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Mismatch between intact electrical excitability and lost heat pain in diabetic neuropathy

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Abstract

Patterns of sensory involvement in diabetic neuropathy vary between studies and diagnostic approaches. Although some report early thermal deficits, others find predominant large-fiber changes, and hypersensitivity in early disease is inconsistently observed. Elevated heat pain thresholds (HPTs) may indicate either selective loss of heat transduction or advanced peripheral denervation of polymodal nociceptors. We examined whether thermal and mechanical pain functions align with psychophysical axonal excitability by combining German Research Network on Neuropathic Pain-quantitative sensory testing with slow depolarizing transdermal electrical stimulation of polymodal C-fibers in 66 adults with diabetes mellitus. Neuropathy was assessed by Toronto Consensus Criteria, quantitative sensory testing (QST), questionnaires, and serum neurofilament light chain (NfL) as a marker of axonal damage. Mechanical pain sensitivity correlated with electrically evoked pain ($r \approx 0.60$-0.62, both $P < 0.0001$), consistent with parallel changes in mechanical transduction and axonal excitability, whereas HPT did not correlate with electrical pain. Many individuals with elevated HPT still exhibited strong electrically evoked pain responses, suggesting impaired heat transduction despite preserved superficial axonal excitability. Participants with sensory loss in QST showed reduced sensitivity to electrical stimuli and higher detection and pain thresholds, consistent with more advanced afferent dysfunction. NfL levels generally correlated with sensory impairment, although at low electrical intensities, higher NfL values were associated with stronger pain ratings, indicating intensity-dependent links between axonal pathology and nociceptor hyperexcitability. Combining QST with C-fiber–targeted electrical testing refines phenotyping of small-fiber dysfunction in diabetic neuropathy by revealing dissociation between thermal and electrical pain modalities and capturing the heterogeneous course from preserved function to selective thermal hypoalgesia and eventual sensory loss.

Keywords: Diabetic sensorimotor polyneuropathy, Slow depolarizing electrical stimuli, Quantitative sensory testing, Phenotypic transitions, Sensory loss

1. Introduction

Diabetes mellitus (DM) represents a major global health challenge, with diabetic neuropathy (DN) affecting up to 50% of individuals over the course of their disease.[6,17,25] Among such individuals, 20% to 30% also experience neuropathic pain.[7] Diabetic neuropathy manifests in several forms, with distal symmetric polyneuropathy (DSPN) being the most common subtype.[10,39,58] Distal symmetric polyneuropathy may predominantly involve small fibers, large fibers, or both.[17,39] Longitudinal studies indicate that neuropathy tends to gradually deteriorate rather than remain stable or improve.[5,32] Although signs of small-fiber neuropathy (SFN) may occur early, differences in test sensitivity must be considered, and DSPN does not necessarily begin with SFN.[57] Consistent with this heterogeneity, several cross-sectional and prospective studies report early large-fiber involvement and lack of early hypersensitivity.[5,15,19,22,32,48,57] For small-fiber assessment, intraepidermal nerve fiber density (IENFD) from skin biopsies is widely regarded as the diagnostic reference standard.[16] Corneal confocal microscopy (CCM) is increasingly used as an additional structural modality,[16] but does not provide absolute counts of individual fibers and shows variable sensitivity; importantly, both

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IENFD and CCM are structural and lack direct functional characterization.

Quantitative sensory testing (QST) offers a noninvasive, psychophysical approach to evaluating sensory function and is increasingly used for screening and monitoring sensory neuropathies.[3,34,52] The German Research Network on Neuropathic Pain (DFNS) protocol assesses 13 parameters across small- and large-fiber modalities.[34] Combining the measures, distinct QST-derived sensory phenotypes have been proposed to reflect different pathophysiological profiles of neuropathic pain. However, because these phenotypes are defined by QST variables, comparison between phenotypes and the same QST measures entails a degree of methodological circularity and should be interpreted as associative rather than mechanistic. Focusing on small-fiber function (thermal detection and heat pain), a recent longitudinal study has reported early impairment of thermal sensation followed by a later loss of mechanical pain sensitivity, leading to characteristic phenotype shifts over time.[52] By contrast, other datasets—particularly in type 2 diabetes—describe early large-fiber impairment (touch/vibration) with relatively preserved mechanical pain, and no universal early hypersensitivity,[5,15,19,22,32,48,57] underscoring that such trajectories depend on cohort and modality.

A-delta and C-nociceptors contribute differentially to heat pain.[56] A-delta fibers mediate the first pain and can be studied by laser-evoked[8] or contact heat-evoked potentials at steeper ramps, whereas polymodal C-nociceptors contribute predominantly under slow ramp-like thermal stimuli used in QST, with some A-delta contribution remaining.[36,51] Elevated heat pain thresholds (HPTs) could therefore reflect either distal nociceptor denervation or impaired heat transduction with preserved nociceptive axons. Electrical stimulation can be used to probe residual axonal excitability; slow depolarizing transdermal sinusoidal stimulation has been reported to preferentially activate polymodal and mechano-insensitive ("silent") C-nociceptors in human skin.[18,23,24,43]

In this study, we combined DFNS-QST with slow depolarizing transdermal electrical stimulation oriented toward C-fiber activation to examine the relationship between heat pain function and psychophysical excitability in individuals with DSPN. We considered 2 nonexclusive explanatory patterns for elevated HPT: (1) epidermal denervation (predicting higher detection/pain thresholds and reduced or absent electrically evoked pain) and (2) impaired thermal transduction with preserved axons (predicting retained electrically evoked pain despite hypoalgesia). This combined approach aimed to refine small-fiber phenotyping in DSPN by linking thermal and electrically evoked pain measures as complementary indicators of superficial axonal function.

2. Methods

2.1. Study cohort

Data were drawn from the Heidelberg Study of Diabetes and its Complications (HEIST-DiC), a monocentric observational cohort designed to track the incidence and progression of diabetic complications in individuals with type 1 diabetes, type 2 diabetes, and pre-diabetes.[14,27] The protocol was approved by the ethics committee of Heidelberg University Hospital (Approval No. S-383/2016), registered at ClinicalTrials.gov (NCT03022721), and conducted in accordance with the principles of the Declaration of Helsinki.

Between January and October 2023, all clinic attendees with diabetes mellitus were screened for participation. Several individuals declined informed consent; additional exclusions followed predefined contraindications, including cardiac pacemaker (n = 1) and neurological conditions unrelated to diabetic neuropathy (eg, lumbar disc herniation with nerve compression; n = 5). In total, 66 participants fulfilled all inclusion criteria and completed the full protocol. Participants were a consecutively enrolled series during the predefined recruitment window; no post hoc selection was applied.

2.2. Clinical examination and neuropathy assessment

All individuals received a standardized clinical evaluation, including tendon reflexes and sensory testing. Neuropathy assessment comprised questionnaires and bedside examinations, including Neuropathy Deficit Score (NDS) and Neuropathy Symptom Score (NSS),[27] as well as perception of standardized stimuli applied with a TipTherm pen (20-23°C), 10-g-monofilament, a Rydel-Seiffer tuning fork (64 Hz, 8/8 scale), and a reflex hammer. Nerve conduction studies (NCS) were performed on the peroneal, tibial, and sural nerves using a Viking IV electromyography system (Nicolet).[12] Clinical profiling followed the Toronto Consensus Criteria, which incorporate neurological signs and symptoms together with at least 1 abnormal confirmatory test (eg, NCS or QST)[47]; diagnostic categories (possible, probable, confirmed) are reported.

Quantitative sensory testing was performed according to the DFNS protocol,[42] which comprises 13 parameters covering small- and large-fiber-mediated functions: cold detection threshold (CDT), warm detection threshold (WDT), thermal sensory limen (TSL), cold pain threshold (CPT), HPT, pressure pain threshold, mechanical pain threshold (MPT), mechanical pain sensitivity (MPS), wind-up ratio, mechanical detection threshold (MDT), vibration detection threshold (VDT), dynamic mechanical allodynia (DMA), and paradoxical heat sensation (PHS).[27,42] Complete QST profiles were obtained in the dorsum of the foot (primary test region) and additionally at the dorsum of the hand.

The results for each QST parameter were converted to z-scores against age- and sex-adjusted DFNS reference values (means and SDs), following Rolke et al.[42] PHS and DMA were treated categorically (not z-standardized). Individuals with DSPN were classified based on right-foot QST into phenotypes—"normal sensory response (NSR)," "thermal hyperalgesia (TH)," "mechanical hyperalgesia (MH)," or "sensory loss (SL)"—as described by Vollert et al., 2017.[55] To capture clinical pain characteristics, the painDETECT questionnaire was administered to participants reporting lower-extremity symptoms (eg, pain, cramps, burning, numbness, fatigue). For subgroup analyses, participants reporting lower-extremity symptoms were stratified into predominantly positive sensory symptoms (eg, burning, pain) vs negative sensory symptoms (eg, numbness, tingling) according to painDETECT item profiles.

2.3. Clinical chemistry

Fasting blood and morning urine were collected and analyzed in the Central Laboratory of the Heidelberg University Hospital. Measurements included hemoglobin A1c (HbA1c), lipid profile (low-density lipoprotein, high-density lipoprotein, total cholesterol, triglycerides), urine albumin/creatinine ratio, and estimated glomerular filtration rate (eGFR) calculated using the Chronic Kidney Disease Collaboration formula.[30] Additional metabolic markers included homeostasis model assessment for insulin resistance (HOMA-IR) and beta-cell function (C-peptide). Serum neurofilament light chain (NfL) was quantified as a biomarker for axonal damage.[35]

2.4. Slow depolarizing electrical C-fiber stimulation

Participants first completed a familiarization session with slow depolarizing electrical stimuli and Numeric Rating Scale (NRS) pain ratings (0-10) at the same sites used for QST (dorsum of the right hand and foot). Training exposures comprised sinusoidal pulses at 4 Hz for 2.5 seconds at randomized intensities (0.05, 0.1, 0.2 mA) and single half-sinusoidal pulses (0.5 seconds) at 0.2 to 1.0 mA in 0.2 mA steps with 10-second interstimulus intervals. Training data were not analyzed.

Stimulation was delivered using bipolar platinum electrodes (0.4 mm diameter; 2 mm interelectrode distance).[29] A constant-current stimulator (Digitimer DS5, Welwyn Garden City, United Kingdom) controlled by a DAQ (NI USB-6221, National Instruments, Austin, TX) generated sinusoidal and half-sinusoidal pulses through Dapsys8 software (Brian Turnquist, Bethel University). For comfort and safety, participants manually positioned—and could remove—the electrode on the dorsomedial area of the right hand and foot under investigator supervision.

The test protocol comprised 5 parts, each performed twice; duplicates were averaged for analysis: (1) single half-sine stimuli (500 ms) presented in random order (0.2-1.0 mA); (2) sinusoidal 4-Hz, 2.5-second stimuli with incremental intensity to determine the detection threshold (lowest current detected); (3) further current increase to the pain threshold, defined as the lowest intensity eliciting NRS $\geq$ 2; (4) sinusoidal 4-Hz, 2.5-second stimuli at randomized intensities (0.025-0.4 mA) with NRS ratings; (5) continuous (60 seconds) suprathreshold sinusoidal stimulation at 4 Hz, using an individualized intensity defined a priori as the current that produced NRS = 3 (mild pain) in part (4); if NRS = 3 was not reached within 0.025 to 0.4 mA, the highest tolerated intensity within that range was used. The Numeric Rating Scale was recorded at 5, 15, 30, 45, and 60 seconds. The 60-second stimulation in (5) was then repeated at a fixed 0.2 mA for all participants, providing a common reference across individuals irrespective of individualized suprathresholds.

This stimulation paradigm is oriented toward preferential activation of unmyelinated nociceptors based on prior reports, while recognizing that exclusive C-fiber selectivity cannot be demonstrated in a psychophysical setup.[18,23,24,43]

2.5. Statistics

Statistical analyses were carried out using GraphPad Prism version 10.0. The Shapiro-Wilk test was used to evaluate the normality of the data. For data that followed a normal distribution, group comparisons were made using ordinary 1-way ANOVA, followed by either Tukey post hoc test, Fisher Least Significant Difference (LSD) test, or Scheffé post hoc for pairwise comparisons. When the data did not meet normality assumptions, the Kruskal-Wallis test was applied for group comparisons, with the Dunn post hoc test used for subsequent pairwise analysis. Bivariate correlations between variables were examined using the Spearman rank correlation coefficient. All tests were 2-sided with $\alpha = 0.05$; multiplicity was addressed by the specified post hoc procedures. No imputation was performed, and complete-case analyses were used.

3. Results

3.1. Participant characteristics, clinical examination, and neuropathy assessment

Using the Toronto Consensus Criteria, 51/66 participants met diagnostic criteria for DSPN: 19 possible, 21 probable, and 11 confirmed. Quantitative sensory testing-based sensory phenotyping (right foot) yielded 4 groups: 19 normal sensory response (NSR), 9 thermal hyperalgesia (TH), 18 mechanical hyperalgesia (MH), and 20 SL. Mean age differed across phenotypes, with the NSR group significantly younger than all other groups ($P < 0.01$). Groups also differed in diabetes duration, metabolic parameters (BMI, HOMA-IR, C-peptide, HbA1c), lipid profile, and neuropathy severity (NDS, NSS, nerve conduction), as summarized in **Table 1**.

3.2. Quantitative sensory testing profiles at the foot and hand

At the foot dorsum, z-scores were significantly lower in the SL group than in the other phenotypes for 9 parameters—CDT, WDT, TSL, CPT, HPT, MDT, MPT, MPS, and VDT (all $P < 0.05$; **Fig. 1A**), which reflect single QST measures rather than composite scores. In this cohort, mechanical pain sensitivity did not differ significantly among NSR, MH, and TH. The MH phenotype was distinguished from TH by an additional reduction in thermal sensitivity. Compared with NSR, thermal sensitivity in TH was generally lower, except for cold pain thresholds, which were significantly sensitized (**Fig. 1A**). At the hand dorsum, sensory impairment was less pronounced overall (**Fig. 1B**). Reduced thermal sensitivity was already evident in SL and MH, whereas mechanical and electrical pain sensitivity were less affected at this site.

3.3. Electrical stimulation outcomes

Sensitivity to slow depolarizing electrical stimulation was reduced in SL, evidenced by right-shifted dose–response curves for half-sine and 4-Hz sinusoidal stimuli (**Fig. 2A**) and by higher currents required for detection and for pain threshold (NRS $\geq$ 2; **Fig. 2B**). No significant differences were observed between MH and TH for these measures (**Figs. 2A and B**). When sinusoidal stimuli were delivered for 60 seconds at individualized suprathreshold intensities (defined in Methods as the lowest current producing NRS = 3 in part [iv]), electrically evoked pain was comparable among NSR, MH, and TH (**Fig. 2C**). A similar pattern was observed at a fixed 0.2 mA; pain ratings tended to be higher in TH and MH than NSR, but differences were not significant.

3.4. Mismatch between sensory functions of polymodal nociceptors and their electrical excitability

If reduced sensory function were solely due to loss of innervation, electrical pain responses would be expected to decrease in parallel. Mechanical pain sensitivity correlated with electrically evoked pain for both half-sine stimuli (which preferentially activate polymodal nociceptors)[41] and sinusoidal stimuli (which can activate both polymodal and "silent" nociceptors)[21] (Pearson r = 0.60 and r = 0.62, respectively; both $P < 0.0001$; **Fig. 3**). By contrast, HPT did not correlate with electrically evoked pain. Robust electrical pain ratings were observed even in individuals with markedly elevated HPT, indicating that, despite impaired heat pain function, nociceptive axonal excitability at superficial sites could be retained. Although most SL participants clustered separately (**Fig. 3**), NSR, TH, and MH overlapped. Taken together, this pattern is consistent with preserved superficial nociceptor excitability despite diminished heat pain function, without asserting a specific mechanism.

3.5. Correlations between neurofilament light chain and sensory function

Serum NfL, a biomarker of axonal damage,[33,35] correlated negatively with most sensory test results (**Fig. 4A**). Correlations

Table 1

Demographic factors and clinical characteristics of individuals with diabetes mellitus.

Characteristic	NSR	TH	MH	SL
Age, y	55.4 ± 3.6	**61.6 ± 3.9****	**72.1 ± 2.0****	**59.7 ± 2.7****
Sex, F/M	3/5	3/6	4/10	5/15
Duration since diabetes diagnosis, y	19.5 ± 2.9	12.9 ± 2.2	18.8 ± 4.1	21.5 ± 2.7
Diabetes type, 1/2	3/5	1/8	4/10	5/15
Pain, yes/no	6/2	3/6	2/12	4/16
BMI, kg/m^2	28.7 ± 1.8	31.1 ± 1.8	27.6 ± 1.4	29.3 ± 0.9
Mean corpuscular volume (MCV), fL	89.9 ± 1.9	88.9 ± 0.8	87.4 ± 0.9	88.9 ± 0.6
Albumin, g/L	44.4 ± 1.1	44.9 ± 0.7	44.9 ± 0.6	44.8 ± 0.4
Creatinine, mg/dL	0.7 ± 0.1	0.8 ± 0.1	0.9 ± 0.1*	1.1 ± 0.2**
eGFR, mL min^{-1} $(1.73)^{-2}$	98.8 ± 4.8	90.4 ± 4.6	81.1 ± 3.4**	82.8 ± 4.0*
Urine albumin/creatinine ratio (mg/mmol creatinine)	0.8 ± 0.4	1.4 ± 0.9	3.4 ± 1.5	4.4 ± 1.9
Total cholesterol, mg/dL	169.4 ± 14.1	165.3 ± 7.6	173.8 ± 9.1	175.2 ± 7.2
HDL cholesterol, mg/dL	54.9 ± 8.1	47.7 ± 2.2	53.3 ± 3.4	48.8 ± 2.4
LDL cholesterol, mg/dL	79.9 ± 10.7	99.5 ± 7.0	96.3 ± 6.8	99.2 ± 6.4
Triglycerides, mg/dL	173.4 ± 43.1	144.9 ± 24.7	136.0 ± 16.6	134.4 ± 14.4
Troponin T, pg/mL	15.1 ± 4.0	9.5 ± 1.6	13.2 ± 1.7	15.6 ± 4.7
Fasting glucose, mg/dL	165.6 ± 19.6	143.3 ± 12.2	143.3 ± 14.7	142.5 ± 10.5
HbA1c, %	7.7 ± 0.3	6.7 ± 0.5	7.1 ± 0.3	6.5 ± 0.2
C-peptide, ng/mL	1.8 ± 0.6	2.1 ± 0.3	1.9 ± 0.3	1.7 ± 0.2
HOMA-IR	8.8 ± 4.0	8.5 ± 4.8	6.8 ± 2.1	7.4 ± 1.8
Neurofilament light chain (NfL) in serum, pg/mL	8.3 ± 1.6	8.6 ± 1.9	13.9 ± 1.8	14.4 ± 2.1
Neuropathy Deficit Score, /10	3.3 ± 0.5	2.8 ± 0.7	4.9 ± 0.6	4.4 ± 0.7
Neuropathy symptom score, /10	4.0 ± 0.8	2.1 ± 0.8	3.1 ± 0.9	2.2 ± 0.5
Amplitude N. suralis right leg, μV	8.4 ± 3.4	5.9 ± 2.0	5.7 ± 1.7	**2.3 ± 0.5***
Amplitude N. tibialis right leg, μV	13.9 ± 4.9	10.5 ± 3.5	7.1 ± 2.0	**6.6 ± 1.5***
Amplitude N. peroneus right leg, μV	7.1 ± 2.5	4.4 ± 1.5	3.7 ± 1.0	**1.5 ± 0.3****

Individuals were grouped according to the previously suggested sensory QST phenotypes of the foot dorsum as "normal sensory phenotype," "thermal hyperalgesia (TH)," "mechanical hyperalgesia (MH)," or "sensory loss (SL)." Data are mean ± SEM. Statistical significance was assessed using the Kruskal-Wallis 1-way analysis of variance and the Dunn multiple-comparison test, comparing each phenotype to the normal sensory phenotype denoted by asterisks (*$P < 0.05$, **$P < 0.01$).

BMI, body mass index; eGFR, estimated glomerular filtration rate; F, female; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein; M, male; HOMA-IR, homeostasis model assessment of insulin resistance; NSR, normal sensory phenotype. Electrical paradigms: HS, half-sine stimulation (500 ms); SIN, 4-Hz sinusoidal stimulation (2.5 seconds, 10 pulses); SIN 1 minute, continuous 4-Hz sinusoidal stimulation for 1 minute; QST, quantitative sensory testing.

were weak for C-fiber–weighted measures such as HPT and WDT, but reached significance for thinly myelinated (eg, CDT) and myelinated (MDT/VDT) modalities, with the strongest negative association for VDT, consistent with greater involvement of large, myelinated fibers. For electrical stimulation, pain ratings during 4-Hz sinusoidal stimuli tended to correlate negatively with NfL at higher intensities (**Fig. 4B**). At lower amplitudes (0.025-0.05 mA), pain ratings increased with higher NfL, suggesting intensity-dependent associations between axonal injury markers and psychophysical excitability.

3.6. Positive vs negative symptom profiles in distal symmetric polyneuropathy

Among participants with DSPN, those reporting positive sensory symptoms (burning, pain; n = 17) exhibited greater electrically evoked pain than those with predominantly negative symptoms (numbness; n = 16). Differences were evident for 500-millisecond half-sine stimuli at 0.4, 0.6, 0.8, and 1.0 mA (**Fig. 5A**) and for 4-Hz sinusoidal bursts (10 pulses, 2.5 seconds) at 0.2 and 0.4 mA (both $P < 0.01$; **Fig. 5B**). During 60-second continuous sinusoidal stimulation, pain ratings were markedly higher in the positive-symptom group (**Figs. 5C and D**). Consistently, detection and pain thresholds for sinusoidal stimuli were lower in the positive-symptom group (both $P < 0.05$; **Figs. 5E and F**). The observed symptom-linked elevation in electrically evoked pain and lower threshold points to greater superficial excitability in the positive-symptom group.

4. Discussion

Diabetic neuropathy progressively involves small and large fibers, resulting in sensory deficits and neuropathic pain. Quantitative sensory testing provides psychophysical readouts useful for staging DN; however, because QST-derived sensory phenotypes are defined by the same QST variables, comparisons between phenotypes and those variables entail methodological circularity. Accordingly, phenotype differences should be interpreted as

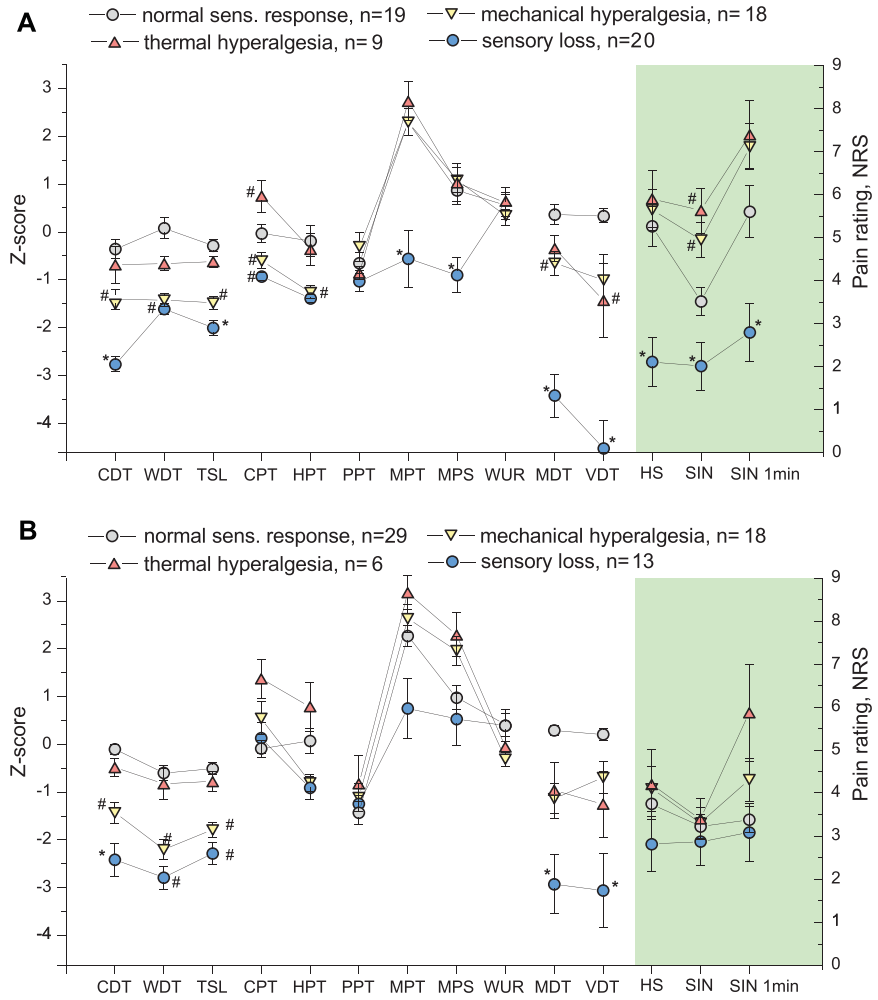

Figure 1. QST z-scores and electrically evoked pain at the foot (A) and hand (B). At the foot, only the sensory loss (SL) phenotype shows consistently lower z-scores across modalities, accompanied by lower pain ratings to electrical stimulation (upper panel). The mechanical hyperalgesia (MH) phenotype differs from thermal hyperalgesia (TH) by exhibiting reduced thermal sensitivity. Note that mechanical pain sensitivity is higher in NSR and comparable to TH/MH. Data are mean ± SEM. *$P < 0.05$ vs all other phenotypes; #$P < 0.05$ vs NSR (1-way ANOVA, Fisher LSD post hoc). CDT, cold detection threshold; CPT, cold pain threshold; HPT, heat pain threshold; HS, half-sine stimulation (500 ms); MDT, mechanical detection threshold; MPS, mechanical pain sensitivity; MPT, mechanical pain threshold; PPT, pressure pain threshold; QST, quantitative sensory testing; SIN 1 minute, continuous 4-Hz sinusoidal stimulation for 1 minute; SIN, 4-Hz sinusoidal stimulation (2.5 seconds, 10 pulses); TSL, thermal sensory limen; VDT, vibration detection threshold; WDT, warm detection threshold; WUR, wind-up ratio.

associations rather than direct evidence of underlying mechanisms. Sensory loss may arise from impaired transduction (nociceptive terminals present but functionally silent) or from denervation (retracted terminals). Distinguishing these patterns matters for pathophysiological and treatment considerations. Accordingly, DFNS-QST was combined with C-fiber–oriented slow depolarizing transdermal electrical stimulation to examine whether heat and mechanical pain functions align with, or dissociate from, psychophysical indices of superficial axonal excitability.

In our cohort, concurrent reductions in mechanical pain and electrically evoked pain were compatible with loss of polymodal nociceptor input (gradual denervation). By contrast, elevated heat pain thresholds (HPTs) despite preserved electrically evoked pain suggested impaired heat transduction with retained superficial axonal excitability. These inferences are associative; alternative contributors (eg, stimulus depth, spatial heterogeneity of receptor endings, potential A-fiber co-activation at higher currents) may also contribute. Consistent with this interpretation, symptom-based stratification showed higher electrically evoked pain and lower sinusoidal detection and pain thresholds in participants with positive sensory symptoms than in those with predominantly negative symptoms.

4.1. Mechanical and heat pain vs electrical stimulation (C-fiber–oriented)

We related responses to C-fiber–oriented electrical stimulation to the mechanical and heat sensitivity of cutaneous nociceptors. Although pinprick pain latency is largely determined by A-delta conduction,[59] polymodal C-nociceptor contributions have been reported.[54] Under differential A-fiber block, QST-derived mechanical pain measures can be sensitized ($z > 1$ for mechanical pain sensitivity; $z > 2$ for mechanical pain threshold), consistent with a C-nociceptor role in pinprick pain processing.[54] Polymodal C-nociceptors have low mechanical activation thresholds (~3 g von Frey)[44] and encode mechanical pain,[26] while punctate hyperalgesia after capsaicin is strongly linked to A-delta nociceptors.[59]

In our data, mechanical pinprick pain correlated with electrically evoked pain ($r \approx 0.5$-0.6), suggesting that dysfunction affecting transduction and axonal excitability can progress in

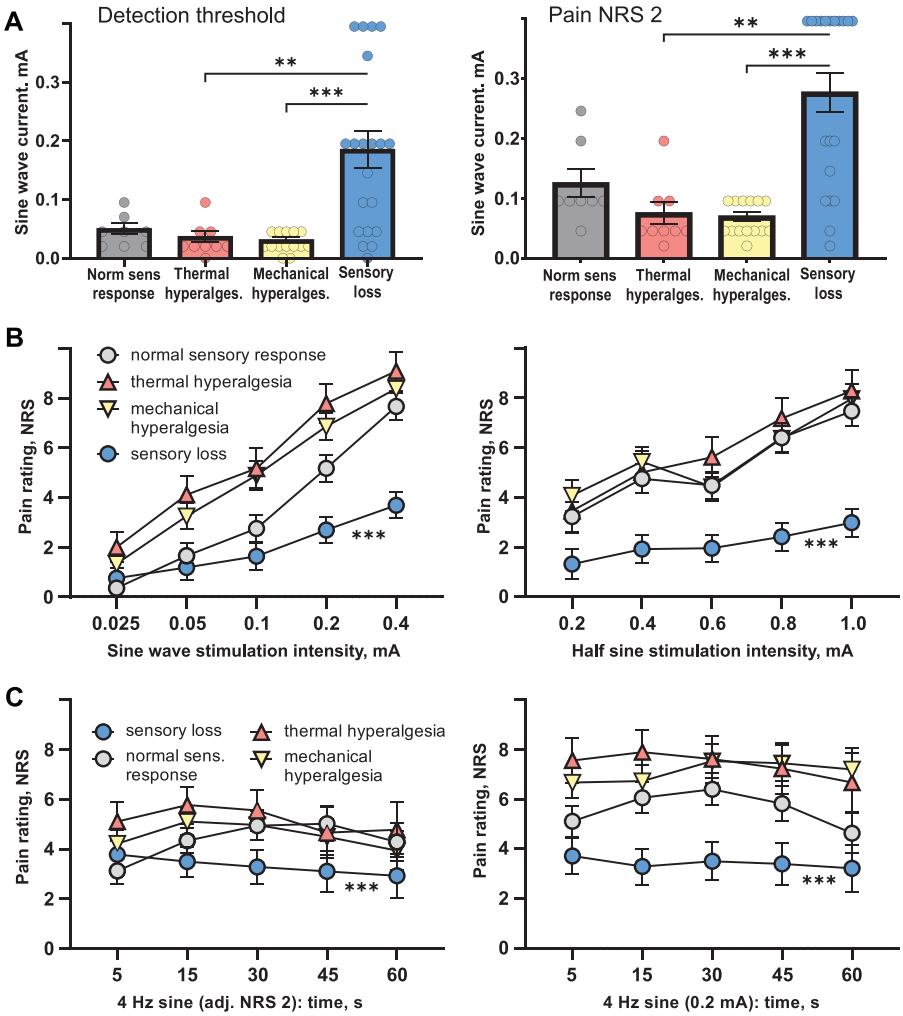

Figure 2. C-fiber–oriented electrical stimulation at the foot dorsum. Mean detection thresholds (left) for 4-Hz sinusoidal stimuli and pain thresholds (right; lowest current eliciting NRS ≥ 2) across sensory phenotypes (A). Intensity–response curves for electrically evoked pain to 4-Hz sinusoidal (left) and 500-millisecond half-sine (right) stimuli. Only SL shows significantly lower pain ratings than all other phenotypes (B). Pain ratings during 1-minute sinusoidal stimulation at an individualized suprathreshold intensity (defined in Methods as the lowest current producing NRS = 3 in part iv; left) and at a fixed 0.2 mA (right) (C). Only SL shows significantly lower pain ratings than all other phenotypes. ***$P < 0.001$ (1-way ANOVA, Scheffé post hoc). NRS, Numeric Rating Scale; SL, sensory loss.

parallel; progressive terminal retraction in DN could be 1 compatible explanation.[41] C-nociceptors also contribute to tonic heat pain.[28,51] If half-sine stimulation (preferential for polymodal C-nociceptors)[43,45,46,53] and heat pain primarily reflect the same terminal population, reduced HPT would be expected to co-occur with reduced electrically evoked pain. Instead, we observed a dissociation: individuals with markedly impaired heat pain (HPT $z < -1$) could still report high electrical pain (eg, NRS > 6) to half-sine stimuli, whereas such a dissociation was not seen for mechanical pain (**Fig. 3**). This pattern is consistent with preserved superficial nociceptor excitability despite diminished heat pain function, without asserting a specific mechanism.

The slow heating rate in the QST protocol (~1°C/second) likely engages transduction sites at ~100 to 300 μm depth,[50] approximating epidermal thickness at hand (~100 μm) and foot dorsum (~170 μm).[31] Terminal retraction would be expected to elevate HPT; however, a parallel reduction in electrical responses would also be anticipated given the depth-dependent decline of stimulus potential.[40] By contrast, local thermal buffering (core 37°C, perfusion) steepens temperature gradients across skin,[13] potentially making HPT more sensitive to distal retraction than electrical responses—1 plausible contributor to the dissociation. In addition, terminal Schwann cells modulate mechanical but not heat activation of polymodal nociceptors in mice[1,37]; if a similar division applies to humans, preserved Schwann-cell support could help maintain mechanical and electrical responses despite impaired heat pain. These mechanistic links are hypotheses that require targeted studies.

4.2. Sensory phenotypes

Longitudinal studies suggest a gradual decline of sensory function in diabetes.[5,32,52] When focusing on small-fiber function, thermal sensitivity typically declines early, whereas, as illustrated in **Figure 6**, mechanical pain sensitivity remains relatively preserved in the initial stages.[5,52] Such progression patterns are difficult to reconcile with the concept of multiple stable sensory phenotype clusters. In our dataset, nociceptive functions overlapped broadly across proposed phenotypes, with a clearer separation only for SL—in line with prior analyses where principal components separated loss from non-loss rather than multiple discrete clusters.[55] Notably, the MH phenotype showed reduced thermal rather than increased mechanical pain, and TH exhibited slightly reduced heat pain relative to NSR. Overall, the data support SL as

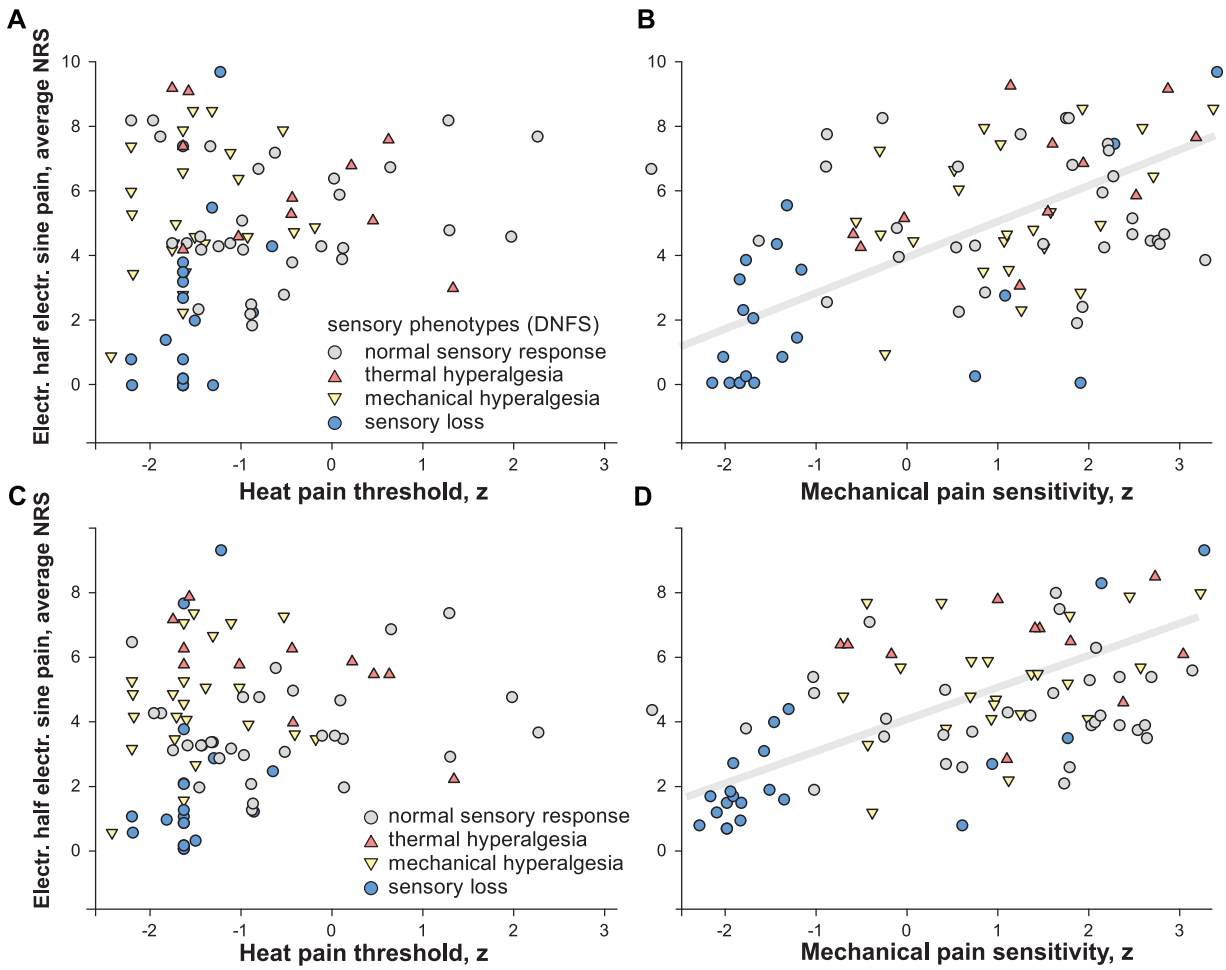

Figure 3. Correlations between electrically evoked pain and mechanical/heat pain functions. Average pain ratings to half-sine stimulation (500 ms; 0.2-1.0 mA) are plotted against HPT (A) and mechanical pain sensitivity (MPS) (B). Average pain ratings to 4-Hz sinusoidal bursts (10 pulses; 0.05-0.4 mA) are plotted against HPT (C) and MPS (D). Points show individual participants, colored by QST phenotype. High electrical pain ratings despite elevated HPT (heat hypoalgesia) are evident, whereas mechanical pain sensitivity correlates closely with electrically evoked pain. Best-fit linear regressions (gray) are shown for reference. HPT, heat pain threshold; NRS, Numeric Rating Scale; QST, quantitative sensory testing, DNFS, Deutscher Forschungsverbund Neuropathischer Schmerz, German Research Network on Neuropathic Pain.

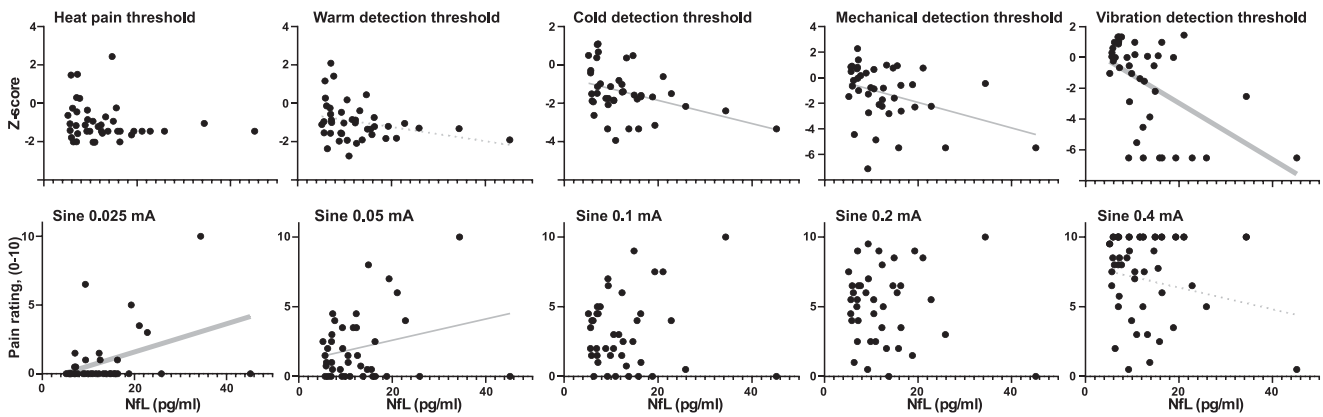

Figure 4. Serum neurofilament light chain (NfL) and sensory function. Correlations between serum NfL and individual QST measures (HPT, WDT, CDT, MDT, VDT). Significant negative correlations are most pronounced for non-nociceptive detection thresholds (CDT, MDT, VDT) (*upper panels*). Correlations between NfL and electrically evoked pain during 4-Hz sinusoidal bursts (10 pulses) across intensities 0.025 to 0.4 mA. Pain ratings show a positive association with NfL at the lowest intensities, but a negative association at higher intensities (*lower panels*). Pearson linear correlation: $^{*}P < 0.05$, $^{***}P < 0.001$; dashed regression lines denote trends ($0.05 \leq P < 0.06$). CDT, cold detection threshold; HPT, heat pain threshold; MDT, mechanical detection threshold; QST, quantitative sensory testing; VDT, vibration detection threshold; WDT, warm detection threshold.

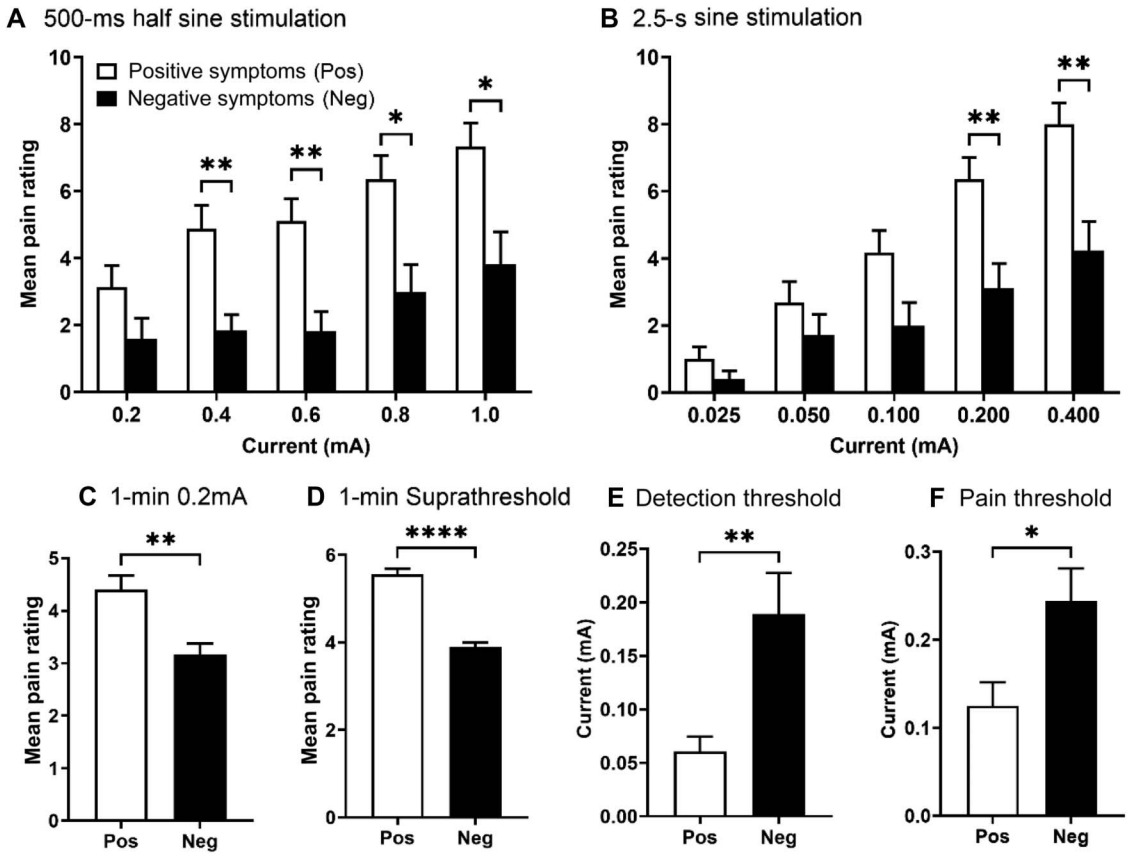

Figure 5. Positive vs negative symptom profiles in DSPN: electrical outcomes. Mean pain ratings (0-10) across 5 current intensities during 500-millisecond half-sine (A) and 2.5-second sinusoidal (B) stimulation. Mean pain ratings during 1-minute sinusoidal stimulation at 0.2 mA (C) and at individualized suprathreshold intensity (D; lowest current producing NRS = 3 in part iv of the protocol). Group differences in sinusoidal detection thresholds (E) and pain thresholds (F). Data are mean ± SEM. Independent-samples Mann–Whitney *U* tests: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. DSPN, distal symmetric polyneuropathy; NRS, Numeric Rating Scale.

a later stage within a continuum of decline, while cautioning against over-interpreting additional discrete phenotypes—particularly given the circularity inherent to QST-defined groupings.

4.3. Neurofilament light chain

Serum neurofilament light chain (NfL) is increasingly recognized as a biomarker of axonal injury. Yet, its role in diabetic neuropathy remains a subject of active investigation, with studies reporting both clear associations with severity and limitations in diagnostic utility.[33,35] In line with our multimodal approach, we included NfL as a complementary serum marker to contextualize structural axonal degeneration, particularly of larger, myelinated fibers, alongside our functional psychophysical measures. We sought to explore its potential relationship with pain phenotypes and nociceptor excitability, prompted by longitudinal observations suggesting a link between elevated NfL and future pain risk.

Most sensory tests correlated negatively with serum NfL, consistent with its role as a biomarker of axonal injury.[35] Associations were strongest for large, myelinated functions (eg, VDT) and thinner myelinated modalities (eg, CDT), whereas correlations with C-fiber–weighted measures (HPT, WDT) were weaker (**Fig. 4A**). Prior work linked higher NfL to nociceptor hyperexcitability and future pain risk.[35] Similarly, in our data, low-intensity 4-Hz sinusoidal stimulation showed higher pain ratings with higher NfL, whereas higher intensities showed the expected negative relationship (**Fig. 4B**). As slow sinusoidal stimuli can elicit single spikes per cycle[23] and C-fibers' long membrane time constant[38] may be further prolonged by increased membrane resistance in neuropathy,[49] small currents could suffice to produce pain in sensitized axons. These observations suggest that NfL may index both degeneration and intensity-dependent aspects of hyperexcitability; interpretations remain associative.

4.4. Clinical implications

Absent heat pain in DN should not be taken as evidence of epidermal denervation unless accompanied by loss of mechanical and electrical pain. Patients with preserved mechanical/electrical pain but impaired heat pain likely retain superficial nociceptive axons and might be candidates for topical therapies acting peripherally (eg, lidocaine[4] or capsaicin).[2,20] Conversely, absence of both mechanical and electrical pain responses may reflect more advanced denervation where centrally acting agents (eg, gabapentinoids or tricyclic antidepressants) could be prioritized.[11] Beyond symptom control, early metabolic interventions (glycemic optimization, lifestyle modification) may help preserve nociceptor function and delay small-fiber involvement.[9] These implications are hypothesis-generating and warrant prospective validation.

4.5. Limitations

This single-center, cross-sectional study captures 1 time point only; longitudinal datasets combining DFNS-QST with slow depolarizing electrical stimulation are needed to map trajectories. The sample size was modest, which may limit generalizability.

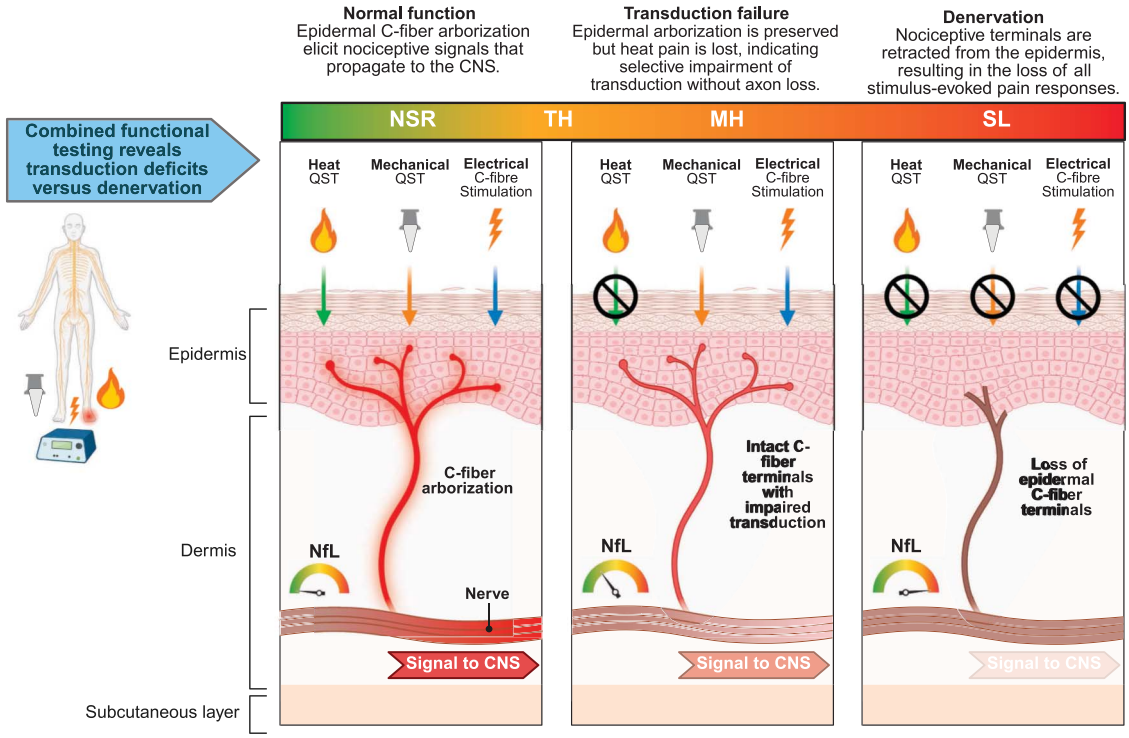

Figure 6. Visual representation of the progressive continuum of C-fiber dysfunction in diabetic neuropathy. This figure depicts the progressive decline of C-fiber nociceptive function in individuals with diabetic neuropathy. The panels illustrate predefined sensory phenotypes—NSR (normal sensory response), TH (thermal hyperalgesia), MH (mechanical hyperalgesia), and SL (sensory loss)—and their associated structural and functional changes in nociceptors. The progression begins with reduced thermal pain sensitivity despite preserved intraepidermal C-fiber endings, suggesting early transduction deficits. This is followed by impairment of mechanical pain perception, characterized by partial transduction failure and limited structural loss. In the final stage (SL), pronounced denervation occurs, with C-fiber terminals retracting from the epidermis and complete loss of stimulus-evoked nociceptive signaling. SL, sensory loss.

Pain was not prevalent in our cohort, which limited formal comparisons between participants with and without neuropathic pain. Psychophysical outcomes are inherently subjective; to mitigate expectancy effects, participants completed a training session, and those data were excluded. C-fiber selectivity of electrical stimulation cannot be proven in this setup. Phenotypes are QST-defined, introducing circularity when compared back to QST variables. Finally, serum NfL can be influenced by age and renal function; intensity-dependent associations observed in this study require confirmation in larger, adjusted analyses.

5. Conclusion

Combining DFNS-QST with C-fiber–oriented electrical stimulation revealed a dissociation between heat pain function and electrical pain responses in a subset of individuals with DN. This pattern is compatible with impaired heat transduction in the presence of retained superficial axonal excitability, whereas concurrent reductions in mechanical and electrical pain are more consistent with terminal loss (**Fig. 6**). These findings challenge the interpretation of multiple discrete QST phenotypes and support a continuum culminating in sensory loss. They also raise the possibility that NfL reflects not only axonal injury but intensity-dependent elements of hyperexcitability at low stimulus amplitudes. Prospective multimodal studies integrating structural and functional measures should test these hypotheses, refine risk stratification, and inform treatment selection in DN.

Conflict of interest statement

The authors have no conflict of interest to declare.

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