Measurement of cortical, nerve, and muscle excitability in early phase clinical drug development

Ruijs, T.Q.

CHAPTER 4

EFFECTS OF MEXILETINE AND LACOSAMIDE ON NERVE EXCITABILITY IN HEALTHY SUBJECTS: A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, CROSSOVER STUDY

Clin Pharmacol Ther, 112: 1008-1019. https://doi.org/10.1002/cpt.2694

Titia Q. Ruijs[1,2], Ingrid W. Koopmans[1,2], Marieke L. de Kam[1], Michiel J. van Esdonk[1], Martin Koltzenburg[3], Geert Jan Groeneveld[1,2], Jules A.A.C. Heuberger[1]

1. Centre for Human Drug Research, Leiden, NL
2. Leiden University Medical Centre, Leiden, NL
3. National Hospital for Neurology and Neurosurgery, London, UK

ABSTRACT

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, three-way crossover study, effects of single oral doses of 333 mg mexiletine and 300 mg lacosamide were compared to placebo. On each study visit, motor- and sensory nerve excitability measurements of the median nerve were performed (pre-dose; 3- and 6-hours post-dose) using Qtrac. Treatment effects were calculated using an ANCOVA with baseline as covariate. Mexiletine and lacosamide had significant effects on multiple motor- and sensory nerve excitability variables. Depolarizing threshold electrotonus (TEd_{40} (40-60ms)) decreased by mexiletine (estimated difference (ED) -1.37% (95% confidence interval: -2.20, -0.547); p=0.002) and lacosamide (ED -1.27% (-2.10, -0.443); p=0.004) in motor nerves. Moreover, mexiletine and lacosamide decreased superexcitability (less negative) in motor nerves (ED 1.74% (0.615, 2.87); p=0.004, and 1.47% (0.341, 2.60); p=0.013, respectively). Strength-duration time constant decreased after lacosamide in motor- (ED -0.0342 ms (-0.0571, -0.0112); p=0.005) and sensory nerves (ED -0.0778 ms (-0.116, -0.0399); p<0.001).

Concluding, 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.

INTRODUCTION

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 non-invasive 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 to 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 to 45 years old, with body mass index (BMI) between 18-30 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. Subjects with conditions considered to influence electrophysiological measurements were excluded. Use of medication, dietary supplements, CYP450 iso-enzyme modulating products, alcohol, caffeine, and nicotine was 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 seven days. On each visit, three motor- and sensory NETT measurements were performed: pre-dose, and three- and six-hours post-dose. Blood samples for pharmacokinetic (PK) analysis were drawn pre-dose, and before and after each post-dose 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 endpoints, and variability was expressed in coefficients 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 first subject, first dose.

STUDY DRUGS Mexiletine (Namuscla, 167 mg, Lupin Europe GMBH) capsules and lacosamide (Vimpat, 100 mg, UCB Pharma S.A.) 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 three 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] Mean 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 by a statistician uninvolved in the clinical study conduct. Subjects were randomly assigned to one of six treatment sequences in a balanced study design. Randomisation numbers were assigned to participants sequentially after medical screening by blinded study staff.

PK ANALYSIS Plasma concentrations of the study drugs were analysed using a validated LC-MS/MS 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 (LLOQ) was 0.06 mg/L for mexiletine and 0.75 mg/L for lacosamide. Reproducibility of the assays was in line with the EMA bioanalytical method development guideline, with CV%s <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, USA), 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 (CMAP) were recorded from the abductor pollicis brevis, using a belly-tendon montage (Disposable Tab Electrodes, Natus Medical, Pleasanton, USA). Sensory nerve action potentials (SNAP) were recorded antidromically using ring electrodes (Disposable Wide Ring Electrode, Natus Medical, Pleasanton, USA) on digit three. When no

SNAP could be recorded from digit three, digit two was used. CMAP and SNAP signals were amplified using an EMG amplifier (D440-2, DigiTimer, Hertfordshire, UK), gain 10.000 for sensory measurements and 300 for motor measurements, bandpass filter 3 to 3000 Hz. Signals were digitized using an analog-digital convertor (NI-USB-6341, National Instruments, Austin, USA). Hum Bug (Quest Scientific Instruments, North Vancouver, 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, Cincinnati, USA) 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, London, UK). This paradigm and corresponding variables were described previously.[2,3] Each NETT measurement consists of four protocols:[10] stimulus response curve (relationship between stimulus current and amplitude of the muscle/sensory action potential); strength-duration relationship (relationship between stimulus duration and stimulus charge); 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 recovery cycle (threshold changes due to supramaximal conditioning pulses at interstimulus 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 endpoints. 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, London, UK) was used to process data and generate the following endpoints (description 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} (90-100ms)), accommodation half-time (time when TEd_{40} is halfway between TEd_{40} peak and TEd_{40} (90-100ms)), TEh_{40} (X-Xms) (mean threshold decrease due to 40% and 20% hyperpolarizing currents, with conditioning stimulus latencies between brackets (X-Xms)), fanning (sum of values of TEd_{40} (190-200ms) and TEh_{40} (190-200ms)), 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), 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, 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 intra-subject variability

and inter-subject variability of NETT, expressed in CV%s, 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 ALS patients in a similar cross-over design.

CONCENTRATION-EFFECT RELATIONSHIPS For analysis of concentration-effect relationships, PK data was linked to PD measurements, based on closest available clock-time. Each variable was modelled with an intercept only, a linear concentration-effect relationship and non-linear (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 non-linear relationships were compared with the intercept only model with an analysis of variance, fits of linear and non-linear 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).

RESULTS

The clinical phase of the study ran from September 2019 to February 2020. Eighteen subjects were enrolled, demographics are listed in *Supplementary Table 1*. *Supplementary Figure 1* shows individual – and mean ± standard deviation-plasma concentrations of mexiletine and lacosamide. No observations in the absorption phase are available. Mean concentrations ranged between 5.88 mg/L and 4.83 mg/L for lacosamide, and 0.903 mg/L and 0.639 mg/L for mexiletine. The summary plasma concentrations by protocol time are listed in *Supplementary Table 2*. 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; super-excitability, accommodation half-time and minimum- and hyperpolarizing I/V slope from one measurement; refractoriness from three measurements; and all threshold electrotonus variables from five measurements.

Raw baseline excitability variables before administration of the study drugs, and post-dose estimated means, are shown in *Supplementary Table 3*. Test-retest reliability (CV%) is listed in *Supplementary Table 4*.

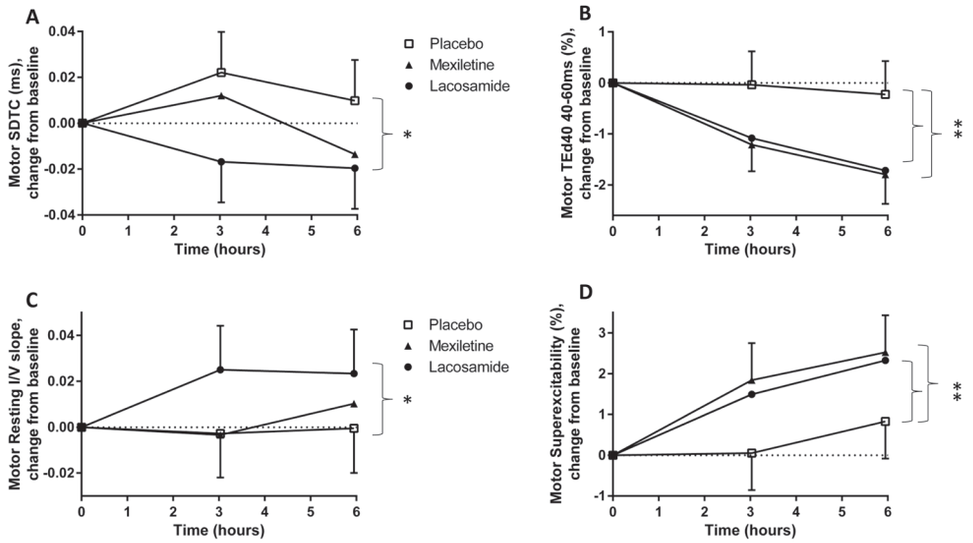
PHARMACODYNAMIC EFFECTS ON MOTOR NERVE EXCITABILITY Effects of mexiletine and lacosamide on motor nerve excitability are listed in *Table 1*. A representative selection of significant variables from each NETT paradigm is shown in *Figure 1*, depicted as the estimated mean change from baseline. Furthermore, to visualize effects on NETT recordings, average recordings of three- and six-hours post-dose (treatment vs. placebo, without baseline correction) are shown in *Figure 2* and *Figure 3*, for mexiletine and lacosamide respectively.

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} (40-60ms) (*Figure 1B*); TEd_{40} (90-100ms); TEd_{40} (190-200ms)). 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.

LACOSAMIDE SDTC was significantly shortened by lacosamide compared to 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} (10-20ms); TEd_{40} (40-60ms) (*Figure 1B*); TEd_{40} (90-100ms); TEd_{40} (190-200ms)).

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) TEd40 (40-60ms), C) Resting I/V slope, 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 versus placebo in the treatment period are highlighted with an asterisk. N=18.

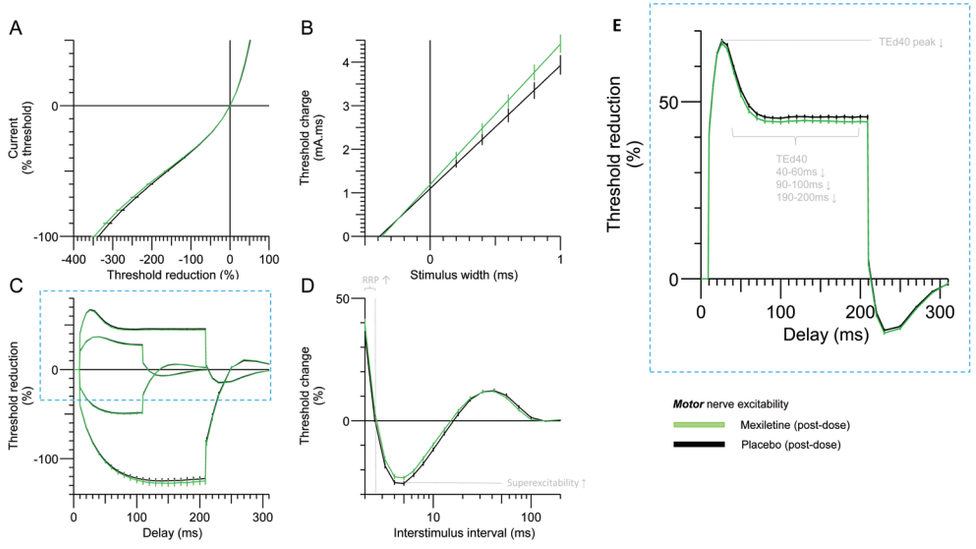


Accommodation half-time and S2-accommodation were significantly reduced by lacosamide. Furthermore, lacosamide had significant effects on threshold electrotonus with 20% depolarizing currents (TED_{20}): TED_{20} peak and TED_{20} (10-20ms) were lowered compared to placebo.

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

DRUG 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 post-dose NETT recordings (treatment vs. placebo, without baseline correction), are shown in *Figure* 5 and *Figure* 6, for mexiletine and lacosamide respectively.

FIGURE 2 The average post-dose (three and six 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; 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 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.



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} (10-20ms) (*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. Furthermore, lacosamide decreased refractoriness at ISI 2ms and subexcitability.

FIGURE 3 The average post-dose (three and six 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; 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 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.

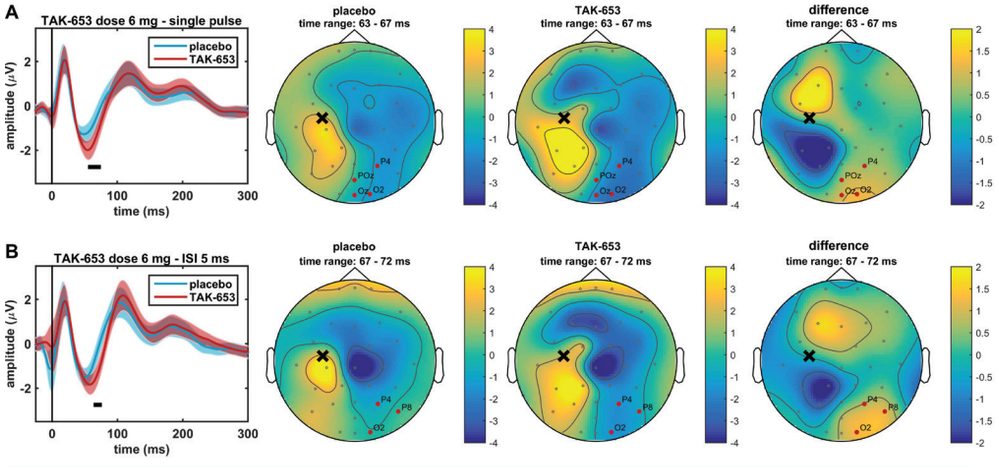


FIGURE 4 Estimated mean change from baseline of sensory nerve excitability threshold tracking variables. Every graph shows one selected variable with significant treatment effects from each threshold tracking paradigm: A) strength duration time constant (SDTC), B) TEd40 (10-20ms), C) Hyperpolarizing I/V slope, 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 versus placebo in the treatment period are highlighted with an asterisk. N=18.

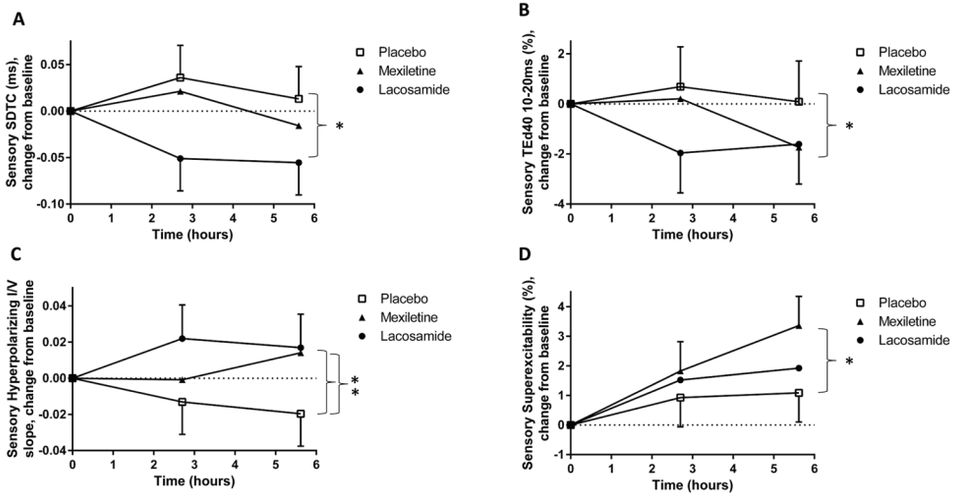


FIGURE 5 The average post-dose (three and six hours) sensory nerve excitability threshold tracking recordings of placebo (black) vs. mexiletine (green). Variables that were significantly affected by mexiletine are highlighted with ↑ (for increase) and ↓ (for decrease). Subgraphs of excitability recordings are as follows: A) I/V relationship; B) strength-duration relationship; C) threshold electrotonus; 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 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.

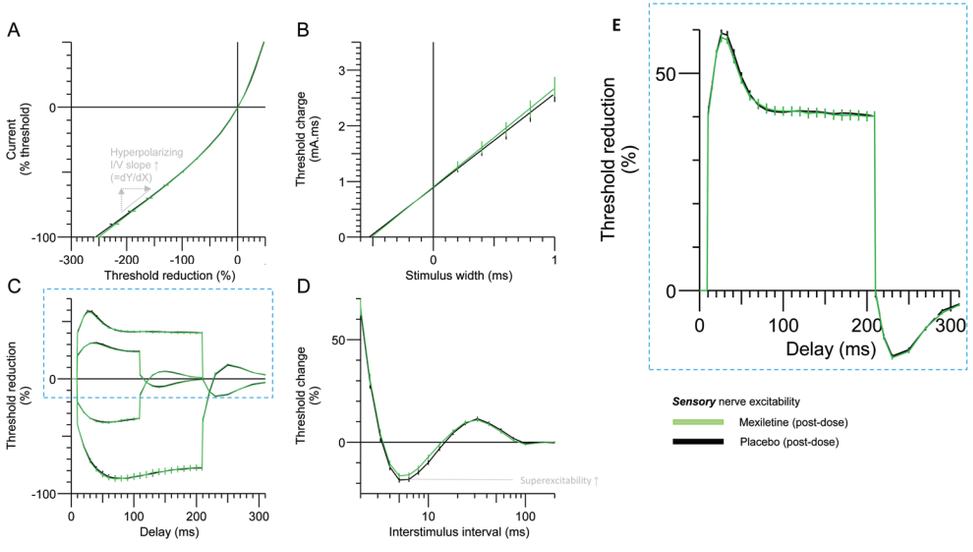


FIGURE 6 The average post-dose (three and six hours) sensory nerve excitability threshold tracking recordings of placebo (black) vs. lacosamide (red). Variables that were significantly affected by lacosamide are highlighted with ↑ (for increase) and ↓ (for decrease). Subgraphs of excitability recordings are as follows: A) I/V relationship; B) strength-duration relationship; C) threshold electrotonus; 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 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.

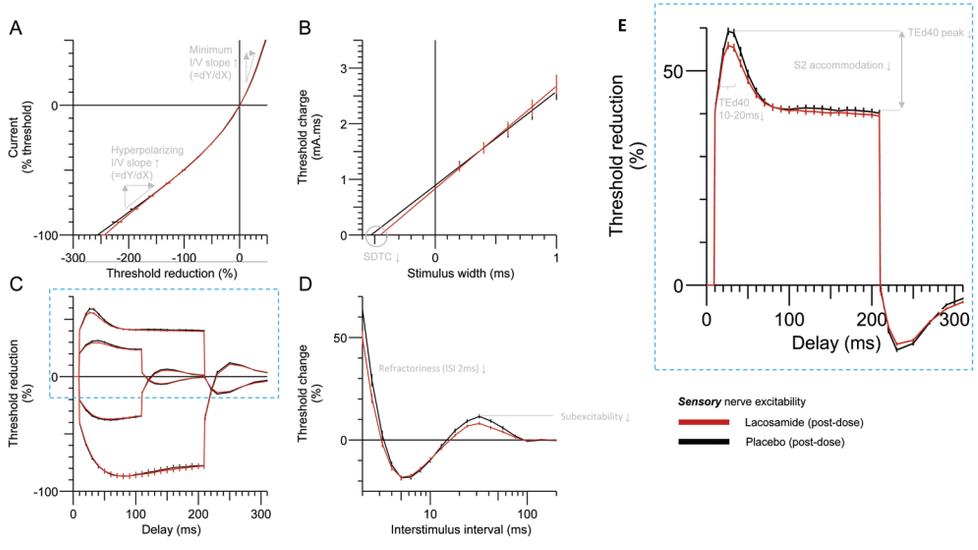


TABLE 1 Treatment effects of mexiletine vs. placebo, and lacosamide vs. placebo, on motor- and sensory nerve excitability threshold tracking endpoints (estimated mean difference with placebo, 95% CI, p-value).

		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} (10-20ms) (%)	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} (40-60ms) (%)	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} (90-100ms) (%)	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} (190-200ms) (%)	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
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} (10-20ms) (%)	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

(*Continuation Table 1*)

		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
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} (10-20ms) (%)	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} (90-100ms) (%)	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} (190-200ms) (%)	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
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

CI, *confidence interval*; CMAP, *compound muscle action potential*; ISI, *interstimulus interval*; SNAP, *sensory nerve action potential*.

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 modelling.[12] 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. Lastly, 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 [12] 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. Above-described differences between the TTX-model and mexiletine and lacosamide (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,[12] whereas mexiletine binds to the inner pore and exhibits a state-dependent Na_V-block.[13] 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.[14,15] More recent findings suggest that lacosamide does bind to fast-inactivated state of sodium channels, but with slow binding and unbinding kinetics.[16] 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.[17] 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.[12]

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 investigating Na_V-blocking effects on NETT in humans available 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)[17] and human intoxication with TTX[12] have been previously evaluated. After the conduction block of anaesthetic 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 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 modelling that (indirect) effects on membrane potential and other channels contributed to the observed lidocaine effect.[12,17] Of course, this setting 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 three 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, 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.[18,19] There are differences in expression of persistent Na_V-channels between motor- and sensory nerves.[20] Moreover, within each group there are further differences of motor axons innervating fast or slow muscles, whereas cutaneous sensory neurons contain 4 types of afferents which could be differentially affected by Na_V-blockade. This could include:

- differences in resting membrane potential
- expression differences of transporters such as the sodium/potassium ATPase pump
- qualitative and quantitative differential ion-channel expression profiles.[19]

There may also be technical limitations that could explain these differences: recording of SNAPs is more challenging than CMAPs. However, the CV%s 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 towards 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 number of healthy subjects, significant effects of Na_V-blockade can be detected at plasma concentrations within the therapeutic range. Moreover, NETT has favourable characteristics for a PD biomarker. It is non-invasive 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%s (estimated from the statistical model) were below 10% for most variables, which indicates high test-retest reliability (*Supplementary Table 4*). 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 anaesthetic nerve blocks.[21] Also, 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.

SUPPLEMENTARY TABLE 1 **Demographics.**

Demographics	N	18
Age (years)	Mean	25
	SD	5
	Median	24
	Range	19, 36
Height (cm)	Mean	184
	SD	8
	Median	184
	Range	170, 202
Weight (kg)	Mean	80
	SD	13
	Median	80
	Range	60, 100
BMI (kg/m²)	Mean	23
	SD	3
	Median	23
	Range	19, 30

BMI = *body mass index*; SD = *standard deviation*.

SUPPLEMENTARY TABLE 2 **Plasma concentrations (mg/L) of mexiletine and lacosamide at the scheduled sampling times (minutes after dosing). Additionally, this table lists median concentration in µM, based on molecular weight 250.29 g/mol for lacosamide[1] and molecular weight 179.26 g/mol for mexiletine.[2]**

	Time after dosing (minutes)	Mean plasma concentration (mg/L)	Standard deviation	Median plasma concentration (mg/L)	Median plasma concentration (µM)
Lacosamide (300 mg)	0	0	0	0	0
	160	5.88	1.18	5.89	23.51
	204	5.69	1.12	5.70	22.75
	335	4.98	1.01	4.94	19.72
	379	4.83	0.988	4.87	19.44
Mexiletine (333 mg)	0	0.00	0.00	0.00	0.00
	160	0.903	0.214	0.855	4.77
	204	0.835	0.195	0.787	4.39
	335	0.653	0.155	0.618	3.45
	379	0.639	0.187	0.590	3.29

1. *National Center for Biotechnology Information. PubChem Compound Summary for CID 219078, Lacosamide. 2005-08-09 updated 2022-01-29. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Lacosamide.*
2. *National Center for Biotechnology Information. PubChem Compound Summary for CID 4178, Mexiletine 2005 06-24 updated 2022-01-29. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Mexiletine.*

SUPPLEMENTARY TABLE 3 Raw baseline and estimated means (three- and six-hours post-dose) of excitability variables for placebo, mexiletine and lacosamide.

		Motor nerve excitability			Sensory nerve excitability		
		Raw mean baseline	Estimated mean +3h	Estimated mean +6h	Raw mean baseline	Estimated mean +3h	Estimated mean +6h
CMAP (mV)/ SNAP (µV)	Placebo	13.0	13.5	13.4	37.8	43.8	44.6
	Mexiletine	12.5	13.1	13.0	40.3	41.7	36.8
	Lacosamide	12.6	13.7	13.7	41.3	46.0	42.3
Threshold for 50% CMAP/ SNAP (mA)	Placebo	4.34	4.16	4.20	3.02	2.65	2.63
	Mexiletine	4.81	4.20	4.45	2.88	2.77	2.71
	Lacosamide	4.23	4.14	4.14	2.96	2.74	2.80
Rheobase (mA)	Placebo	3.06	2.59	2.56	1.86	1.54	1.55
	Mexiletine	3.41	2.65	2.61	1.74	1.67	1.67
	Lacosamide	2.94	2.56	2.52	1.81	1.71	1.74
Strength-duration time constant (ms)	Placebo	0.379	0.401	0.388	0.520	0.549	0.526
	Mexiletine	0.374	0.390	0.365	0.514	0.534	0.497
	Lacosamide	0.383	0.362	0.359	0.505	0.462	0.457
TEd_{40} 10-20ms (%)	Placebo	65.7	66.2	65.7	58.1	58.4	57.8
	Mexiletine	66.5	65.3	64.5	57.9	57.9	55.9
	Lacosamide	65.6	63.9	63.6	57.0	55.7	56.1
TEd_{40} 40-60ms (%)	Placebo	49.6	49.5	49.3	46.9	46.1	45.5
	Mexiletine	49.6	48.3	47.7	45.9	45.6	44.4
	Lacosamide	49.4	48.4	47.8	45.7	45.3	45.7
TEd_{40} 90-100ms (%)	Placebo	45.8	45.3	45.6	42.4	41.6	41.5
	Mexiletine	45.7	44.3	44.5	42.0	40.9	40.6
	Lacosamide	45.7	44.0	44.4	41.8	40.7	42.2
TEd_{40} 190-200ms (%)	Placebo	45.6	45.7	45.9	41.5	40.4	41.0
	Mexiletine	45.6	44.5	44.4	40.6	40.3	39.6
	Lacosamide	45.7	43.7	43.8	41.6	39.5	39.9
TEd_{40} peak (%)	Placebo	64.9	65.3	64.9	58.4	58.4	57.8
	Mexiletine	65.5	64.4	63.5	58.1	57.6	55.6
	Lacosamide	64.7	63.0	62.5	56.9	55.7	56.1
TEd_{40} accommodation half-time (ms)	Placebo	19.4	20.2	19.6	16.1	17.1	16.3
	Mexiletine	20.1	20.1	19.2	16.2	16.6	15.3
	Lacosamide	19.3	19.0	18.3	15.2	14.9	14.1
S2 accommodation (%)	Placebo	19.1	20.0	19.2	16.1	16.8	16.3
	Mexiletine	19.8	20.0	18.9	16.1	16.8	15.0
	Lacosamide	19.0	19.1	18.1	15.2	15.0	13.9
TEd_{20} 10-20ms (%)	Placebo	33.8	33.9	34.0	29.7	-32.2	-31.9
	Mexiletine	34.3	33.9	33.7	29.6	-32.0	-30.9
	Lacosamide	34.0	33.0	33.1	29.1	-31.0	-32.2
TEd_{20} peak (%)	Placebo	36.2	36.5	36.4	31.4	31.8	31.6
	Mexiletine	37.0	36.1	35.7	31.2	31.4	30.0
	Lacosamide	36.3	35.0	34.8	30.7	30.0	30.4

(Continuation Supplementary Table 3)

		Motor nerve excitability			Sensory nerve excitability		
		Raw mean baseline	Estimated mean +3h	Estimated mean +6h	Raw mean baseline	Estimated mean +3h	Estimated mean +6h
TEh_{40} 10-20ms (%)	Placebo	-73.0	-73.9	-73.4	-66.5	-66.5	-65.5
	Mexiletine	-73.6	-74.4	-73.6	-66.3	-66.5	-65.3
	Lacosamide	-73.7	-72.2	-72.5	-66.2	-63.7	-65.8
TEh_{40} 90-100ms (%)	Placebo	-123	-125	-122	-89.7	-85.8	-84.7
	Mexiletine	-124	-124	-123	-87.4	-86.9	-87.2
	Lacosamide	-122	-121	-122	-89.0	-84.6	-86.5
TEh_{40} 190-200ms (%)	Placebo	-124	-124	-123	-81.0	-78.7	-78.8
	Mexiletine	-127	-124	-123	-79.9	-78.6	-79.1
	Lacosamide	-123	-121	-121	-81.3	-76.8	-79.1
Fanning sum of TEd_{40}- and TEh_{40} 190-200 ms	Placebo	169	170	168	122	119	120
	Mexiletine	173	169	167	121	119	119
	Lacosamide	169	164	165	123	116	119
Hyper-polarizing I/V-slope	Placebo	0.347	0.356	0.334	0.334	0.325	0.318
	Mexiletine	0.356	0.338	0.323	0.330	0.337	0.352
	Lacosamide	0.364	0.346	0.348	0.349	0.360	0.355
Minimum I/V-slope	Placebo	0.240	0.241	0.240	0.304	0.311	0.307
	Mexiletine	0.236	0.236	0.232	0.305	0.316	0.319
	Lacosamide	0.249	0.249	0.246	0.316	0.335	0.320
Resting I/V-slope	Placebo	0.588	0.579	0.582	0.725	0.771	0.765
	Mexiletine	0.585	0.579	0.592	0.742	0.777	0.779
	Lacosamide	0.575	0.607	0.605	0.775	0.779	0.742
Relative refractory period (ms)	Placebo	2.52	2.59	2.56	3.18	3.38	3.28
	Mexiletine	2.56	2.65	2.61	3.24	3.37	3.33
	Lacosamide	2.52	2.56	2.52	3.15	3.27	3.09
Refractor-iness at ISI 2 ms (%)	Placebo	32.2	36.5	33.6	55.2	66.5	62.6
	Mexiletine	33.8	40.6	35.6	56.3	65.2	60.5
	Lacosamide	31.1	33.2	28.8	53.2	54.2	47.5
Sub-excitability (%)	Placebo	11.9	11.7	11.5	9.72	10.5	10.2
	Mexiletine	11.1	12.1	12.1	8.87	10.7	10.6
	Lacosamide	10.4	11.5	10.8	9.20	8.09	7.40
Super-excitability (%)	Placebo	-24.9	-24.7	-23.9	-19.5	-18.6	-18.4
	Mexiletine	-24.4	-22.9	-22.2	-19.1	-17.7	-16.2
	Lacosamide	-24.9	-23.2	-22.4	-19.9	-17.6	-18.0

SUPPLEMENTARY TABLE 4 Inter- and intrasubject coefficient of variation (CV%) based pre-dose values at each visit, and intrasubject CV% based on the statistical model.

	Motor nerve excitability			Sensory nerve excitability		
	Inter-subject CV (%)	Intra-subject CV (%)	Model-based intrasubject CV (%)	Inter-subject CV (%)	Intra-subject CV (%)	Model-based intrasubject CV (%)
CMAP (mV)/SNAP (µV)	26.7	12.8	8.9	34.3	16	16.9
Threshold for 50% CMAP/SNAP (mA)	32.9	31.2	12.7	33.9	32.9	18.20
Rheobase (mA)	35.8	34.4	14.1	38.9	37.5	22.10
Strength-duration time constant (ms)	22.9	13	10.1	19.7	16.2	13.9
TEd_{40} (10-20ms) (%)	6	3.7	3	8	5.5	5.4
TEd_{40} (40-60ms) (%)	7.3	3.6	2.7	9.8	5.1	6.6
TEd_{40} (90-100ms) (%)	8.1	4.1	3.2	10.1	5.4	7.9
TEd_{40} (190-200ms) (%)	8.2	4.2	3.2	10.9	5.4	7.6
TEd_{40} peak (%)	6	3.4	2.5	8.2	4.8	5.2
TEd_{40} accommodation half-time (ms)	12.3	6.5	7.9	16.1	10.6	11.4
S2-accommodation (%)	12.4	7	8.4	16	9.7	12.1
TEd_{20} (10-20ms) (%)	6.6	4.6	3.2	9.1	6.8	8.6
TEd_{20} peak (%)	8	5	3.3	9.1	6	8.2
TEh_{40} (10-20ms) (%)	7.2	4.5	3.8	6.5	4.1	5.3
TEh_{40} (90-100ms) (%)	15.3	8.6	5.9	16.1	7.2	5.9
TEh_{40} (190-200ms) (%)	18.1	10.1	6.1	16.2	6.7	5.8
Fanning	14.6	7.7	4.8	13.5	5	5.6
Hyperpolarizing I/V-slope	15.8	8.5	9.90	16.3	10.2	10.1
Minimum I/V-slope	17.3	8.4	6.1	14.2	7.9	7.3
Resting I/V-slope	12.7	6.5	5.8	15.5	9.3	11.60
Relative refractory period (ms)	8.2	4.3	3.5	15.9	6.3	7.7
Refractoriness at ISI 2 ms (%)	40.5	22.6	19.1	38.9	17.7	19.2
Subexcitability (%)	35.7	24	20.1	43.6	16.3	26.2
Superexcitability (%)	21	7.7	7.9	31.4	8.7	9.6

SUPPLEMENTARY TABLE 5 Concentration-effect relationship relationships of excitability threshold tracking endpoints. The slope and p-value of the linear effect relations are reported. Baseline was estimated as a separate variable in the model.

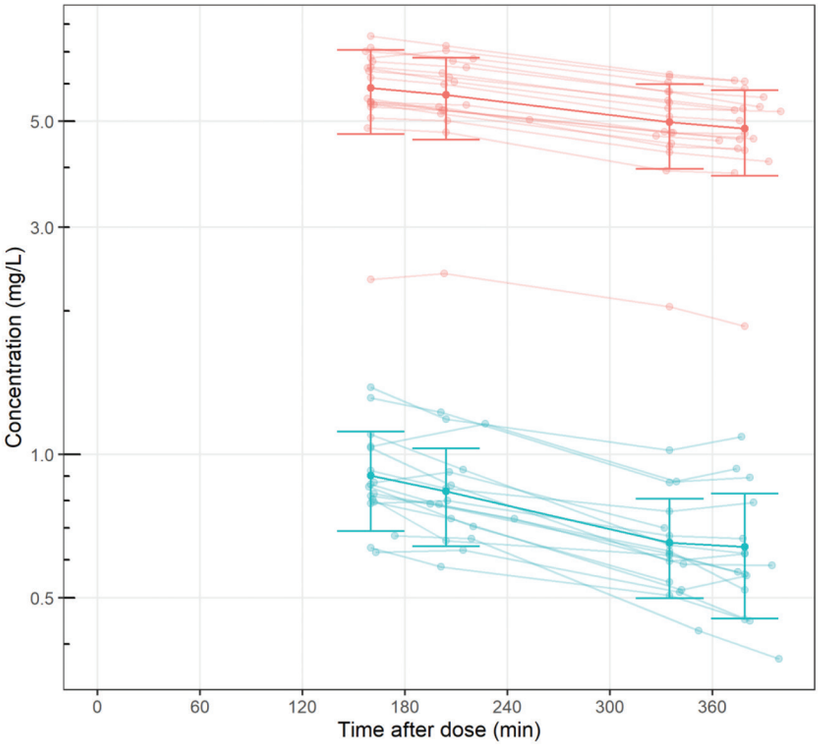
		Motor nerve			Sensory nerve		
		Estimated population baseline	Slope (/ug/L)	P value	Estimated population baseline	Slope (/ug/L)	P value
CMAP (mV)/SNAP (µV)	Mexiletine	13.14			40.38		
	Lacosamide	13.13	0.15	0.001	40.9	0.862	0.004
Threshold for 50% CMAP/ SNAP (mA)	Mexiletine	4.39			2.89		
	Lacosamide	4.12			2.92		
Rheobase (mA)	Mexiletine	3.08			1.75		
	Lacosamide	2.88			1.78		
Strength-duration time constant (ms)	Mexiletine	0.38			0.53		
	Lacosamide	0.39	-0.004	0.002	0.52	-0.011	<0.001
TEd_{40} (10-20ms) (%)	Mexiletine	65.68			57.8		
	Lacosamide*	65.7	1.02/-2.56	<0.001	57.8	-0.385	<0.001
TEd_{40} (40-60ms) (%)	Mexiletine	49.46	-1.732	<0.001	46.14	-1.508	0.037
	Lacosamide	49.43	-0.248	<0.001	45.87		
TEd_{40} (90-100ms) (%)	Mexiletine	45.61	-1.582	<0.001	41.84	-1.502	0.037
	Lacosamide	45.64	-0.282	<0.001	41.63		
TEd_{40} (190-200ms) (%)	Mexiletine	45.58	-1.39	<0.001	40.73	-1.487	0.038
	Lacosamide	45.7	-0.352	<0.001	41.2	-0.24	0.002
TEd_{40} peak (%)	Mexiletine	64.99	-0.893	0.043	57.84		
	Lacosamide	64.82	-0.40	<0.001	57.89	-0.433	<0.001
TEd_{40} accommodation half-time (ms)	Mexiletine	19.76			16.48		
	Lacosamide	19.44	-0.167	0.001	16.05	-0.285	<0.001
S2-accommodation (%)	Mexiletine	19.5			16.38		
	Lacosamide	19.19	-0.122	0.022	15.99	-0.29	0.001
TEd_{20} (10-20ms) (%)	Mexiletine	33.93			29.29		
	Lacosamide	33.84	-0.171	<0.001	29.29		
TEd_{20} peak (%)	Mexiletine	36.35			31.32		
	Lacosamide*	36.29	1.57/-2.01	<0.001	31.37	-0.239	0.005
TEh_{40} (10-20ms) (%)	Mexiletine	-73.58			-66.23		
	Lacosamide	-73.41	0.193	0.025	-66.27	0.313	0.016
TEh_{40} (90-100ms) (%)	Mexiletine	-124.36			-87.01		
	Lacosamide	-122.07			-88.12	0.59	0.001
TEh_{40} (190-200ms) (%)	Mexiletine	-124.64			-79.81	2.37	0.037
	Lacosamide	-123	0.635	0.042	-80.27	0.503	<0.001
Fanning	Mexiletine	169.87			120.48	-3.721	0.017
	Lacosamide	168.71	-0.989	0.003	121.5	-0.763	<0.001
Hyperpolarizing I/V-slope	Mexiletine	0.34			0.33		
	Lacosamide	0.35			0.33	0.004	0.002

(Continuation Supplementary Table 3)

		Motor nerve			Sensory nerve		
		Estimated population baseline	Slope (/ug/L)	P value	Estimated population baseline	Slope (/ug/L)	P value
Minimum I/V-slope	Mexiletine	0.24	-0.008	0.025	0.31		
	Lacosamide	0.24	0.001	0.029	0.31	0.004	<0.001
Resting I/V-slope	Mexiletine	0.59			0.75	0.051	0.025
	Lacosamide	0.58	0.004	0.002	0.76	0.007	0.019
Relative refractory period (ms)	Mexiletine	2.56	0.102	<0.001	3.29	0.155	0.007
	Lacosamide	2.54			3.23		
Refractoriness at ISI 2 ms (%)	Mexiletine	34.14	5.963	0.007	60.05	5.675	0.035
	Lacosamide	32.21			57.85	-1.102	0.019
Subexcitability (%)	Mexiletine	11.95			9.91		
	Lacosamide	11.26			10.06	-0.443	<0.001
Superexcitability (%)	Mexiletine	-24.37	2.421	<0.001	-18.81	2.418	<0.001
	Lacosamide	-24.66	0.311	<0.001	-19.26	0.256	<0.001

*E_{max} *variables presented as* EC50/E_{max}, EC50 *is reported as* ug/L.

SUPPLEMENTARY FIGURE 1 Individual and mean ± standard deviation plasma concentrations of mexiletine and lacosamide, at all four post-dose sampling timepoints before and after the nerve excitability threshold tracking measurements at three and six hours after dosing. N=18.



REFERENCES

1 Alsaloum M, Higerd GP, Effraim PR, Waxman SG. Status of peripheral sodium channel blockers for non-addictive pain treatment. *Nature reviews Neurology*. Dec 2020;16(12):689-705. doi:10.1038/s41582-020-00415-2

2 Bostock H, Cikurel K, Burke D. Threshold tracking techniques in the study of human peripheral nerve. Muscle & nerve. Feb 1998;21(2):137-58. doi:10.1002/(sici)1097-4598(199802)21:2<137::aid-mus1>3.0.co;2-c

3 Kiernan MC, Bostock H, Park SB, *et al.* Measurement of axonal excitability: Consensus guidelines. *Clinical neurophysiology; official journal of the International Federation of Clinical Neurophysiology*. Jan 2020;131(1):308-323. doi:10.1016/j.clinph.2019.07.023

4 Burke D, Kiernan MC, Bostock H. Excitability of human axons. *Clinical neurophysiology; official journal of the International Federation of Clinical Neurophysiology*. Sep 2001;112(9):1575-85. doi:10.1016/s1388-2457(01)00595-8

5 Kuwabara S, Misawa S, Tamura N, *et al.* The effects of mexiletine on excitability properties of human median motor axons. *Clinical neurophysiology; official journal of the International Federation of Clinical Neurophysiology*. Feb 2005;116(2):284-9. doi:10.1016/j.clinph.2004.08.014

6 Pringle T, Fox J, McNeill JA, *et al.* Dose independent pharmacokinetics of mexiletine in healthy volunteers. *British journal of clinical pharmacology*. Mar 1986;21(3):319-21. doi:10.1111/j.1365-2125.1986.tb05196.x

7 Svendsen T, Brodtkorb E, Baftiu A, Burns ML, Johannessen SI, Johannessen Landmark C. Therapeutic Drug Monitoring of Lacosamide in Norway: Focus on Pharmacokinetic Variability, Efficacy and Tolerability. *Neurochemical research*. Jul 2017;42(7):2077-2083. doi:10.1007/s11064-017-2234-8

8 Reimers A, Berg JA, Burns ML, Brodtkorb E, Johannessen SI, Johannessen Landmark C. Reference ranges for antiepileptic drugs revisited: a practical approach to establish national guidelines. *Drug design, development and therapy*. 2018;12:271-280. doi:10.2147/dddt.s154388

9 Cawello W, Mueller-Voessing C, Fichtner A. Pharmacokinetics of lacosamide and omeprazole coadministration in healthy volunteers: results from a phase I, randomized, crossover trial. *Clinical drug investigation*. May 2014;34(5):317-25. doi:10.1007/s40261-014-0177-2

10 Kovalchuk MO, Heuberger J, Sleutjes B, *et al.* Acute Effects of Riluzole and Retigabine on Axonal Excitability in Patients With Amyotrophic Lateral Sclerosis: A Randomized, Double-Blind, Placebo-Controlled, Crossover Trial. *Clinical pharmacology and therapeutics*. Dec 2018;104(6):1136-1145. doi:10.1002/cpt.1096

11 Tomlinson S, Burke D, Hanna M, Koltzenburg M, Bostock H. *In vivo* assessment of HCN channel current (Ih) in human motor axons. *Muscle & nerve*. 2010;41(2):247-256. doi:https://doi.org/10.1002/mus.21482

12 Kiernan MC, Isbister GK, Lin CS, Burke D, Bostock H. Acute tetrodotoxin-induced neurotoxicity after ingestion of puffer fish. *Annals of neurology*. Mar 2005;57(3):339-48. doi:10.1002/ana.20395

13 Nakagawa H, Munakata T, Sunami A. Mexiletine Block of Voltage-Gated Sodium Channels: Isoform- and State-Dependent Drug-Pore Interactions. *Molecular pharmacology*. Mar 2019;95(3):236-244. doi:10.1124/mol.118.114025

14 Errington AC, Stöhr T, Heers C, Lees G. The investigational anticonvulsant lacosamide selectively enhances slow inactivation of voltage-gated sodium channels. *Molecular pharmacology*. Jan 2008;73(1):157-69. doi:10.1124/mol.107.039867

15 Curia G, Biagini G, Perucca E, Avoli M. Lacosamide: a new approach to target voltage-gated sodium currents in epileptic disorders. *CNS drugs*. 2009;23(7):555-68. doi:10.2165/00023210-200923070-00002

16 Jo S, Bean BP. Lacosamide Inhibition of Nav1.7 Voltage-Gated Sodium Channels: Slow Binding to Fast-Inactivated States. *Molecular pharmacology*. Apr 2017;91(4):277-286. doi:10.1124/mol.116.106401

17 Moldovan M, Lange KH, Aachmann-Andersen NJ, Kjær TW, Olsen NV, Krarup C. Transient impairment of the axolemma following regional anaesthesia by lidocaine in humans. *The Journal of physiology*. Jul 1 2014;592(13):2735-50. doi:10.1113/jphysiol.2014.270827

18 Kiernan MC, Lin CS, Andersen KV, Murray NM, Bostock H. Clinical evaluation of excitability measures in sensory nerve. *Muscle & nerve*. Jul 2001;24(7):883-92. doi:10.1002/mus.1085

19 Fujimaki Y, Kanai K, Misawa S, *et al.* Differences in excitability between median and superficial radial sensory axons. *Clinical neurophysiology; official journal of the International Federation of Clinical Neurophysiology*. Jul 2012;123(7):1440-5. doi:10.1016/j.clinph.2011.11.002

20 Bostock H, Rothwell JC. Latent addition in motor and sensory fibres of human peripheral nerve. *The Journal of physiology*. Jan 1 1997;498 (Pt 1)(Pt 1):277-94. doi:10.1113/jphysiol.1997.sp021857

21 Moldovan M, Alvarez S, Rothe C, *et al.* An *in vivo* Mouse Model to Investigate the Effect of Local Anesthetic Nanomedicines on Axonal Conduction and Excitability. *Frontiers in neuroscience*. 2018;12:494. doi:10.3389/fnins.2018.00494