Research Paper

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Pattern of neuropathic pain induced by topical capsaicin application in healthy subjects

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Abstract

Human experimental pain models are widely used to study drug effects under controlled conditions, but they require further optimization to better reflect clinical pain conditions. To this end, we measured experimentally induced pain in 110 (46 men) healthy volunteers. The quantitative sensory testing (QST) battery (German Research Network on Neuropathic Pain) was applied on untreated ("control") and topical capsaicin-hypersensitized ("test") skin. Z-transformed QST-parameter values obtained at the test site were compared with corresponding values published from 1236 patients with neuropathic pain using Bayesian statistics. Subjects were clustered for the resemblance of their QST pattern to neuropathic pain. Although QST parameter values from the untreated site agreed with reference values, several QST parameters acquired at the test site treated with topical capsaicin deviated from normal. These deviations resembled in 0 to 7 parameters of the QST pattern observed in patients with neuropathic pain. Higher degrees (50%-60%) of resemblance to neuropathic QST pattern were obtained in 18% of the subjects. Inclusion in the respective clusters was predictable at a cross-validated accuracy of 86.9% by a classification and regression tree comprising 3 QST parameters (mechanical pain sensitivity, wind-up ratio, and z-transformed thermal sensory limen) from the control sites. Thus, we found that topical capsaicin partly induced the desired clinical pattern of neuropathic pain in a preselectable subgroup of healthy subjects to a degree that fuels expectations that experimental pain models can be optimized toward mimicking clinical pain. The subjects, therefore, qualify for enrollment in analgesic drug studies that use highly selected cohorts to enhance predictivity for clinical analgesia.

Keywords: Human, Experimental pain, Models, Drug development, Bioresponses

1. Introduction

Experimental induction of pain in healthy subjects is widely used to study the physiology and pathophysiology of human nociception and analgesia.[9] During analgesic drug development, experimental human pain models are a cost-reducing alternative to clinical trials. However, as with animal models,[19] their utility is controversial because of a perceived poor translation of their outcome to clinical settings. A recent analysis, however, showed that this is only partially true. By using the most suitable experimental human model,[15] an acceptable degree of prediction of clinical analgesic drug efficacy can already be obtained.[20]

Further optimization of experimental pain models to achieve better reflection of clinical pain remains a goal of human pain research. Because drugs under development have to demonstrate efficacy in clinical pain settings, for example, through assessment of their efficacy with standardized clinical pain tests, this study was performed to assess experimentally induced pain under similar standardized conditions. An assumption made was that development of successful experimental pain models should mimic clinical pain. Therefore, the well-established experimental pain model of capsaicin sensitization[22] was submitted to an assessment with a standardized clinical test battery[24] for neuropathic pain.

The working hypothesis was that the pattern of clinical neuropathic pain is inducible, at least to a certain extent, in healthy human subjects using the well-established hypersensitization procedure of topical capsaicin application. Because it was expected that only a subgroup of healthy subjects would demonstrate this complementarity, criteria were sought that could be used to identify subjects displaying the highest degree of inducibility of neuropathic pain patterns. The goal was to provide a quantitative basis for the preselection of subjects in future experimental studies to assess the efficacy of drugs for the treatment of neuropathic pain in a highly selected cohort to enhance predictivity for clinical settings.

2. Methods

2.1. Subjects and study design

Healthy volunteers of white ethnicity by self-assignment (n = 110, 46 men), aged 18 to 36 years (mean ± SD 25.1 ± 3.2 years), were enrolled after having provided informed written consent. Exclusion criteria were drug intake during the previous week, except for oral contraceptives and vitamin or hormone-substituting drugs

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(eg, L-thyroxin), a current clinical condition involving pain, and current diseases according to questioning and medical examination. Before the experimental tests, all subjects completed training sessions with pain tests applied to an area different from the planned test and control areas. The study protocol complied with the Declaration of Helsinki on Biomedical Research Involving Human Subjects and was approved by the Ethics Committee of the Medical Faculty of the Goethe University, Frankfurt am Main, Germany.

Quantitative sensory testing (QST) was performed twice in each subject, once on untreated limb skin, defined as the "control" site ("Control Data"), and again contralaterally on the comparable limb but hypersensitized area, defined as the "test" site ("Test Data"). The body area to be tested was randomly assigned to the subjects; possible sites were the dorsal sides of the hand in the dermatome of N. radialis or of the foot in the dermatome of N. fibularis profundus. Experimental hyperalgesia was induced at the test site by applying 150 mg capsaicin cream (0.2%, manufactured by the local hospital pharmacy) onto a 3- × 3-cm^2 skin area and covering it with plaster for 30 minutes before testing.[22] This model was chosen because it is well established in experimental pain research. Results in this system agree, for several drug classes, with their analgesic effects against neuropathic pain. This was recently shown in an analysis of available evidence,[15] suggesting that capsaicin-induced hyperalgesia is a suitable experimental approach to neuropathic pain in human subjects.

2.2. Quantitative sensory testing and raw data processing

Resemblance of clinical pain to experimental hyperalgesia was assessed using a clinically established QST test battery proposed by the German Research Network on Neuropathic Pain.[24,25] This battery includes thermal and mechanical stimuli grouped into 7 tests of sensory perception and pain (**Table 1**). These are administered in the order: thermal detection thresholds, the so-called "thermal sensory limen," cold and heat pain thresholds, mechanical detection threshold, mechanical pain threshold (MPT), mechanical pain sensitivity (MPS), dynamic mechanical allodynia, temporal pain summation, vibration detection threshold, and pressure pain threshold. The room temperature was kept at 20°C to 25°C while testing. Measurements were taken by trained investigators fully adhering to the published instructions,[23–25] which are therefore only briefly recapitulated in **Table 1**.

The 7 tests provided a total of 13 different QST parameters, which were processed according to the instructions in,[23–25] including log-transformation of some parameter values as specified in **Table 1**, the column "Basic data processing." Subsequently, also as proposed, each QST parameter value was mapped onto the distribution of the reference group that consists of a total of 180 healthy subjects, in whom a data set of 1080 values has been obtained. This serves as the reference for all QST-based diagnoses.[17] Therefore, according to the QST standard procedure, 11 of the individual QST parameter values were z-transformed as $Z_{QST,individual} = \frac{QST_{individual} - QST_{reference}}{\text{standard deviation}_{reference}}$, where the QST reference values and SDs were the published values,[17] with regard to the sex, age, and tested body site of the actual subject. The signs of the z-scores were adjusted to denote that a z-score >0 indicates high sensitivity and z-score <0 indicates low sensitivity, according to the standard instructions. These z-values served as the basis for further analyses. Data processing differed from this procedure for 2 of the 13 QST parameters, ie, the paradoxical heat sensations and the dynamic mechanical allodynia, because these parameters were not available in the reference publication for the present analysis,[18] and therefore could not be included.

2.3. Data analysis

The extent to which the QST pattern of neuropathic pain could be induced in healthy subjects was analyzed by comparison with the pattern of the 11 z-transformed QST parameters reported from 1236 patients with neuropathic pain.[18] Plotting the distribution of z-transformed QST parameters from test and control sites in this study (**Fig. 1**) suggested similarity to a similar plot of neuropathic pain vs reference parameters (in Fig. 2 of Ref. 18). This encouraged further analyses, which were performed using the Matlab (MathWorks, Natick, MS) software packages.

Data analysis passed through 4 main steps comprising (1) verification of consistency of the QST parameter values obtained at the untreated control site of the present subjects with those obtained in the "Normal" group of, Ref. 18 (2) establishment of Bayesian decision rules using the data published in Ref. 18 to allow assignment of QST parameter values obtained at the test site in the present subjects to either normal or neuropathic group. This individual pattern of response to capsaicin application, obtained with respect to its similarity to neuropathic pain, permitted (3) common types (clusters) in these individual response patterns to be identified as a basis for (4) the establishment of preselection criteria for subjects with a highly capsaicin-inducible pattern of neuropathic pain.

First, the means and SDs of the QST parameters of the normal group from Appendix B of Ref. 18 (n = 180 taken from Ref. 17) were used for a quality check of the QST values obtained at the control sites in the present subjects (n = 110). Unpaired *t* tests resulted in *P* values greater than 0.47 for all QST parameters, indicating that with correct application of the QST protocol, the data agree with the reference findings. Accordingly, deviations from reference values observed at the test sites in the present subjects could be regarded as capsaicin-induced effects and were not due to failed measurements.

Second, the parameters of the normal (n = 180) vs the group of patients with neuropathic pain from Appendix B of Ref. 18 (n = 1099… 1236) were used to calculate the Bayesian posterior probabilities as $P(\text{neuropathic}|x) = \frac{P(x|\text{neuropathic}) \cdot P(\text{neuropathic})}{P(x)}$ using maximum likelihood estimation. This generated, for each QST parameter, the probability P(neuropathic|*x*) that an observed value × fits within the distribution of neuropathic QST values, provided that this QST parameter has been measured in a particular subject, given the prior probability P(neuropathic) of observing a neuropathic QST value and the prior probability P(*x*) of observing this particular numerical QST parameter value. The Bayesian decision limits obtained (**Table 2**) were used to assign individual QST parameter values, determined at the test sites of the 110 study participants, to either neuropathic (1) or normal (0) groups. This yielded a 110 × 11 matrix (110 subjects, 11 QST parameters) filled with zeroes or ones according to the assignment of the individual QST values to normal or neuropathic (**Fig. 2**).

Third, following the expectation that the pattern of neuropathic pain may be inducible by topical capsaicin application only in a subgroup of healthy subjects, the similarity matrix generated was used to classify subjects with respect to the resemblance of their individual QST pattern to those of neuropathic pain derived in the previous analytical step. Specifically, Bayesian posteriors ranging from 0 (=not assigned to neuropathy-typical QST

Table 1

Components of the quantitative sensory testing (QST) battery of the German research network on neuropathic pain.[24,25]

Test	QST parameter	Assessed sensory dimension	Basic data processing
Thermal testing	CDT	Application of cold stimuli on a 3- × 3-cm^2 skin area, baseline t° = 32°C, decreasing temperature ramp of 1°C/s, TSA 2001-II (MEDOC, Ramat Yishai, Israel)	Difference from baseline 32°C of the mean of 3 measurement repetitions; *log transformation*
	WDT	Application of warm stimuli to a 3- × 3-cm^2 skin area, baseline t° = 32°C, increasing temperature ramp of 1°C/s, TSA 2001-II (MEDOC, Israel)	Difference from baseline 32°C of the mean of 3 measurement repetitions; *log transformation*
	TSL	Application of alternating cold and warm stimuli to a 3- × 3-cm^2 skin area, baseline t° = 32°C, temperature ramp of 1°C/s, TSA 2001-II (MEDOC, Israel)	Difference in the means of the 3 warmth and the 3 cold detection thresholds; *log transformation*
	CPT	Application of cold stimuli to a 3- × 3-cm^2 skin area, baseline t° = 32°C; decreasing temperature ramp 1°C/s, TSA 2001-II (MEDOC, Israel)	Mean of the 3 measurement repetitions
	HPT	Application of warm stimuli to a 3- × 3-cm^2 skin area, baseline t° = 32°C, increasing temperature ramp of 1°C/s, TSA 2001-II (MEDOC, Israel)	Mean of the 3 measurement repetitions
Pressure pain threshold	PPT	Application of blunt pressure stimuli to musculus thenar for the hand area and musculus abductor hallucis for the foot area, Commander Algometer, JTECH Medical, Midvale, Utah (1 cm^2 probe area)	Mean of the 3 measurement repetitions; *log transformation*
Mechanical pain threshold	MPT	Application of pinprick stimuli (forces 8-512 mN; contact area 0.2 mm) following stare-case paradigm, starting force of 8 mN, The Pin-Prick, MRC Systems GmbH, Heidelberg, Germany	Geometric mean of the 5 ascending and 5 descending stimuli; *log transformation*
Stimulus–response function	MPS	Application of pinprick stimuli and tactile stimuli in a balanced order, pain rating of each pinprick stimulus on a 0-100 numerical rating scale ("0" = "no pain," "100" = "strongest pain imaginable"), The Pin-Prick, MRC Systems GmbH, Heidelberg, Germany	Geometric mean of the pain ratings of the 35 pinprick stimuli; *log transformation*
Wind-up	WUR	Temporal summation of pinprick stimuli, application of single pinprick stimulus (force 256 mN) followed by train of 10 pinprick stimuli (force 256 mN, 1/s repetition rate) over skin area of 1- × 1-cm^2, pain rating of the train on a 0-100 numerical rating scale ("0" = "no pain," "100" = "strongest pain imaginable"), The Pin-Prick, MRC Systems GmbH, Heidelberg, Germany	Ratio of the mean pain ratings of the 5 series of stimuli and the mean pain ratings of the 5 single stimuli; *log transformation*
Mechanical detection threshold	MDT	Application of von Frey hairs (forces 0.25-512 mN, diameter 0.5 mm) following stare-case paradigm, starting force of 16 mN, Optihair2-Set, MARSTOCK nervtest, Schriesheim, Germany	Geometric mean of the 5 ascending and 5 descending stimuli; *log transformation*
Vibration detection threshold	VDT	Application of descending vibration stimuli (Rydel–Seiffer tuning fork, 64 Hz, 8/8 scale) to the processus styloideus radii for the hand area and malleolus medialis for the foot area	Mean of the 3 measurement repetitions

CDT, cold detection threshold; CPT, cold pain threshold; HPT, heat pain threshold; MDT, mechanical detection threshold; MPS, mechanical pain sensitivity for pinprick stimuli; MPT, mechanical pain threshold; PPT, pressure pain threshold; TSL, thermal sensory limen; TSL, z-transformed thermal sensory limen; VDT, vibration detection threshold; WDT, warm detection threshold; WUR, wind-up ratio.

parameters) to 1 (=assigned to neuropathy) were treated as a single point in an 11-dimensional Euclidean vector space (data space, n = 11 dimensions for the 11 QST parameters). The analysis followed the approach previously described.[16] To obtain clusters in this vector space, the data were projected onto a 2-dimensional plane. Because classical projection algorithms, such as principal component analysis or multidimensional scaling, cannot preserve complex cluster structures, the Emergent Self-Organizing Maps (ESOM/U-Matrix) method was used.[31] Using ESOM, data were projected onto a 2-dimensional grid (map space) of 50 × 82 = 4200 units ("neurons"). The map space is toroid[29] and therefore borderless, ie, opposite edges are connected. The projection is neighborhood preserving,[11] ie, points that are neighbors in the high dimensional data space are also neighbors on the map space. Each neuron holds, in addition to the input vector from the 11-dimensional space, a further vector carrying "weights" of the same dimensions as the 11 input dimensions. The weights initially were randomly drawn from the range of the data variables. Subsequently, they were adapted to the data (learning phase, 50 epochs). After learning was complete, data from the trained ESOM were presented on the 2-dimensional toroid map. On this map, a cluster structure could be visualized by adding a third dimension, consisting of the average distance of the weight vector of a neuron to the weight vectors of its direct neighbors, which is known as the U-Matrix.[31] A geographical map analogy with watersheds was used to indicate borders of data clusters. The process was performed using the ESOM toolbox,[30] publicly available at

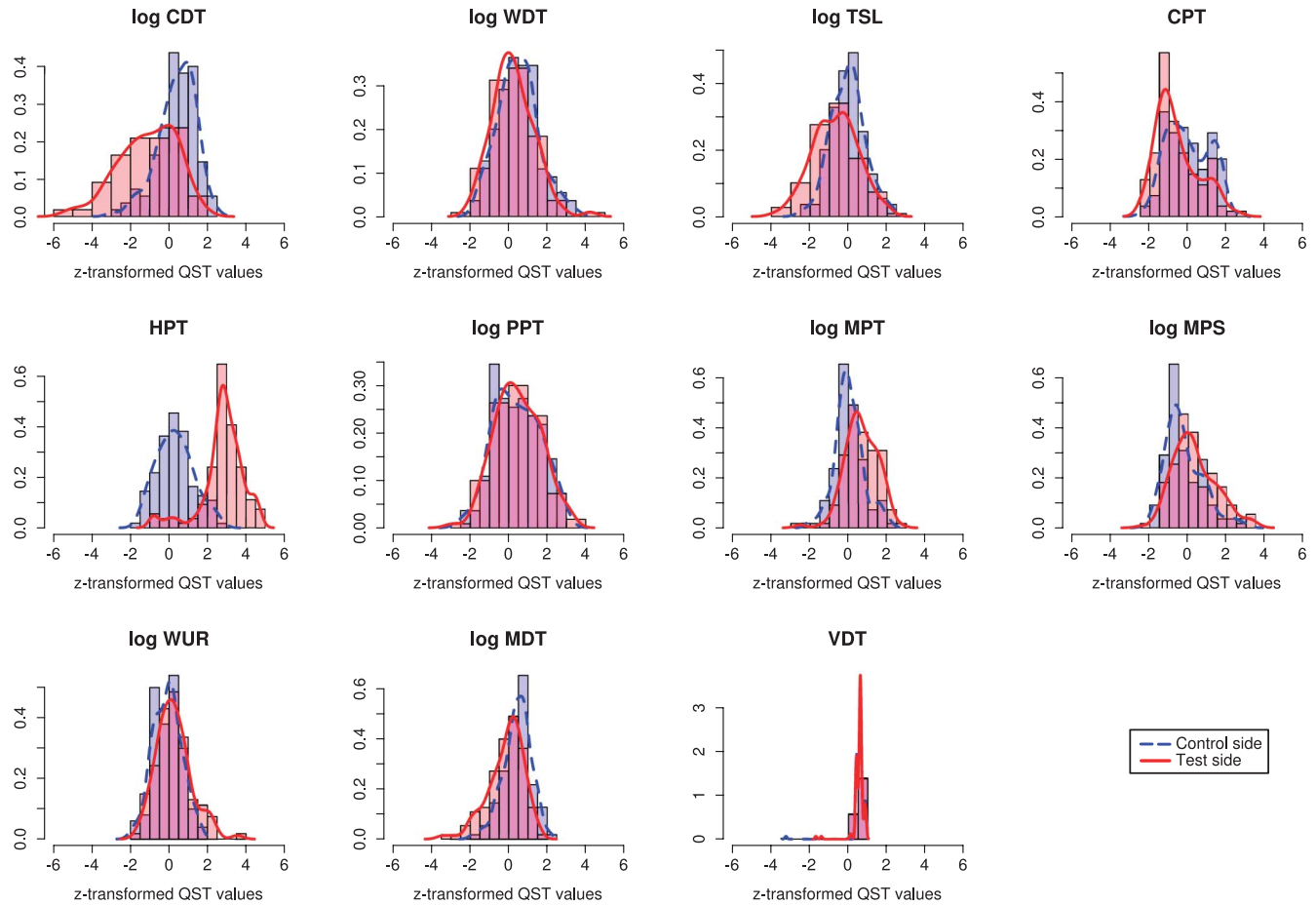

Figure 1. Distribution of the QST parameters after z-transformation; comparable to Figure 2 in Ref. 18. Histograms are shown of the capsaicin-treated test sites (red) of all 98 healthy subjects in comparison with the untreated control sites (blue) and superimposed probability density functions. Note that z-transformation against an age and sex-matched control cohort[24] eliminated differences due to test site, gender, and age among the reference values. The y-axis indicates the probability density of the respective z-values at test and control sites against the published reference cohort. The modus of these curves at the control sites is always very close to zero indicating that the data correspond to the published control data as expected from healthy subjects. CDT, cold detection threshold; CPT, cold pain threshold; HPT, heat pain threshold; MDT, mechanical detection threshold; MPS, mechanical pain sensitivity for pinprick stimuli; MPT, mechanical pain threshold; PPT, pressure pain threshold; QST, quantitative sensory testing; TSL, z-transformed thermal sensory limen; VDT, vibration detection threshold; WDT, warm detection threshold; WUR, wind-up ratio.

http://www.uni-marburg.de/fb12/datenbionik/software (accessed on June 30, 2014). This procedure provides advantages over alternative methods such as K-means or Ward because these methods impose prior assumptions on the shape of the clusters (spherical). The ESOM/U-matrix projection and clustering method does not require such prior assumptions about the shape of the clusters.

Fourth, following clustering of the subjects with respect to the degree of successful induction of QST pattern of neuropathic pain, predictors were sought to identify those subjects in which this procedure was successful. As a quantitative criterion for the agreement with the expected QST pattern of neuropathic pain for each individual, a "neuropathy inducibility score," (NIS) was calculated from the row sums of the similarity matrix as $NIS = \sum(QST \in neuropathy)$. If the possible maximum of NIS = 11 was reached, in this particular subject, the QST parameter pattern totally agreed with that published for neuropathic pain. Success in the induction of a neuropathic pain pattern was considered to have been achieved when the subject had an NIS that lies within the upper half of the NIS distribution. Therefore, preselection rules for inclusion in clusters comprising these subjects were sought. A classification and regression tree (CART) analysis[4] was performed to derive comprehensible and easily applicable selection algorithms. As candidate factors for preselection criteria, the 11 QST parameters at the control site, together with the subject's age and sex, were used. Accuracy of the identified rule was assessed by a 10-fold cross-validation.

3. Results

The QST parameter values obtained at the control site of the subjects in our study agreed with the reference values from the QST test battery (unpaired *t* tests: *P* values always >0.47), supporting the supposition that deviations from reference observed at the test sites could be regarded as capsaicin effects and were not due to failed measurements. Several QST parameters acquired from the capsaicin-treated test site displayed deviations in their distribution from the control site (**Fig. 1**).

According to the Bayesian decision limits (**Table 2**) generated from the published data (Appendix B of Ref. 18), a 110 × 11 matrix (**Fig. 2**) was obtained filled with ones or zeroes indicating for each subject (n = 110) whether or not, respectively, each QST parameter (n = 11) measured at the capsaicin-treated side resembled QST parameters assessed in patients with neuropathic pain.

Table 2

Bayesian decision limits calculated from the parameters for the normal (n = 180) vs the patient with neuropathy group from appendix B of Ref. 18 (n varying between 1099 and 1236) using the Bayesian posterior probabilities and the maximum likelihood estimation.

QST parameter	Bayesian decision limit
CDT	−0.9735
WDT	−0.8314
TSL	−0.8209
CPT	0.65
HPT	1.01
PPT	1.13
MPT	1.19
MPS	1.03
WUR	0.61
MDT	−1.4659
VDT	−1.4335

A z-transformed QST value beyond (the mean being close to zero for normal values) this decision limit, ie., higher when the difference between patients with pain and controls in appendix B of Ref. 18 was positive and lower in the opposite case, or alternatively, when referring to probability, higher than the probability not to belong to a neuropathy-like value, is regarded as typical neuropathic pain.
CDT, cold detection threshold; CPT, cold pain threshold; HPT, heat pain threshold; MDT, mechanical detection threshold; MPT, mechanical pain threshold; MPS, mechanical pain sensitivity; PPT, pressure pain threshold; TSL, z-transformed thermal sensory limen; VDT, vibration detection threshold; WDT, warm detection threshold; WUR, wind-up ratio.

The ESOM projection of the successes in inducing neuropathy-like QST parameters by means of capsaicin application and the subsequent visualization of the cluster distances provided a U-Matrix (**Fig. 3**), in which individuals were located close together who shared a pattern resembling neuropathy across the 11 QST parameters. The result was the identification of 7 clusters in the capsaicin-induced QST pattern resembling typical neuropathy values. Neuropathy inducibility score levels from 0 to 7 were observed in the measurements obtained at the test site (**Fig. 4**). For the subjects included in the 2 clusters, in which the highest number of subjects, relative to the total cluster size, displayed high NIS values (cluster #2 and #6 in **Table 3**), prediction criteria were sought.

Prediction of inclusion in the clusters comprising subjects with the highest probability of capsaicin-inducible QST pattern of neuropathic pain (**Fig. 5**) could be made with a CART rule that included QST parameters, MPS, wind-up ratio (WUR), and z-transformed thermal sensory limen (TSL), obtained at the control site. Specifically, assignment to clusters #2 and #6 (**Table 3**), in which the highest number of subjects relative to the total cluster size displayed high NIS values, can be expected to be met by the QST values obtained without capsaicin treatment under the following conditions: The QST-conform z-transformed values for the MPS to pinprick stimuli must be greater than 0.054. In addition, the z-transformed QST value for the MPT must either be greater than 0.4615, or the TSL must be smaller than −0.5115. In a 10-fold cross-validation, this rule provided a total accuracy of 86.9% for correct assignment of subjects to these clusters. Thus, using the CART decision tree, on the basis of testing MPS, WUR and TSL at n = 20, 18% of all subjects can be preselected with a probability that 80% of them show a response pattern that is consistent with the pattern observed in neuropathic pain.

4. Discussion

The results described here support the hypotheses underlying this investigation. Specifically, according to the first study

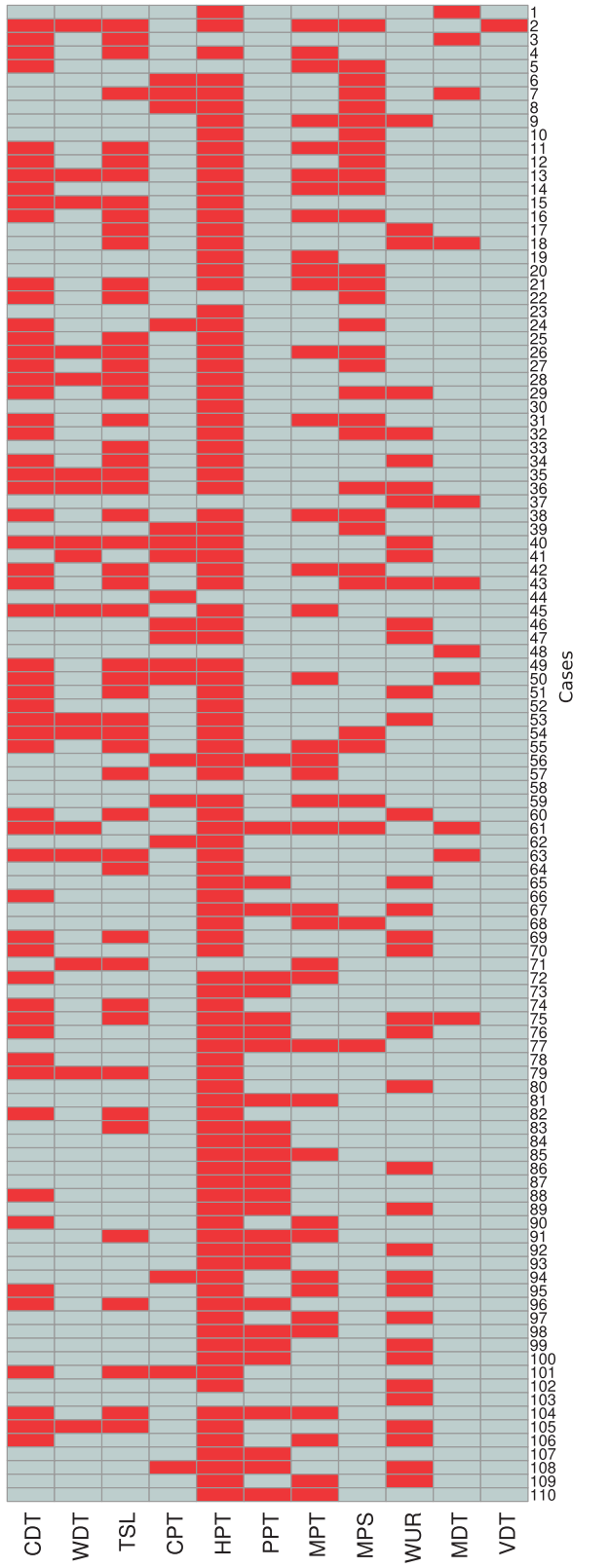

Figure 2. Bayesian matrix (110 subjects, 11 QST parameters) constituted by zeroes (gray) or ones (red) according to the assignment of the individual QST values (Bayes posteriors >0.5), measured following topical capsaicin application, to normal or neuropathic based on the Bayesian decision limits in Table 2. CDT, cold detection threshold; CPT, cold pain threshold; HPT, heat pain threshold; MDT, mechanical detection threshold; MPS, mechanical pain sensitivity; MPT, mechanical pain threshold; PPT, pressure pain threshold; TSL, z-transformed thermal sensory limen; VDT, vibration detection threshold; WDT, warm detection threshold; WUR, wind-up ratio.

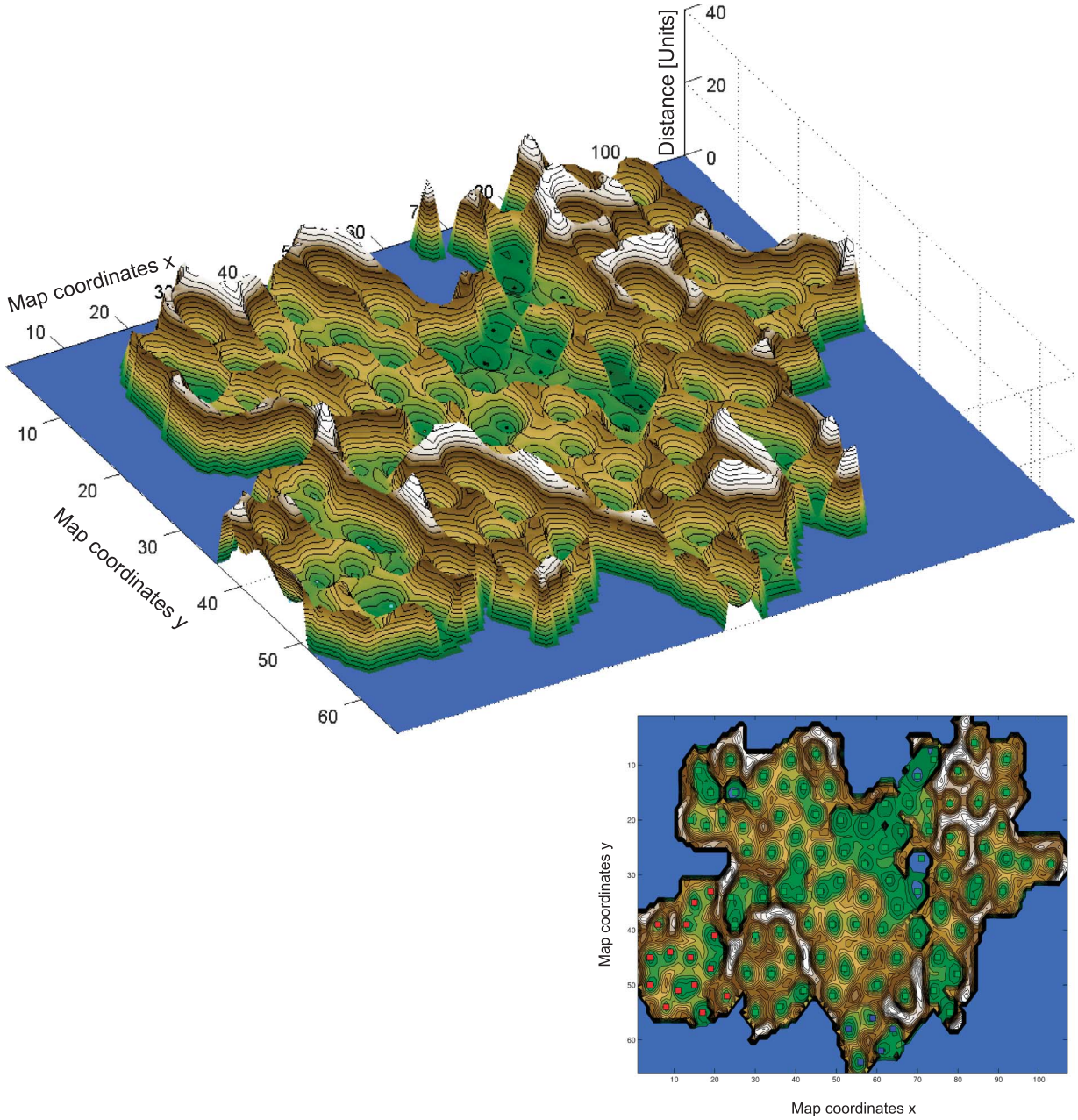

Figure 3. U* matrix 3-dimensional view (top), intended for a geographical interpretation (for technical details of the presentation, see http://www.uni-marburg.de/fb12/datenbionik/forschung/esom), showing the clustering of subjects with comparable quantitative sensory testing (QST) parameter pattern to that of neuropathic pain, following topical capsaicin application (Fig. 2). The clusters were visualized using a U-Matrix,[12] which is a representation of the distances in data space on top of a map space. The U-Matrix was cut from a tiled display of the Emergent Self-Organizing Maps (ESOM) to remove duplicate representation of the data. It was colored as a geographical map with brown or snow-covered heights and green valleys. High "walls" in a U-Matrix indicate large distances between the QST responses to capsaicin application in the 110 subjects. Points represent subjects and their coordinates in the toroid map space are used to address them when querying information. Points, or "persons," lying together in a valley of the U-Matrix indicate that these persons have a common response type pattern of QST parameters to topical capsaicin application. Thus, valleys indicate clusters of similar response types. The watersheds of the U-Matrix indicate borderlines of clusters. To enhance readability, at the bottom, a bird's view of the same U* matrix shows the projection of data points onto this map, in which the clusters #2 and #6 (compare Table 3), which contain the highest fraction of subjects with high inducibility of neuropathy-like QST pattern, are colored in XX and YY, respectively.

hypothesis, a QST pattern of clinical neuropathic pain can be induced in healthy subjects by topical capsaicin application. According to the second study hypothesis, the success of this procedure varied among subjects. Approximately 20% of a random sample of healthy subjects showed an enhanced resemblance of the induced pain to clinical neuropathic pain, which was promising with respect to the possibility of conducting analgesic drug studies that use highly selected cohorts in which clinical pain can be partly mimicked to enhance predictivity of analgesic drug efficacy in clinical pain settings.

The present assessments and analyses demonstrated that experimentally induced pain may resemble clinical pain as assessed by a standardized clinical pain test (QST). The pattern of neuropathic pain reported from 1236 patients[18] was also inducible in healthy subjects, reproducing 64% of the full pattern (7 of 11 QST parameters). The complete QST battery includes 13

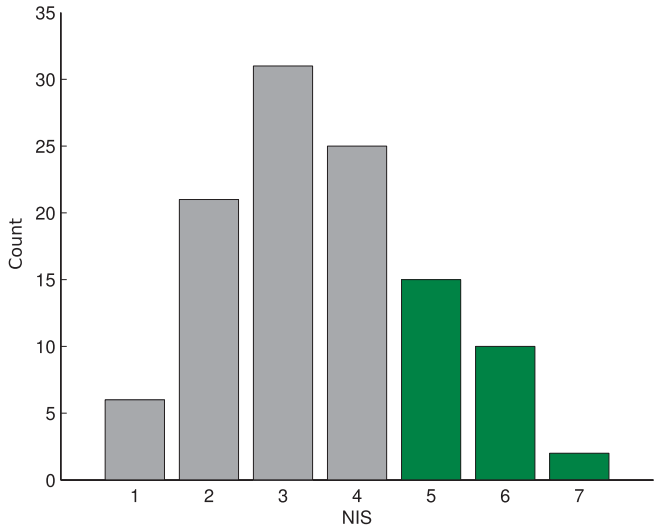

Figure 4. Distribution of the neuropathy inducibility score (NIS), calculated as NIS = $\sum$(QST $\in$ neuropathy), across the 110 subjects. The NIS corresponds to the row sums in the similarity matrix in Figure 2. The higher the NIS, the more quantitative sensory testing (QST) parameters, following capsaicin application, were assigned to the typical neuropathic QST values using the Bayesian decision limits (Table 2), calculated from the parameters of normal subjects and patients with neuropathy obtained from appendix B of Ref. 18. Subjects with NIS in the upper half of the NIS distribution (green bars) were in the focus of subsequent analysis, which indicated comparatively high inducibility of neuropathic pain patterns.

parameters, but 2 could not be analyzed because their distributions were not available from the reference publication.[18] At the very least, more than 50% of a pattern typical for neuropathic pain was inducible.

The present analyses further indicate that the QST pattern of neuropathic pain is not induced by capsaicin application in any randomly chosen subject. This is emphasized by the z-score distributions of the QST data (**Fig. 1**). Those obtained at the control site, on normal (untreated) skin, closely match the distributions of the different QST-parameters in the patients' normal skin areas (Fig. 2 in Ref. 18). The capsaicin model yielded gain or loss of function, expressed as right or left shifts in the distributions, respectively, which on average across the whole sample (**Fig. 6**) differed to some extent from the respective changes observed in patients with neuropathic pain,[18] although the direction of the average differences agrees in most tests with that reported from patients with neuropathic pain (compare Fig. 3 in Ref. 18). However, patients often displayed a broader distribution, with both gain and loss as potential signs of deafferentation or central sensitization,[3,8] while the healthy subjects showed a more homogenous response indicated by narrower distributions (**Fig. 1**). Differences from patients with pain may be due to, for example, the acute testing before desensitization of afferents leading to loss of function. Hence, the capsaicin model applied to a random sample of healthy subjects might favor patients with plus symptoms (hyperalgesia, allodynia) more likely to be targeted during drug development than patients with minus symptoms. Nevertheless, this analysis provided criteria for the selection of a subsample suited for drug development that comprises mainly subjects in whom these differences were comparatively small.

Table 3

Cluster contingency table of the neuropathy inducibility score (NIS), calculated as NIS = $\sum$(QST $\in$ neuropathy), separated according to the clusters of similar NIS pattern obtained by means of Emergent Self-Organizing Maps, ESOM/U-Matrix analysis (Fig. 3).

CART	NIS								Sum
	0	1	2	3	4	5	6	7	
1	0 (0)	0 (0)	2 (13)	2 (13)	5 (33)	4 (27)	2 (13)	0 (0)	15
2	0 (0)	1 (7)	1 (7)	1 (7)	6 (40)	2 (13)	3 (20)	1 (7)	15
3	0 (0)	1 (17)	3 (50)	2 (33)	0 (0)	0 (0)	0 (0)	0 (0)	6
4	0 (0)	2 (9)	4 (17)	6 (26)	9 (39)	2 (9)	0 (0)	0 (0)	23
5	1 (3)	1 (3)	6 (18)	15 (45)	8 (24)	1 (3)	1 (3)	0 (0)	33
6	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (40)	2 (40)	1 (20)	5
7	0 (0)	0 (0)	0 (0)	5 (38)	5 (38)	3 (23)	0 (0)	0 (0)	13
Sum	1	5	16	31	33	14	8	2	110

The cells indicate the number of subjects assigned to the respective 1 of 7 clusters, with the relative number (percentage) per total inclusions in the respective cluster given in parentheses after the number of subjects. The bottom and right margins of this table display the column and row sums, respectively. Clusters #2 and #6 contained the highest relative contribution from subjects with a high NIS.
CART, classification and regression tree.

The assessment of agreement with neuropathy-like pattern, ie, the subject's position inside or outside the desired clusters, was based on 11 QST parameters without further critical selection. These also included, according to the standardized protocol and reference information,[18] the WUR, although the WUR has never been tied systematically to the presence or absence of neuropathic pain. In the reference publication,[18] an abnormal WUR was observed mainly in patients with postherpetic neuralgia and central pain. However, WUR might not present a specific sign of neuropathic pain but rather of central sensitization under chronic pain conditions.[5] Because the present data cannot serve to judge the place of WUR within the QST test battery, the obtained selection criteria were based on the current standard, yet might have to be altered if in future the WUR were to be excluded from the QST test battery following a critical clinical reevaluation of its specificity. However, because WUR contributed only 9.7% to the global NIS (**Fig. 6**), no major consequences for the present selection criteria may be expected. Moreover, the selection rules for suitable subjects, in the present context, were obtained without WUR (right branch of the decision tree in **Fig. 5**) and indeed, a CART constructed without including WUR as a candidate factor, still provided a comparable overall accuracy of cluster assignment of 84.5%.

Given these differences between experimental pain induced in healthy volunteers and clinical settings for patients with neuropathic pain, and in agreement with the current prestudy exceptions, it is noteworthy that >50% resemblance of the induced pain to clinical neuropathic pain, could be observed only in a subgroup of healthy subjects, whereas in other subjects, the resemblance was less than 10% of the QST parameters (**Table 3**). However, analyses also showed that healthy subjects displaying the highest attainable degree of capsaicin-inducible pattern of neuropathic pain can be identified based on their responses to 3 QST tests, MPS, WUR, and TSL, at the control site without sensitization. These can be easily used as suitable preselection criteria applied during recruitment of subjects for cost-effective experimental human pain studies, in earlier phases of clinical drug development. This could form the basis for enhanced predictive planning of preclinical drug studies when performed in highly selected cohorts, with the clinical drug target in mind, and offers a further improvement of the utility of experimental human pain models for the prediction of clinical analgesic drug efficacy.[15,20]

Clustering the subjects with respect to their pattern of agreement between capsaicin-inducible QST pattern and neuropathic QST pattern suggests that those displaying a high comparability of capsaicin-inducible pattern to neuropathic pain share a common (patho-) physiological background. Because it is known that after nerve injury, neuropathic pain also only develops in some patients, the presently proposed target group may

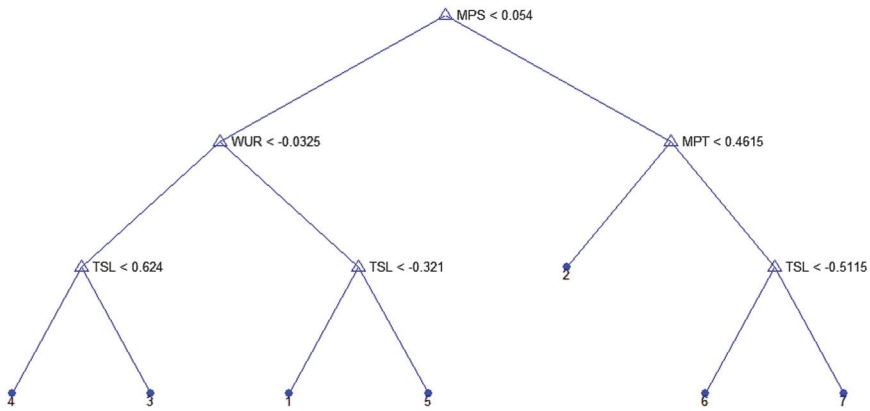

Figure 5. Classification and regression tree (CART) visualizing the decision algorithm for assignment of subjects to the 7 clusters of subjects with comparable quantitative sensory testing (QST) parameter patterns resembling neuropathic pain, following topical capsaicin application, obtained by Emergent Self-Organizing Maps, ESOM/U-Matrix analysis (Fig. 3). When the condition noted at each decision node applies, the tree is followed to the left, elsewhere to the right. Accordingly, CART analysis identified the QST parameters, mechanical pain sensitivity for pinprick stimuli (MPS), mechanical pain threshold (MPT), wind-up ratio (WUR), and z-transformed thermal sensory limen (TSL), obtained at the control site, as the basis for cluster assignment with a total accuracy of 86.9%. Assignment to clusters #2 and #6 (Table 3), in which the highest number of subjects, relative to the total cluster size, displayed high neuropathy inducibility score values, can be obtained with the parameters MPS, MPT, and TSL (right branch of the tree) and can be expected to be met, by the QST values obtained without capsaicin treatment, under the following conditions: The QST-conform z-transformed[17] values of the MPS must be greater than 0.054. In addition, the z-transformed QST value of the mechanical pain threshold (MPT) must either be greater than 0.4615, or the TSL must be smaller than −0.5115. This identifies subjects who, according to the present hypothesis, should be recruited for experimental studies aimed at predicting the efficacy of analgesic drugs against neuropathic pain.

represent subjects who are more prone to development of neuropathic pain under pathological clinical conditions. In this case, the proposed highly selected cohort for experimental assessment of analgesia would be well suited as representatives of the clinical condition. However, based on the present predictors, we can only conclude that the (patho-) physiological background shared by these subjects involves a particular pain sensitivity phenotype. This agrees with previous analysis of complex pain phenotypes that consistently indicated that subgroups of subjects obtained by means of distribution analysis identify high, average, and low pain phenotype groups,[6] and that classical cluster analyses[10] or ESOM/U-Matrix analyses identify even more complex phenotype groups.[16] Additional characteristics, possibly shared by these subjects, comprise psychological mechanisms shown to explain interindividual differences in the development of chronic pain[13,14] or a common complex genetic background that analogously underlies distinct pain phenotypes[16] Further possible factors may include epigenetic or biochemical parameters that might provide potential alternative or complementary preselection criteria for subject enrollment in analgesic drug studies.

Only a fraction of healthy subjects was expected after topical capsaicin application to show a similar pattern in the QST test battery to that reported for patients with neuropathy. Applying the 3 QST subtests, identified as being sufficiently distinctive for these subjects to be able to enroll a highly selected study cohort suitable for the assessment of drug effects on neuropathic pain, seems manageable even in a small laboratory setting. Typical pharmacological studies enroll 16 to 50 subjects (eg, Refs. 2,7,21,26–28). When expecting that 18% of a random sample of healthy subjects will qualify, based on their response to 3 QST subtests, 89 to 278 subjects need to be tested to obtain the intended sample. This seems practically achievable and will comprise a singular effort to provide a pool of potential candidates for future studies, pending evidence that the inducibility of neuropathy-like pattern of QST parameters is a stable trait. This offers the possibility of exploitation of advantages of phase 1 drug tests in healthy volunteers rather than using patients, ruling out the control of confounders such as clinically indicated medications related or unrelated to pain, avoiding the need to change or interrupt clinically indicated medications and the ethical requirement for a placebo condition. Pain or its inhibition by medications is suitable for studies in healthy subjects, according to various guidelines for drug studies such as Ref. 1, which states that the decision as to whether a phase 1 trial should be done in healthy subjects or patients should be made on a case-by-case basis. Pain clearly qualifies for studies in healthy volunteers as the risks of novel medications may be acceptable in healthy subjects, the molecular target is usually present in healthy subjects, and variability is likely to be much lower than in patients with pain.[1] The broad and, importantly, successful[15,20] use of experimental pain models supports the utility of the present approach that is ultimately aimed at optimizing the predictivity of experimental pain studies in humans.

The assessment of neuropathy-likeness, as the main focus of this study, in the pattern of QST parameters induced by topical capsaicin application was possible as a result of the availability of numerical information on this pattern from an extensive analysis in 1236 patients with pain,[18] obtained in conformity to the instructions of the standardized QST test battery[24,25] to which the present data acquisition adhered in a similar manner. This allowed the use of the present data as intended by this test battery that has been established as a diagnostic tool, allowing clinicians to test the responses to the above-mentioned stimuli in a single patient and to compare the individual responses to reference values derived from healthy subjects.[17] This is achieved through z-transformation of individually obtained parameter values using standard reference values made available in this test battery, which allows the comparison of test results across study centers and publications. This is analogous to a laboratory value for which a reference value exists and which therefore does not need to be compared repeatedly in a statistical analysis with the values obtained in healthy subjects. The present assessments exploited this feature of the QST test battery to determine, by comparison of the present results with known patterns of neuropathic pain,[18] the extent to which hypersensitization with topical capsaicin induced neuropathy-like pain pattern. This assessment would have been impossible with raw data because this would have provided only differences from the control sites but would not have allowed any decision on the

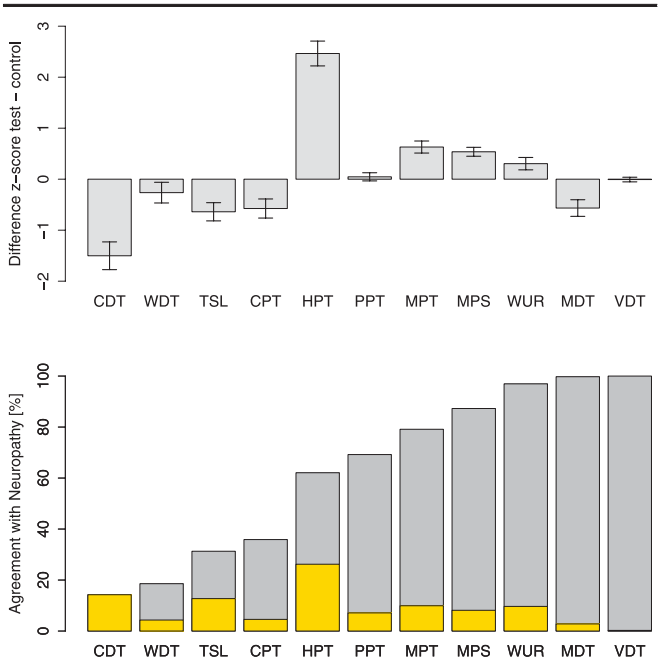

Figure 6. Changes in quantitative sensory testing (QST) parameters and contribution of these differences to the overall neuropathy inducibility score, ie, the inducibility score of neuropathic pain-like results. Top: Absolute sensory difference in QST z-scores between the test area and the control areas for the 11 QST parameters primarily analyzed in the present cohort (for the directions of the differences, compare Fig. 3 in Ref. 18). The columns indicate mean differences (positive or negative) across 110 healthy volunteers following local sensitization with topical capsaicin, with the 95% confidence interval of these differences indicated as error bars. Bottom: Comparative bar plot of absolute (gold bars) and cumulative (gray bars) fractions of inducibility of neuropathic pain-like results in the single QST parameters among the 110 healthy subjects following topical capsaicin applications. CDT, cold detection threshold; CPT, cold pain threshold; HPT, heat pain threshold; MDT, mechanical detection threshold; MPS, mechanical pain sensitivity; MPT, mechanical pain threshold; PPT, pressure pain threshold; TSL, z-transformed thermal sensory limen; VDT, vibration detection threshold; WDT, warm detection threshold; WUR, wind-up ratio.

neuropathy-likeness of the observed pattern. The latter would have required reanalysis of the source data of the previous independent publication; however, this was neither available nor was the present analysis aimed at reevaluating established QST standards.

The present analysis regarded the QST pattern of neuropathic pain as typical for the clinical entity, which is supported by the publication of means and SDs across the 1236 patients with neuropathic pain (Appendix B of Ref. 18). However, these authors pointed out notable differences between the somatosensory profiles for different syndromes causing the neuropathic pain, as the 1236 patients represented different neurological syndromes including polyneuropathy, postherpetic neuralgia, peripheral nerve injury, complex regional pain syndrome, trigeminal neuralgia, central pain, and others (Table 1 of Ref. 18). A substratification of these patients might reveal different patterns of neuropathic pain and require adaptation. However, the present analysis was performed primarily with drug efficacy studies in mind using healthy subjects. Current drug development activities mainly target neuropathic pain as a whole, and development of drugs for specific neuropathies is still not common, supporting the utility of the present results for current drug development. Finally, clustering analysis was performed on a relatively small sample of 110 subjects, which is roughly two thirds the size of the "normal" group used for comparison with neuropathic pain (appendix B of Ref. 18, where the n = 1080 refers to the data points taken from n = 180 subjects analyzed by a study by Magerl et al.[17]).

In this study, induction of the QST pattern of neuropathic pain was approached using the apparently simple yet well-established hypersensitization procedure involving topical capsaicin application.[22] Interestingly, capsaicin-induced hyperalgesia emerged as 1 of the most predictive experimental pain models for clinical analgesic drug efficacy in a recent analysis of successful or unsuccessful empirical predictions of clinical analgesia using experimental pain models[15] This was discovered by statistically analyzing the distribution of published mutual agreements or disagreements between drug efficacy in experimental and clinical pain settings. Capsaicin-induced experimental pain uncovered the efficacy of drugs that had also been observed as being effective in several clinical settings of neuropathic pain, across 2 to 3 different drug classes, in trigeminal neuralgia, mixed neuropathic pain, phantom limb, and diabetic neuropathy.[15] This finding further supports selection of capsaicin hyperalgesia for the assessment of inducibility of neuropathy-like QST pattern, as performed in this study in healthy subjects, as a relevant choice for future human experimental studies testing the efficacy of drugs developed against neuropathic pain in a highly selected cohort of healthy subjects.

This study demonstrated that experimental pain models, which are highly likely to be predictive for clinical pain, can be identified by exploiting original data combined with numerical information available in the literature.[18] This emphasizes the utility of standardized and comprehensive clinical pain phenotyping[17,23–25] also in an experimental context. The desired clinical pattern of neuropathic pain was experimentally inducible, to a degree that fuels expectations that experimental pain models can be optimized toward mimicking clinical pain, in a preselectable subgroup comprising approximately 20% of healthy subjects who therefore qualify for enrollment in analgesic drug studies that use highly selected cohorts to enhance predictivity of analgesic drug efficacy in clinical pain settings, and who can be selected at an accuracy of >80%.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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