high dose of lidocaine (5–6 mL of a 50 mM solution lidocaine) administered as local nerve block (not placebo-controlled)[19] and human intoxication with TTX[14] have been previously evaluated. After the conduction block of anesthetic lidocaine perfusion, when force had recovered, profound effects on nerve excitability were still measured. Consistent results between lidocaine and TTX were a decreased depolarizing threshold electrotonus, SDTC, and superexcitability, which is in line with our findings on these variables. It should be noted, however, that at high concentrations lidocaine decreased hyperpolarizing electrotonus and left-shifted the depolarizing I/V relationship, which was opposite to the effects of TTX poisoning. This discrepancy indicates there may be other factors than Na$_V$ blockade driving these changes, and the authors indeed showed with nerve modeling that (indirect) effects on membrane potential and other channels contributed to the observed lidocaine effect.[14,19] Of course, this setting
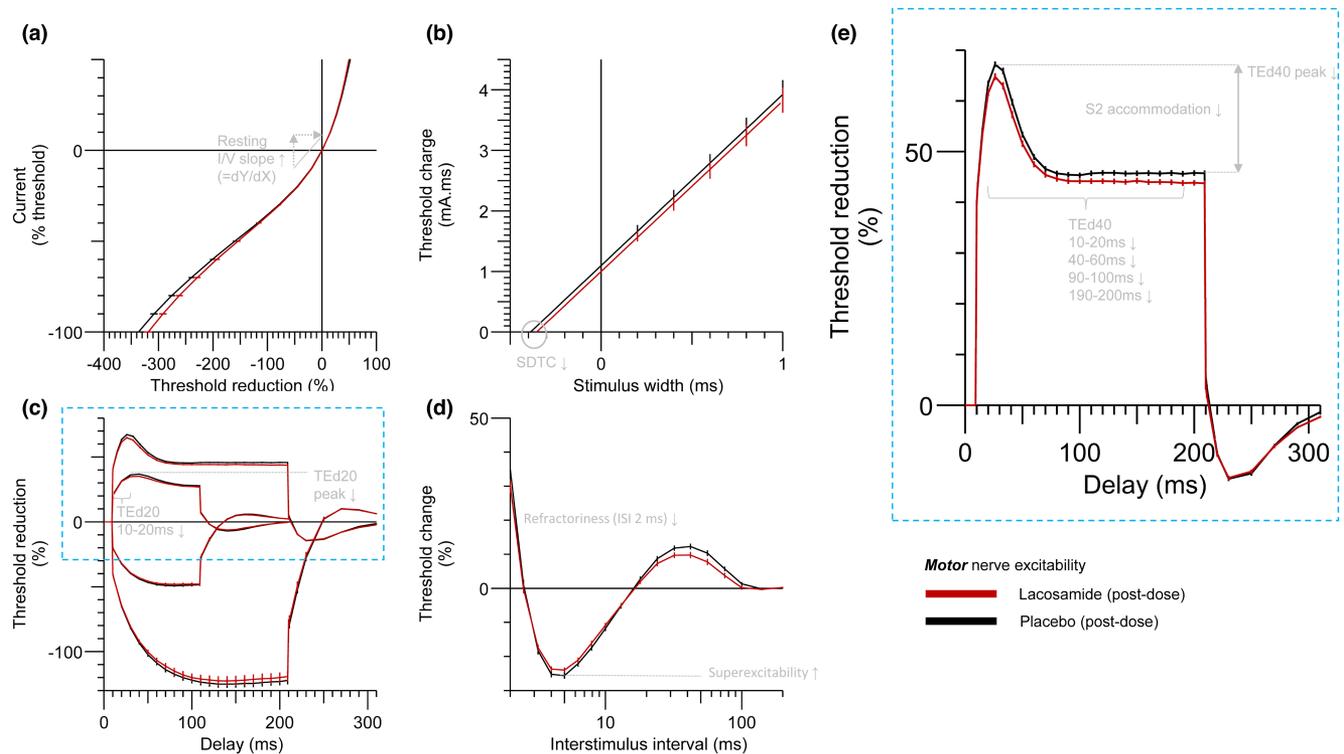
Figure 3 The average postdose (3 and 6 hours) motor nerve excitability threshold tracking recordings of placebo (black) vs. lacosamide (red). Variables that were significantly affected by lacosamide are highlighted with ↑ (for increase) and ↓ (for decrease). Subgraphs of excitability recordings are as follows: (**a**) I/V relationship; (**b**) strength-duration relationship; (**c**) threshold electrotonus; and (**d**) recovery cycles. Graph (**e**) is zoomed in on the depolarizing threshold electrotonus with 40% depolarizing currents. Indication of variables is reproduced from Kiernan et al.[3] Note that these graphs show mean combined postdose measurements for placebo vs. active treatment and baseline measurements are not considered, therefore these do not exactly match the statistical analysis. Moreover, these figures include all measurements including the minimal amount of data excluded in the blinded data review.

with high local drug concentrations might not be fully comparable to our setting with oral administrations.

A final relevant study examined chronic effects of mexiletine in patients with neuropathic pain: mexiletine decreased refractoriness and SDTC after 3 months of use,[5] in line with our reported effects of lacosamide but not mexiletine.

Different effects on motor and sensory nerves

We found different effects of Na_V-blockade on motor vs. sensory nerve excitability. In general, the effects we found on depolarizing threshold electrotonus were more apparent in motor nerves, whereas effects on I/V (hyperpolarizing and minimum I/V slope) were only significantly affected in sensory nerves. These disparate effects may be explained by a physiological difference in nerve excitability profile between motor and sensory axons of the median nerve.[20,21] There are differences in expression of persistent Na_V-channels between motor and sensory nerves.[22] Moreover, within each group there are further differences of motor axons innervating fast or slow muscles, whereas cutaneous sensory neurons contain four types of afferents which could be differentially affected by Na_V-blockade. This could include (i) differences in resting membrane potential, (ii) expression differences of transporters such as the sodium/potassium ATPase pump, and (iii) qualitative and quantitative differential ion-channel expression profiles.[21] There may also be technical limitations that could

explain these differences: recording of SNAPs is more challenging than CMAPs. However, the CV% were not much higher in sensory than motor recordings and it is therefore likely that the observed excitability changes reflect mechanistic differences.

Concentration-effect relationships

The majority (90%) of variables with significant treatment effects also have significant concentration-effect relationships, pointing toward concentration-dependent treatment effects in the studied concentration-range. The fact that we prove drug concentration to be the driver for detected treatment effects encourages the use of NETT as biomarker for pharmacological effects of Na_V modulators. A substantial additional set of 25 variables that did not show significant treatment effects, also had a significant linear concentration-effect relationship. This may hint at an underlying concentration-dependent effect, although not sufficiently robust to be demonstrated in the treatment effect analysis and a larger sample size might be required to identify significant treatment effects on these variables.

Nerve excitability threshold tracking as PD biomarker

A reliable biomarker of Na_V blocking effects for use in early phase clinical drug development is lacking. Given the results of this study, we conclude that NETT is a suitable biomarker for PD effects of Na_V-blockers. Most importantly, in a relatively small
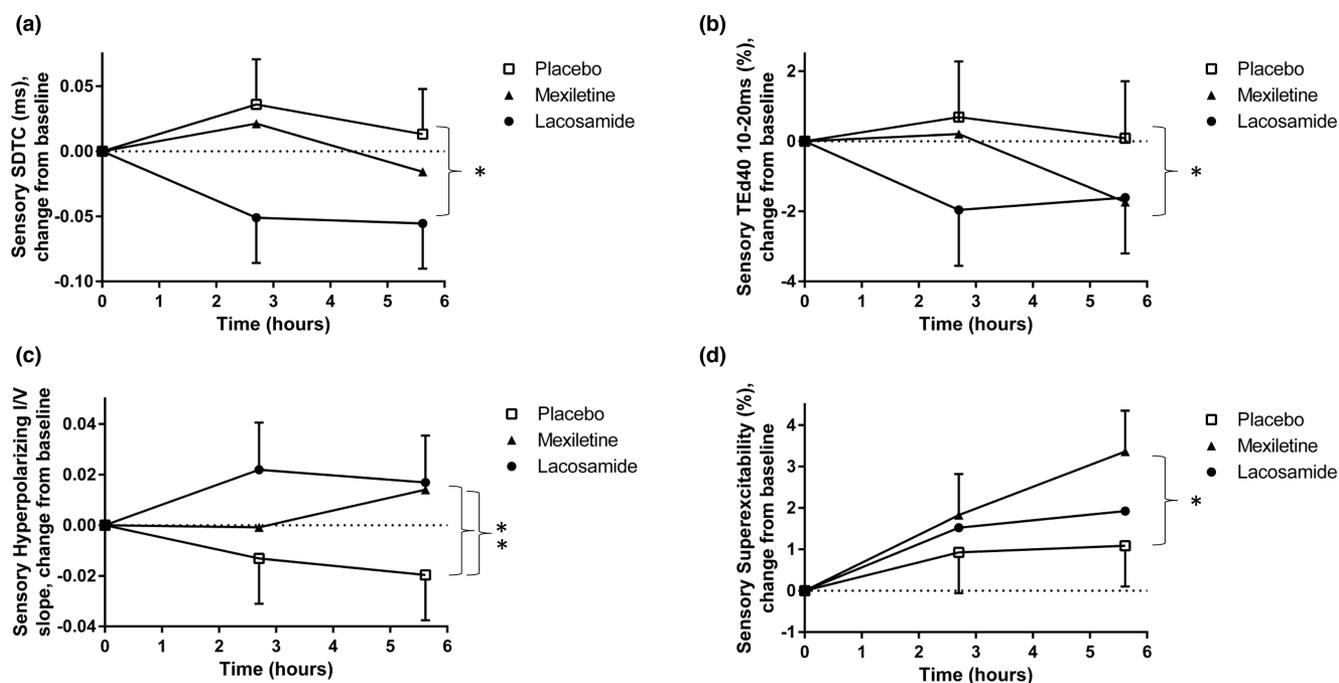
Figure 4 Estimated mean change from baseline of sensory nerve excitability threshold tracking variables. Every graph shows one selected variable with significant treatment effects from each threshold tracking paradigm: (**a**) strength duration time constant (SDTC), (**b**) TEd$_{40}$ (10–20 ms), (**c**) hyperpolarizing I/V slope, and (**d**) Superexcitability. Error bars indicate the 95% confidence interval. The time after dosing (hours) is indicated on the x-axis. Significant effects of mexiletine and/or lacosamide vs. placebo in the treatment period are highlighted with an asterisk. $N = 18$.
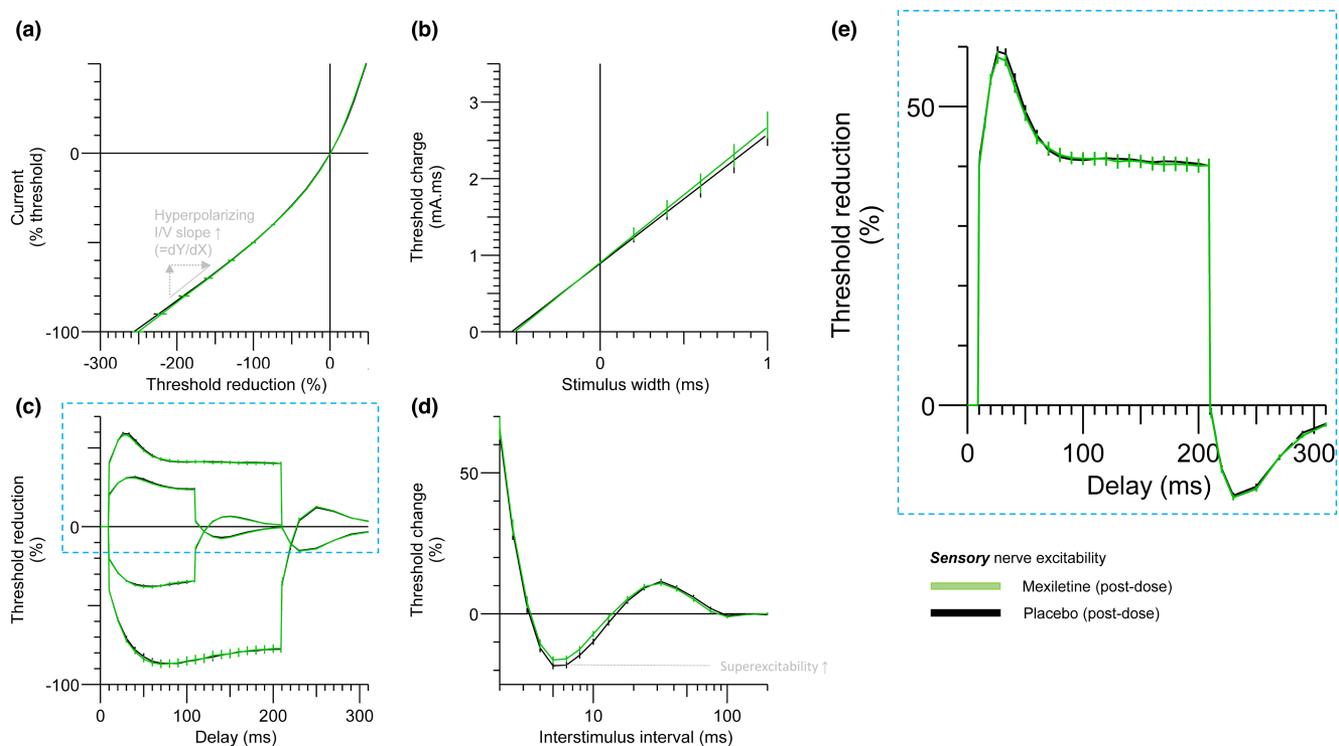
Figure 5 The average postdose (3 and 6 hours) sensory nerve excitability threshold tracking recordings of placebo (black) vs. mexiletine (green). Variables that were significantly affected by mexiletine are highlighted with ↑ (for increase) and ↓ (for decrease). Subgraphs of excitability recordings are as follows: (**a**) I/V relationship; (**b**) strength-duration relationship; (**c**) threshold electrotonus; and (**d**) recovery cycles. Graph (**e**) is zoomed in on the depolarizing threshold electrotonus with 40% depolarizing currents. Indication of variables is reproduced from Kiernan et al.[3] Note that these graphs show mean combined postdose measurements for placebo vs. active treatment and baseline measurements are not considered, therefore these do not exactly match the statistical analysis. Moreover, these figures include all measurements including the minimal amount of data excluded in the blinded data review.

Figure 6 The average postdose (3 and 6 hours) sensory nerve excitability threshold tracking recordings of placebo (black) vs. lacosamide (red). Variables that were significantly affected by lacosamide are highlighted with ↑ (for increase) and ↓ (for decrease). Subgraphs of excitability recordings are as follows: (**a**) I/V relationship; (**b**) strength-duration relationship; (**c**) threshold electrotonus; and (**d**) recovery cycles. Graph (**e**) pis zoomed in on the depolarizing threshold electrotonus with 40% depolarizing currents. Indication of variables is reproduced from Kiernan *et al*.[3] Note that these graphs show mean combined post-dose measurements for placebo vs. active treatment and baseline measurements are not considered, therefore these do not exactly match the statistical analysis. Moreover, these figures include all measurements including the minimal amount of data excluded in the blinded data review.

number of healthy subjects, significant effects of Na$_V$-blockade can be detected at plasma concentrations within the therapeutic range. Moreover, NETT has favorable characteristics for a PD biomarker. It is noninvasive and relatively quick to perform, allowing evaluation of nerve excitability several times a day at different drug plasma concentrations. Intrasubject variability is low, as CV% (estimated from the statistical model) were below 10% for most variables, which indicates high test–retest reliability (**Table S4**). These characteristics indicate that NETT can be considered a valuable tool for determining target engagement in early phase clinical studies in a healthy population. Furthermore, the significant concentration-effect relations found in our study could indicate that the method is suitable for detecting dose-related effects in first-in-human ascending dose studies, as a signal for receptor occupancy. This should be confirmed in future studies. Moreover, the biomarker could potentially be used as a translational tool, for the translation from preclinical (animal) data to human effective doses, as also suggested previously for local anesthetic nerve blocks.[23] In addition, NETT could aid dose finding in the translation from healthy subjects to patients.

Possible limitations

A limitation for the concentration-effect relationship analysis, was the limited number of PD measurements and corresponding PK samples. Because of the long half-life of the study drugs, both measurements were performed at high plasma concentrations. To confirm the potential of NETT to detect concentration-effect relationships, a wider range of plasma concentrations would be desirable.

Statistical analysis performed in our study was not corrected for multiple testing, because of the exploratory nature of the study. However, there is a clear consistency in the significant effects and most significant effects are accompanied by a significant linear concentration-effect relationship, strongly indicating that pharmacological effects are underlying these results.

CONCLUSION

To our knowledge, this is the first published randomized, placebo-controlled trial to evaluate acute effects of Na$_V$-blockers (mexiletine and lacosamide) on NETT in healthy subjects. This study shows that NETT can be used to detect a decrease in peripheral nerve excitability exhibited by both mexiletine and lacosamide. Therefore, NETT can be considered a valuable PD biomarker for effects of Na$_V$-modulation. This could be a useful tool in early phase clinical drug development for proof-of-mechanism, and potentially to assist in dose finding for patient studies.

SUPPORTING INFORMATION

Supporting information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

CONFLICT OF INTEREST
M.K. is director of Neurophysiology Consulting Ltd and QTMS Science Ltd. During the past 5 years, he has been an ad hoc consultant and member of the speaker bureau for Eli Lilly, GSK, Levicept, Marks & Clerk Law, Merck, Neursentis, Pfizer, Richmond Pharmaceuticals Ltd., and Roche. All other authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS
T.R., J.H., and G.J.G. wrote the manuscript. T.R., M.K., G.J.G., and J.H. designed the research. T.R., I.K., and G.J.G. performed the research. M.L.K. and M.E. analyzed the data.

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