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A data science approach to the selection of most informative readouts of the human intradermal capsaicin pain model to assess pregabalin effects

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[Corrections added on 31 October 2020, after online publication: new funder "Fraunhofer Institute for Translational Medicine and Pharmacology ITMP, Theodor-Stern-Kai 7, 60596 Frankfurt am Main" has been added and copyright line has been changed.]

Abstract

Persistent and, in particular, neuropathic pain is a major healthcare problem with still insufficient pharmacological treatment options. This triggered research activities aimed at finding analgesics with a novel mechanism of action. Results of these efforts will need to pass through the phases of drug development, in which experimental human pain models are established components e.g. implemented as chemical hyperalgesia induced by capsaicin. We aimed at ranking the various readouts of a human capsaicin–based pain model with respect to the most relevant information about the effects of a potential reference analgesic. In a placebo-controlled, randomized cross-over study, seven different pain-related readouts were acquired in 16 healthy individuals before and after oral administration of 300 mg pregabalin. The sizes of the effect on pain induced by intradermal injection of capsaicin were quantified by calculating Cohen's *d*. While in four of the seven pain-related parameters, pregabalin provided a small effect judged by values of Cohen's *d* exceeding 0.2, an item categorization technique implemented as computed ABC analysis identified the pain intensities in the area of secondary hyperalgesia and of allodynia as the most suitable parameters to quantify the analgesic effects of pregabalin. Results of this study provide further support for the ability of the intradermal capsaicin pain model to show analgesic effects of pregabalin. Results can serve as a basis for the designs of studies where the inclusion of this particular pain model and pregabalin is planned.

KEYWORDS
analgesia, data science, human pain models, human pharmacology, pain

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1 | INTRODUCTION

Moderate-to-severe persistent pain affects a fifth of European adults and even a third of those older than 70 years.[1,2] Furthermore, neuropathic pain affects approximately 6.9%-10% of the general population.[3] Because of its mechanistic complexity and the often coexisting psychological components, treatment is challenging and often unsatisfying.[4] Indeed, the high prevalence of persistent pain points at insufficient treatment options. Evidence from Cochrane reviews indicates that the available analgesics provide efficacious pain relief, defined as a decrease in pain intensity by at least 50%, lasting for 12 weeks, only in a minority of patients.[5-7] To this adds that today's analgesics often cause side effects that reduce the patients' quality of life and possibly their therapy compliance.[1]

The unsatisfactory situation with the prevalence and treatment options for persistent, including neuropathic, pain raised increasing research activities aimed at analgesics with novel mechanisms of action. Results of these efforts will need to pass through the phases of drug development. Experimental human pain models are established components of this process. They try to mimic the physiology and pathophysiology of nociception, inflammation and analgesia[8] and play an important role in bridging animal and clinical pain studies before initiation of costly clinical trials. Although discussions have been raised about their validity to predict clinical analgesia, analyses suggested an overall satisfactory prediction performance.[9,10]

From a recent comparative computational analysis,[10] human experimental pain models employing chemical hyperalgesia induced by capsaicin emerged with the best record of correct predictions of clinical analgesia. They use activation of thermo-sensitive transient receptor potential (TRP) ion channels, family V, subtype 1 (TRPV1) by capsaicin[11] to evoke pain. Two variants have been established, one using topical application of low-concentration capsaicin cream (0.2%) onto the skin,[12] while the other variant uses an intradermal injection of a small amount of pure capsaicin.[13] Both variants have been frequently applied in human experimental pain research to test analgesics after oral, intravenous or intrathecal application.[14] A PubMed database search on 8 June 2019 for "(((((((intradermal or intracutaneous or subcutaneous)) AND injection) AND capsaicin) AND human) AND study) AND analgesi*) NOT review [Publication Type]" obtained 41 hits (Table 1).

According to this search, the intradermal variant of the capsaicin model has been used more often to assess analgesic drug effects in humans. It also provides a greater variety of readouts (Table 2), such as the intensity of the evoked pain or the size of areas of primary or secondary hyperalgesia. These readouts are used in combination or alone; however, a clear ranking among them with respect to the

TABLE 1 Studies (in order of publication year) using the intradermal capsaicin pain model to assess analgesic/antihyperalgesic/anti-allodynic drug effects in human volunteers. The list was based on a PubMed database search on Mai 8, 2018 for "(((((((intradermal or intracutaneous or subcutaneous)) AND injection) AND capsaicin) AND human) AND study) AND analgesi*) NOT review[Publication Type], followed by curation of the hits

Reference	Individuals (n)	Drug	Antihyperalgesic/anti-allodynic effect [yes/no]
47	12	Ketamine	Yes
		Alfentanil	Yes
		Midazolam	No
55	46	Amitriptyline	No
		Alfentanil	Yes
		Midazolam	Inconclusive
58	12	Alfentanil	Yes
		Ketamine	Yes
62	16	Clonidine	Yes (intrathecal only)
63	25	AMPA/Kainate antag. LY293558	Yes
59	12	Fentanyl	Yes
		Ketamine	Yes
64	24	Clonidine	Yes
65	12	Lidocaine	Yes
		Ketamine	No
66	12	Lidocaine	Yes
		Ketamine	Yes
60	12	Alfentanil	No
		Ketamine	No
67	11	Alfentanil	Yes
		Ketamine	Yes
68	16	Botulinum toxin	No
69	12	Ketamine	Yes
		Lidocaine	Yes
70	16	Procaine	Yes
56	41	Gabapentin	Yes
71	9	Ketamine	Yes
72	18	Neramexane	Yes
		Flupirtine	No
17	20	Morphine	Yes
		Pregabalin	Yes
		Diphenhydramine	No
73	10	Hydrocortisone	Yes
20	18	Pregabalin	No
		Minocycline	No
74	44	AZD1940 (peripheral cannabinoid)	No

(Continues)

TABLE 1 (Continued)

Reference	Individuals (n)	Drug	Antihyperalgesic/ anti-allodynic effect [yes/no]
[75]	16	Clobazam	Yes
		Clonazepam	Yes
		Tolterodine	No
[18]	13	Pregabalin	Yes
[19]	19	Calcium channel blocker ABT-639	No
		Pregabalin	Yes
[76]	18	Ethanol	Yes
[77]		Botulinum-neurotoxin A	No
[78]	21	Diclofenac-methadon Gel	Yes

best detection of analgesic drug effects has not yet been performed. As capsaicin-based pain models are among likeliest candidates to be chosen for the phase-I testing of novel analgesics, such ranking could facilitate the planning of respective studies. This was addressed in the present study.

Specifically, using an analgesic with a sound record of positive effects in the human intradermal capsaicin pain model, several different readouts of pain were acquired and ranked with respect to the most important information about analgesic drug effects. For this purpose, pregabalin was chosen as it figured among the most frequently tested analgesics in the capsaicin pain model (Table 1). Pregabalin is a structural derivative of the inhibitory neurotransmitter gamma-aminobutyric acid. It is a ligand at the $\alpha_2\delta$ subunit of voltage-gated calcium channels and had been established as an anticonvulsant, which was repurposed as an analgesic, anxiolytic and sleep-modulating agent.[15] Nowadays, it is an

TABLE 2 Summary of the parameters acquired to assess hyperalgesia or allodynia induced my means of intradermal injection of capsaicin

Parameter		Assessment
Hyperalgesia		
AreaPin	Size of the area of secondary hyperalgesia	Punctate needle stimulation ("PinPrick," strength 256 mN) at 1 cm steps along 8 linear paths arranged vertically, horizontally, and diagonally around the capsaicin injection site. Quantification of the area connecting the eight points on the paths where a change in sensation (burning, tenderness, more intense prickling) was indicated by the subject.
VasPin	Pain intensity in the area of secondary hyperalgesia	Punctate needle stimulation ("PinPrick," strength 256 mN) at the mid-point of along 8 linear paths, arranged vertically, horizontally and diagonally around the capsaicin injection site, where a change in sensation (burning, tenderness, more intense prickling) was indicated by the subject, and the site of capsaicin injection. Mean of ratings of the painfulness of each stimulation on a 100-mm visual analogue scale ("no pain" to"worst imaginable pain").
Allodynia		
AreaAll	Size of the area of allodynia	Q-tip stimulation at 1 cm steps along 8 linear paths arranged vertically, horizontally, and diagonally around the capsaicin injection site. Quantification of the area connecting the eight points on the paths where a change in sensation (burning, tenderness, more intense prickling) was indicated by the subject.
VasAll	Pain intensity in the area of allodynia	Q-tip stimulation at the mid-point of along 8 linear paths, arranged vertically, horizontally and diagonally around the capsaicin injection site, where a change in sensation (burning, tenderness, more intense prickling) was indicated by the subject, and the site of capsaicin injection. Mean of ratings of the painfulness of each stimulation on a 100 mm visual analogue scale ("no pain" to"worst imaginable pain").
Blood flow		
AreaFla	Size of the area of skin flare around the site of capsaicin injection	Blood flow in the area of capsaicin-treated skin was quantified using high-resolution laser speckle contrast imaging. The laser device was positioned approximately 30 cm above the skin. 2-dimensional colour-coded pictures were processed to calculate the area of capsaicin-induced increase in blood flow.
VasFlare	Mean blood flux intensity in the area of skin flare around the site of capsaicin injection	Blood flow in the area of capsaicin-treated skin was quantified using high-resolution laser speckle contrast imaging. The laser device was positioned approximately 30 cm above the skin. 2-dimensional colour-coded pictures were processed to calculate the mean blood flow in the area of capsaicin-induced increase in blood flow.
Spont. pain		
SponPain	Spontaneous pain induced by the capsaicin injection	100-mm visual analogue scale rating (0, no pain, 100 mm,"worst imaginable pain")

established component of the pharmacological therapy of persistent pain, in particular of neuropathic pain.[5,16] In the human capsaicin-based pain model, pregabalin reduced the area of hyperalgesia,[17] the intensity of pain[18] or hyperalgesia and allodynia.[19] However, the effects of pregabalin on spontaneous pain, flare, allodynia and hyperalgesia were also reported not to differ from placebo.[20]

Based on its frequent use, its generally positive analgesic record and its heterogeneous effects on different parameters acquired with the capsaicin pain model, pregabalin was a suitable drug for the present study aimed at selecting most informative readouts for analgesic drug effects of pregabalin, while its analgesic actions in this model were considered as known. The search for most relevant model readouts turned the project into an explorative data-driven approach, with the hypothesis that the parameters assessed by the capsaicin-based pain model can be ranked for their ability to detect analgesic actions of pregabalin, without a specific hypothesis about particularly useful parameters. This required a data science analysis, while classical statistics was second-line. Specifically, most suitable pain model-derived parameters were approached using methods of feature selection[21] and item categorization for relevance. This data-driven[22] analysis provided finally hints at suitable parameters, which then could be submitted to classical hypothesis-driven[23] statistical testing.

2 | METHODS

2.1 | Study design and setting

This was a placebo-controlled, randomized cross-over study in a human experimental setting and performed in healthy young volunteers. The study followed 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 (protocol number 195/15). Informed written consent was obtained from each participating individual at enrolment. The study was conducted in accordance with the BCPT policy for experimental and clinical studies.[24]

2.2 | Participants and study size

The study cohort consisted of n = 18 healthy men (aged 18-42 years, mean ± standard deviation (SD) 28.4 ± 6.8 years, body-weight 63.3-91.5 kg, mean ± SD, 78.5 ± 7.3 kg). Their actual health was detected by medical history, physical examination including vital signs and routine clinical laboratory test results. Prior to the actual experiments, medications were prohibited for one month, alcohol intake for one day, and food was not allowed for 6 hours.

Considering the data-driven approach aimed at selecting most suitable readouts of the capsaicin pain model rather than pre-defining this, and the aim of providing a basis for planning studies with this model as established in human experimental settings, the sample size of n = 18 was chosen to correspond to those of comparable studies in which intradermal capsaicin injection was used to quantify the effects of pregabalin.[17,18] However, to avoid that this approach led to an underpowered study, it was determined that, at a statistical power of 0.8, n = 14 individuals are needed to establish a reduction of the area of secondary hyperalgesia (average of the 60- and 120-minute time-point observations after capsaicin injection) by 11.6 cm^2 compared to approximately 47 cm^2 under placebo.[17] Hence, 18 subjects were enrolled to account for possible drop-outs.

2.3 | Study medications

Pregabalin (Pregabalin beta 300 mg Hartkapseln, Betapharm Arzneimittel GmbH, Augsburg, Germany) was administered at an oral dose of 300 mg. The dose selection was based on a published meta-analysis (19 studies, 7003 participants) where daily doses of 300-600 mg emerged as effective in the treatment of various types of neuropathic pain and of fibromyalgia, whereas 150 mg was generally ineffective.[5] Moreover, in an experimental setting, 300 mg of oral pregabalin showed significant antinociceptive effects in healthy individuals.[17] The half-maximum effective dose (ED_{50}) of pregabalin on capsaicin-induced pain (including hyperalgesia, tactile and thermal allodynia and their respective areas) in healthy male individuals was calculated to be 252 mg (95% confidence interval 194, 310 mg).[18]

Following overnight fasting, individuals received pregabalin or placebo (hard gelatin capsules, same size and colour as pregabalin capsules, manufactured at the Hospital Pharmacy of the University of Heidelberg, Germany) with 200 mL of lukewarm tap water. Pregabalin has a time-to-peak plasma concentration, T_{max}, of 0.7-1.3 hours after oral administration, an oral bioavailability of approximately 90% and an elimination half-life of approximately 6 hours. Food reduces its absorption.[25] Pregabalin is not metabolized and does not bind at plasma proteins. Its plasma clearance is nearly equivalent to the renal clearance, and 98% of the absorbed dose is renally excreted in its unchanged form.[15] These pharmacokinetic properties, in particular the T_{max} and food dependency of the absorption, were behind the necessity of fasting and of the timing of the pharmacodynamic measurements, which were placed around the expected plasma concentration peak.

To control this, plasma concentrations of pregabalin were analysed. Therefore, venous blood samples (5 mL each) for analysis of the drug concentrations were drawn into lithium heparin tubes at each treatment period from the forearm opposite to the capsaicin injection at −60, −30, −15, 0, 15, 30, 45, 60, 75, 120, 135, 240 and 255 minutes relative to the capsaicin injection, which when corrected by +60 minutes

corresponds to the time-points relative to the administration of pregabalin. Blood samples were centrifuged at 4°C at 1500 *g* for 15 minutes, and following separation of the plasma, samples were stored at −78°C pending analysis. Plasma concentrations of pregabalin were measured after dilution using LC-MS/MS. For this purpose, 10 µL of plasma was added to 1.5 mL of water. Then, 20 µL methanol and 20 µL internal standard (Pregabalin-d6: 625 ng/mL) were mixed. After vortexing (1 minutes) and centrifugation (3 minutes, 20 000 *g*), supernatant was transferred to an autosampler vial. 10 µL of the sample was injected into the LC-MS/MS system. For the chromatographic separation, a Synergi Hydro-RP 2.0 × 150 mm, 4 µm (Phenomenex) column (with a precolumn of the same material) running in gradient elution mode was used. Eluents were A: 1 mM ammonium acetate containing 0.1% acetic acid and B: acetonitrile: methanol: chloroform 46:46:8 (v/v/v) containing 0.4% formic acid. Pregabalin was quantitated using a QTrap 5500 instrument (Sciex) operated in positive ionization mode and multiple reaction monitoring (MRM) for the mass to change transition 160.1 → 55.0. Acquisition was performed using Analyst software v1.6.1 and quantitation, using Multiquant Software v3.0 (both Sciex, Darmstadt, Germany). Calibration curves (200-10 000 ng/mL) and quality control samples (QC, 200, 600, 5000 and 8000 ng/mL) were calculated by least squares regression weighted with $1/x^2$. Accuracy (measured as relative error) and precision (measured as relative standard deviation) of the QC samples were lower than 15% in all cases.

2.4 | Study objectives

The prospective human experimental pain study aimed at finding readouts of the human experimental pain model employing intradermal injection of capsaicin that provided the most relevant information about analgesic effects of pregabalin, based on previously shown efficacy of the drug in this particular pain model. A data-driven approach was chosen for feature selection among candidate readouts.

2.5 | Variables and measurements

2.5.1 | Injection of capsaicin

In each period of the study, participants received a capsaicin injection that consisted of 100 µg capsaicin (Capsaicin USP, Euro OTC Pharma, Bönen, Germany, manufactured for administration in humans by the Pharmacy Department of the University of Leipzig, Germany) administered intradermally in the mid-point of the dominant volar forearm between the wrist and the elbow. Experimental hyperalgesia/allodynia was induced at time-point $t = 0$ minutes, which was 60 minutes after oral administration of pregabalin. This ensured assessments during the highest drug exposure, based on anticipated time courses of plasma concentrations.

2.5.2 | Assessment of hyperalgesia and allodynia

Quantification of capsaicin-induced hyperalgesia or allodynia was performed twice before capsaicin injection, that is at time-point $t = -195$ minutes relative to the capsaicin injection when the individuals had arrived at the laboratory and again at $t = -30$ minutes. Subsequently, measurements were taken at 15, 30, 60, 120 and 240 minutes after capsaicin injection. As preliminary measurements had suggested quick initial changes in the intensity of spontaneous pain, this parameter was additionally rated at 0, 5 and 10 minutes after capsaicin injection. Additional parameters could not be assessed at this short interval. In addition, the subjects were requested to rate selected side effects, including "tiredness," "drowsiness" and "euphoria", on visual analogue scales (VAS) that had a length of 100 mm and ranged from "very weak" to "very strong."

To quantify experimental hyperalgesia and allodynia, seven different parameters were acquired (Table 2). Specifically, the area of hyperalgesia around the site of capsaicin injection (AreaPin) was quantified using a standardized punctate needle stimulator ("PinPrick," strength 256 mN). Specifically, pinprick stimuli were applied at 1-cm steps along 8 linear paths arranged vertically, horizontally and diagonally around the injection site. Stimulation along each path was initiated well outside the hyperalgesic area and continued towards the capsaicin injection site until the subject reports a definite change in sensation (burning, tenderness, more intense prickling). This spot, which reflects the border of the area of hyperalgesia, was marked with a sterile pen. The marks of hyperalgesia were then transferred on a transparency film and connected to form an area. The transparency film was scanned to an IBM-compatible personal computer, and the marked areas on the resulting *.jpg-file of a resolution of 150 dpi were quantified in mm^2 using the software ImageJ 1.50b (National Institutes of Health, Bethesda, Maryland, USA, freely available at https://imagej.nih.gov/ij/index.html,[26]). In addition, the 256 mN needle stimulator was applied to the mid-point between the marked border and the capsaicin injection site at each of the 8 linear paths. The painfulness of each stimulation was assessed by means of a visual analogue scale (VAS) ranging from 0 mm,"no pain," to 100 mm, "worst imaginable pain." The mean of the eight ratings was defined as the pain intensity at the area of hyperalgesia (VASPin). The area and intensity of allodynia around the site of capsaicin injection (AreaAll and VASAll, respectively) were quantified using the same procedure as used to quantify the area of hyperalgesia, except for the needle stimulator that was replaced by a Q-tip.

Capsaicin rapidly produces local neurogenic inflammation (characterized by oedema and erythema) when locally administered to the human skin by stimulating TRPV1 receptors present on dermal sensory nerve endings.[27] The flare response to noxious stimulation of the skin is mediated by polymodal nociceptors of C fibre primary afferent nerves. This has been shown to be associated with a reproducible increase in dermal blood flow.[28] Therefore, in the present study, blood flow in the area of capsaicin-treated skin was quantified before and after capsaicin treatment using high-resolution laser speckle contrast imaging (moorFLPI-2, Moor Instruments GmbH, Remagen, Germany). This non-invasive method visualizes microcirculatory blood flow in tissue instantaneously. The laser device was positioned approximately 30 cm above the skin. The area of increased flow was monitored and the velocity of moving erythrocytes was determined, providing a relative measure of skin perfusion (*laser Doppler flux = velocity · concentration of moving erythrocytes*). The results are visually presented as a 2-dimensional colour-coded picture. The images were analysed by dedicated image-processing software (Moor Instruments), using a cut-off value to distinguish the intensity and area of capsaicin-induced blood flow of that from the physiological blood flow observed at the untreated skin. This cut-off value was defined as the average value + 2 SD of the intensity of the PreDose measurement and was calculated individually for each subject and each treatment period from the respective PreDose measurement.[29] All pixels exceeding the defined threshold were included in the calculation of the flare area (AreaFla) and mean flux intensity change (VASFla).

Spontaneous pain (SponPain) was assessed by means of VAS ranging from 0 mm,"no pain," to 100 mm,"worst imaginable pain." Before the actual experimental tests, all individuals completed training sessions with capsaicin injection and complete hyperalgesia/allodynia-related data acquisition. The capsaicin injections were performed alternately on both arms.

2.6 | Data analysis

Data analysis was performed using the R software package (version 3.4.3 for Linux; http://CRAN.R-project.org/[30]) on an Intel Core i9® computer (operating system: Ubuntu Linux 18.04 64-bit).

2.6.1 | Quantitative variables

The variables included seven different readouts of the capsaicin injection-based human experimental pain model, namely "AreaPin," "VasPin," "AreaAll," "VasAll," "AreaFla," "VasFlare" and "SponPain" (Table 2). Each readout had been acquired at −195, −30, 15, 30, 60, 120 and 240 minutes relative to the capsaicin injection from n = 18 individuals. In addition, variables included descriptive parameters of the pregabalin plasma concentration versus time courses comprising values of peak plasma concentration, C_{max}, and the time to reach the peak, T_{max}, which were read from the data, and the area under the plasma concentrations versus time curve during the observation period.

2.6.2 | Data analysis strategy

Pain model–derived parameters that provided the most relevant information to quantify the analgesic effects of pregabalin were approached using methods of feature selection.[21] Specifically, the effects of pregabalin on readouts of the pain model, averaged across the observation period following capsaicin injection, were quantified using standard effect measures such as Cohen's *d*.[31] Subsequently, among the thus quantitative measures of the model's readouts, those with the highest importance were identified. This was implemented as computed ABC analysis[32] that aims at dividing a set of positive numerical data into three disjoint subsets called "A," "B" and "C." Set "A" should contain the "important few," that is those elements that allow obtaining a maximum of yield with a minimal effort.[33,34]

Thus, the data analysis was performed in three main steps comprising (a) data preprocessing including transformation according to the observed data distributions, which was followed by outlier detection and missing value imputation, (b) the application of feature selection techniques[35] as known from machine learning[22] and (c) the statistical assessment of the selected features using classical methods.

2.6.3 | Data preprocessing

Data preprocessing included data checking, data transformation, statistically adjusting for outliers[36,37] and imputation of missing values. Data distribution was analysed employing Box-Cox transformations,[38] that is $x' = \begin{cases} \frac{(x+c)^{\lambda}-1}{\lambda}, x \neq 0 \\ \log(x+c), x = 0 \end{cases}$ with values of $\lambda = 0$ equalling a log-transformation as $x' = log(x + c)$ where c denotes a constant that was assigned value of 1 to obtain zero-invariant log-transformation, $\lambda = 0.5$ equalling a square root transformation as $x' = \sqrt{x}$, and $\lambda = 1$, which denotes no transformation ($x' = x$). This corresponds to the ladder of power, respectively, of transformations[39] and assures that the data remain interpretable. That is, a log-transformation is in line with both general observations of logarithmic distributions of blood-derived concentration data[40] such as pharmacokinetic data, and the law of Weber and Fechner that describes the logarithmic distribution of sensory data[41] such as hyperalgesia-related data. Similarly, describing an

area of a circular plot by its radius is essentially a square root transformation, which would therefore be the possible expectation for a distribution of area data. However, in the present analysis, the adequate transformation was chosen based on the analysis of the transformed data, x′, for normal distribution by (a) applying Kolmogorov-Smirnov tests[42] and (b) visual inspection of the quantile-quantile plots.

Following data transformation, outliers were removed according to Turkey's method[39] that is based on boxplot statistics (R command using the "boxplot.stats" function implemented in the R core package "grDevices"[30]). The final step of data preprocessing consisted of imputation of missing values. For data acquired at baseline, the means of all measurements were taken to replace missing values. For measurements taken after the intracutaneous injection of capsaicin, a *k* nearest neighbour algorithm was used with $k = 3$[43] applying the weighted average method and Euclidean distance implemented in the "DMwR" R library (https://cran.r-project.org/package=DMwR[44]). Data preprocessing provided a data set consisting of a 32 × 52 sized matrix carrying data related to hyperalgesia or allodynia. This matrix was split into seven separate submatrices, sized 32 × 7 each (16 individuals assessed for the effects of two treatments, seven measurements of the parameters) except for the matrix spontaneous pain with a size of 32 × 10 including the three additional measurements.

2.6.4 | Feature selection

The second analytical step aimed at establishing a numerical criterion that allowed to establish a hierarchy among features with respect to their suitability to quantify the analgesic effects of pregabalin. As a numerical criterion of this hierarchy qualified effects sizes, versus placebo, exerted by the active drug. These effect sizes were quantified by calculating Cohen's *d*[31] that provided standardized treatment differences in parameter means calculated by the difference in means divided by the joint standard deviation. The result was a unit-free number of which, an absolute value of $d = 0.2$ is regarded as a small effect, 0.5 as a medium and >0.8 as a large effect.[31] Cohen's *d* was calculated for each active treatment versus placebo from the individual robust means[45] across the measurements acquired following capsaicin injection, separately for each hyperalgesia-related parameter.

A cut-off value was established in the vector of Cohen's *d* values to define how many features were further analysed. To this end, the values of Cohen's *d* were submitted to computed ABC analysis.[32] This is a categorization technique for the selection of a most important subset among a larger set of items. It has been originally developed in economic sciences to search for the minimum possible effort that gives the maximum yield. Computed ABC analysis aims at dividing a set of data; here, the set of values of Cohen's *d*, into three disjoint subsets called "A," "B" and "C." Subset "A" comprises the profitable values, that is "the important few," whereas subset "C" comprises non-profitable values, that is "the trivial many".[33,34] As ABC analysis requires positive values, all negative Cohen's *d* indicating that hyperalgesia was increased as compared to placebo were regarded as no effect and set at a value of zero. These calculations were done using our R package "ABCanalysis" (http://cran.r-project.org/package=ABCanalysis[32]).

2.6.5 | Statistical assessment of the selected features

The third analytical step aimed at dropping features not providing significant analgesic effects of pregabalin. Thus, following identification of effects that are most relevant for the present explorative analysis of the antihyperalgesic effect of pregabalin in a human capsaicin-based experimental pain model, the statistical significance of the observed changes in the respective hyperalgesia-related parameters was assessed. Data were submitted to analysis of variance for repeated measures (rm-ANOVA), with "treatment" (ie placebo or pregabalin) and "measurement" (ie the time-points of measurement of hyperalgesia-related parameters) as within-subject factors. Calculations were performed using the R command *"aov(ParameterValue ~ Treatment * Measurement + Error(ID/(Treatment * Measurement)),"* with "*Treatment,*" "*Measurement*" and "*ID*" defined as factors, implemented in the R core package "grDevices"[30]). The α-level was set at .05.

3 | RESULTS

3.1 | Participants and descriptive data

Sixteen individuals finished the study while two participants dropped out due to a hypersensitivity to capsaicin. Pregabalin plasma concentrations reached an average maximum of C_{max} = 7925.0 ± 1684.0 ng/mL (mean ± SD) at T_{max} = 79 ± 26 minutes after oral administration. Hence, results of plasma concentration analyses supported the acquisition of the main study parameters during adequate drug exposure in all participants (Figure 1).

3.2 | Main results

3.2.1 | Capsaicin-induced hyperalgesia and allodynia

Following intracutaneous injection of 100 µg capsaicin, all hyperalgesia-related parameters raised quickly (Figure 2). In the first step of the data analysis, data exploration

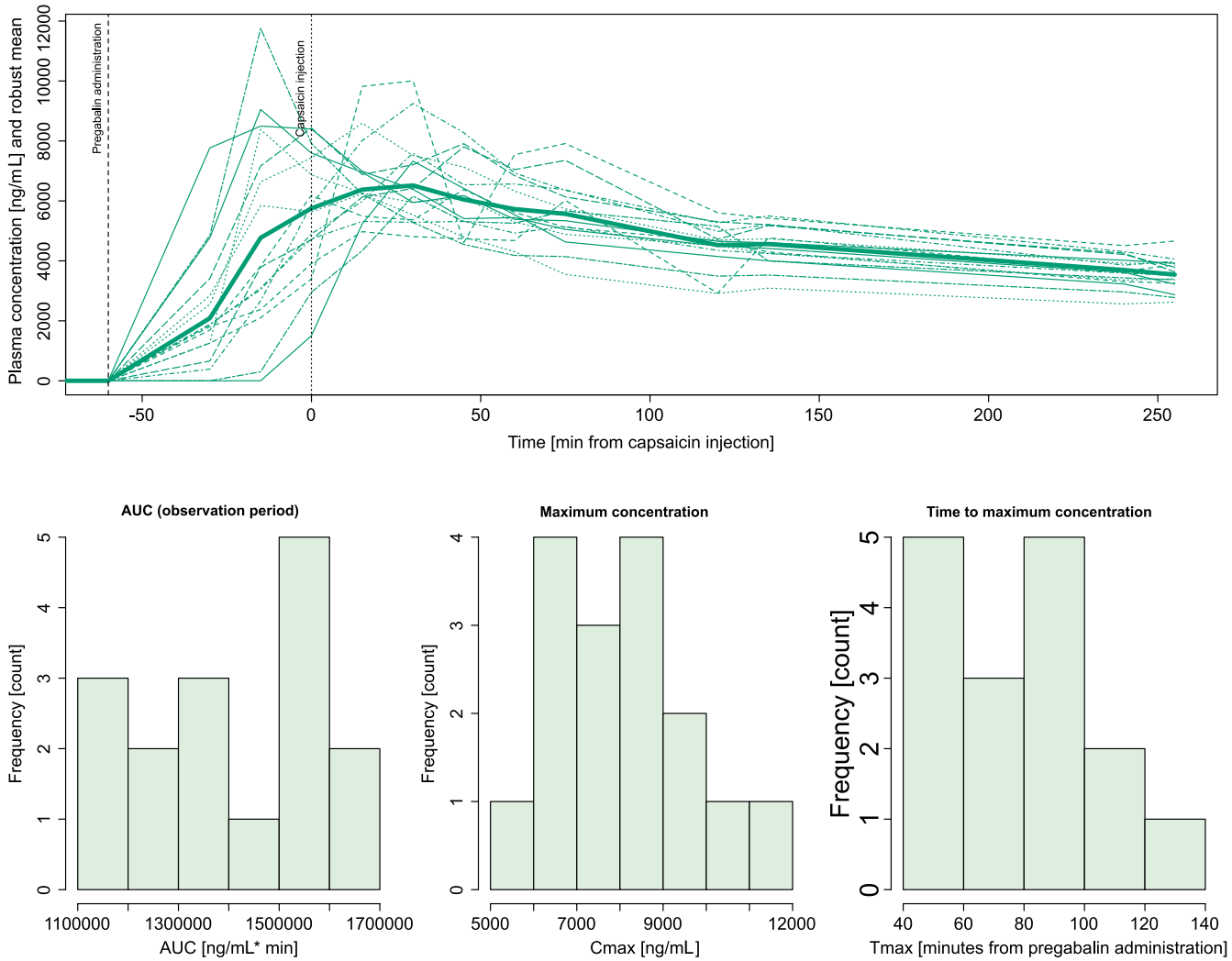

FIGURE 1 Plasma concentrations of the pregabalin and basic descriptive pharmacokinetic parameters. Top: Pregabalin concentrations plotted against the time-points of blood sampling at −60, −30, −15, 0, 15, 30, 45, 60, 75, 120, 135, 240 and 255 minutes relative to the capsaicin injection, which when corrected by +60 minutes corresponds to the time-points relative to the administration of pregabalin. Individual curves ((dotted or dashed lines) and (robust mean bold solid lines)). Bottom: Histograms showing the probability distribution of basic descriptive pharmacokinetic parameters, that is the area under the plasma concentrations versus time curve during the observation period (AUC), the maximum plasma concentrations (C_{max}) and the time to reach the maximum (T_{max}). These pharmacokinetic parameters are reported using a time axis rescaled for pregabalin administration at $t = 0$ minutes. The figure has been created using the R software package (version 3.4.3 for Linuxhttp://CRAN.R-project.org/[30])

using different Box-Cox transformations along the so-called ladder of power, followed by visual inspection of quantile-quantile (QQ) plots, suggested log-transformation (Box-Cox $\lambda = 0$) of most VAS-related data ("VasPin," "VasAll," "VasFlare") and square root transformation (Box-Cox $\lambda = 0.5$) of all area-related data ("AreaPin," "AreaAll," "AreaFla"). This was supported by non-significant Kolmogorov-Smirnov tests, for example $D = 0.070556$, $P = .2279$ for "VasPin" and $D = 0.091104$, $P = .2125$ for "VasAll" after log-transformation (further details not shown). Data transformation was followed by imputation (k nearest neighbour algorithm) of n = 18 missing values (1.08%) and n = 37 outliers (2.22%) in the $32 \times 52 = 1664$ data matrix (16 individuals assessed 2 times, 52 single assessments).

3.2.2 | Pregabalin effects on capsaicin-induced hyperalgesia and allodynia

Following data transformation and missing value/outlier replacement, a 32×52 sized matrix was available for exploring the parameter space with respect to antihyperalgesic or anti-allodynic drug effects. In the second step of the data analysis, Cohen's d was calculated for pregabalin versus placebo from the individual robust means across the measurements acquired following capsaicin injection and separately

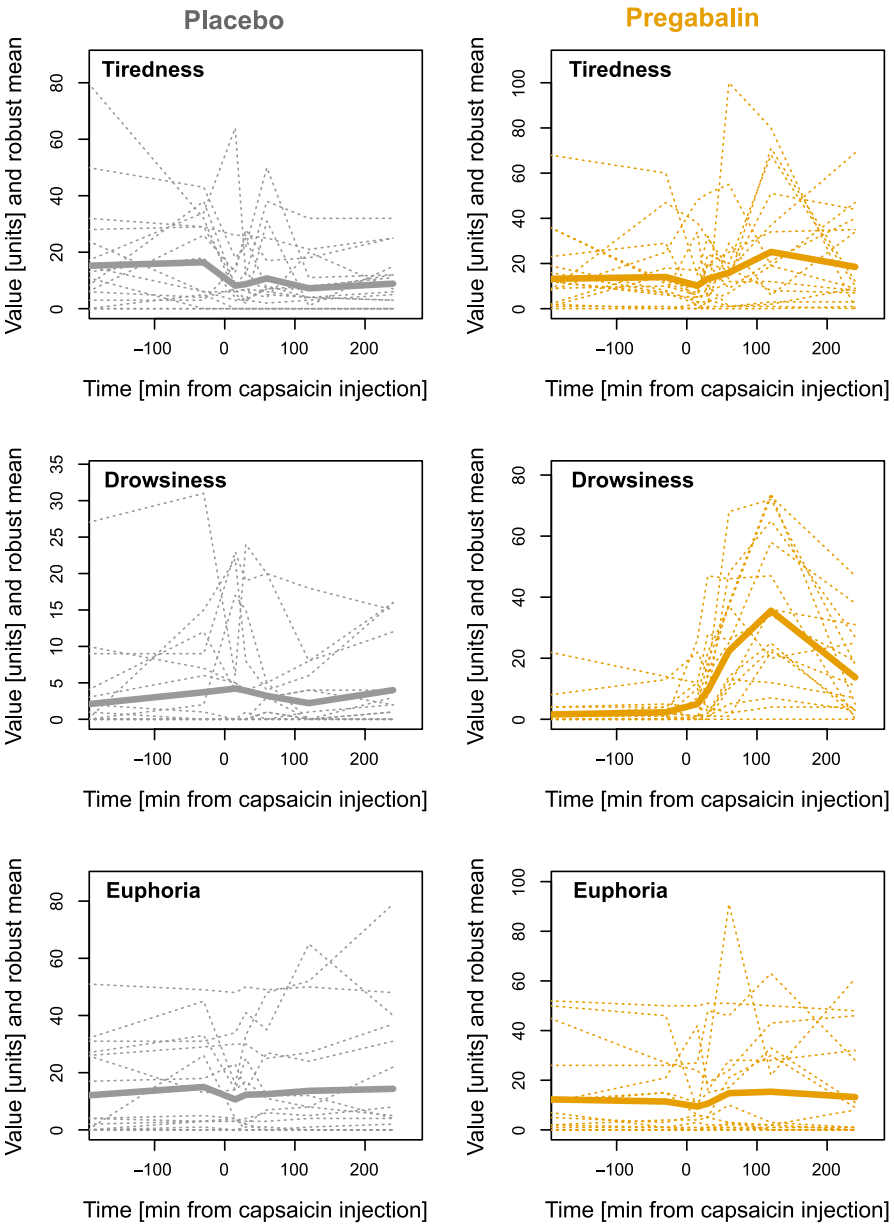

FIGURE 2 Hyperalgesia or allodynia induced following intracutaneous injection of capsaicin. The lines show the time courses of the two most relevant hyperalgesia/allodynia-related parameters (for abbreviations, see Table 2), separately for each study condition (time-points −195, −30, 15, 30, 60, 120, 240 minutes) relative to the capsaicin injection. The panels show these two pain-related parameters observed after administration of placebo (grey lines) along with the same parameter observed after administration of 300 mg pregabalin (orange lines). The thin-dotted lines show the individual time courses, whereas the bold lines show the robust means of the respective observation. The figure has been created using the R software package (version 3.4.3 for Linuxhttp://CRAN.R-project.org/[30])

for each hyperalgesia-related parameter. Among the effects of pregabalin on several different readouts of the human capsaicin pain model (Table 2), four values of Cohen's *d* (Figure 3) exceeded an effect size of 0.2 proposed to indicate a "small effect," as distinct from "no effect".[31]

To identify which of the readouts, judged by the obtained effect size, was most informative of the analgesic effects of pregabalin, computed ABC analysis[32] was applied on the value of Cohen's *d*. This provided a set size "A" of $d = 2$ items, which according to the proposed interpretation of ABC sets, subset "A" comprised the most profitable values.[33,34] Therefore, the parameters belonging to ABC set "A" were considered as the result of the feature selection procedure, that is of the analytical step aimed at choosing only parameters of interest for further analysis while dropping the other candidate parameters. Specifically, items in ABC set "A" (Figure 3) comprised the hyperalgesia-related parameters "VasAll" and "VasPin."

Following the selection of most suitable candidate parameters to quantify pregabalin effects in the capsaicin pain model, the fourth analytical step addressed whether the parameters were able to show analgesic effects of pregabalin statistically significantly. This was addressed by submitting these parameters to rm-ANOVA (Table 3). This resulted in statistically significant effects of the ANOVA factor "measurement" in all parameters supporting hyperalgesic effects of the intradermal injection of capsaicin. Significant effects involving the factor "treatment" were observed only for parameter "VasAll," while for "VasPin" merely a tendency towards such effect could be concluded (Table 3) from an interaction "treatment" by "measurement" that narrowly missed statistical significance (df = 5,75, $F = 1.931$, $P = .0561$).

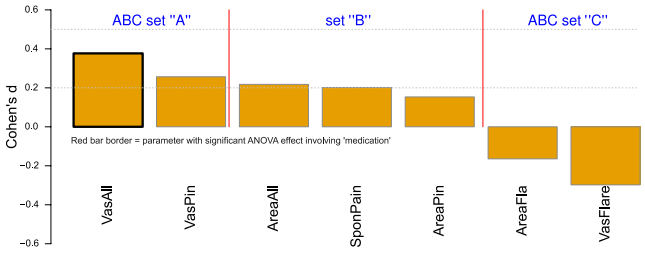

FIGURE 3 Feature selection aimed at detecting the most informative parameters (for abbreviations, see Table 2) indicating antihyperalgesic or anti-allodynic drug effects. **A:** Bar plot of observed effect sizes versus placebo, quantified as Cohen's *d*[31] calculated for each active treatment using the individual robust means across the measurements acquired following capsaicin injection. The bars are colour-coded for medications. Parameters for which a statically significant effect appeared, involving the study parameter "treatment," are highlighted with black frames. The values of Cohen's *d* were submitted to computed ABC analysis.[32] For further details about computed ABC analysis, see Ref. [[32]]. The computed ABC analysis resulted in three disjoint subsets (ABC set "A," "B" and "C"). In line with the proposed interpretation of ABC sets, subset "A" was interpreted to comprise the profitable values.[33,34] Therefore, the parameters belonging to ABC set "A" were considered as the result of the feature selection procedure, that is of the analytical step aimed at choosing only parameters of interest for further analysis while dropping the other candidate parameters. The figure has been created using the R software package (version 3.4.3 for Linuxhttp://CRAN.R-project.org/[30]). In particular, the ABC analysis was performed and plotted using our R package "ABCanalysis" (http://cran.r-project.org/package=ABCanalysis[32])

3.3 | Side effects

Pregabalin was tolerated by all subjects without major side effects requiring medical intervention. However, an increase in the ratings of tiredness and drowsiness was observed. A difference in side effects to placebo is supported by the results of the rm-ANOVA (effect "treatment": df = 1,15, tiredness: $F = 6.185$, $P = .0251$, drowsiness: $F = 20.01$, $P = .000447$; effect "measurement": df = 5,75, tiredness: $F = 0.813$, $P = .544$, drowsiness: $F = 11.44$, $P = 3.2 \times 10^{-8}$; interaction "treatment" by "measurement": df = 5,75, tiredness: $F = 2.083$, $P = .0769$, drowsiness: $F = 15.43$, $P < 2.05 \times 10^{-10}$), whereas "euphoria" was unaffected by medications or time.

4 | DISCUSSION

4.1 | Key results

While the observed analgesic effects were expected and agree with prior reports, in particular with results of three positive studies in which pregabalin was assessed using intradermal injection of capsaicin as a human experimental pain model,[17-19] the present analysis was mainly aimed at providing a ranking of the readouts of the human pain model based on intradermal injection of capsaicin, with respect to the relevance for the detection of analgesic effects. To obtain this ranking, the effects were numerically quantified using Cohen's *d* as previously[17]; however, an item categorization technique recently developed for similar ranking purposes[32] was applied that has not been used so far in the present research context of human experimental drug research. The present results indicate that the rated intensity of pain in the areas of secondary hyperalgesia and allodynia ("VasPin" and "VasAll", respectively) provided the most important information about the analgesics effects of pregabalin. By contrast, the sizes of the areas as frequently used readouts were less responsive to the drug effect. This was observed following oral administration of pregabalin at a dose of 300 mg, which was chosen based on previous evidence.[17-19]

Pregabalin produced, however, only small effects on experimentally induced hyperalgesia and allodynia when judged based on Cohen's *d*. Of the three positive studies assessing effects of pregabalin on capsaicin evoked hyperalgesia,[17-19] only one reported effect sizes.[17] Specifically, compared with placebo, pregabalin produced effect sizes, also calculated as Cohen's *d*, of 0.45-0.53 on the size of the area of hyperalgesia, and effect sizes of 0.19-0.18 on the size of the area of allodynia.[17] On pain intensity of flare related parameters, effect sizes ranged between 0.1 and 0.79. However, of a total of 12 values of Cohen's *d* against placebo reported in this paper, five values indicated no effects (Cohen's $d < 0.2$), two values indicated a weak effect (0.2-0.5), and five further values suggest a strong effect (>0.5). This differs from the present findings while emphasizing that strong effects in the capsaicin pain model cannot safely be expected from pregabalin. Considering that the severity of pain, the area and duration of mechanical hyperalgesia and the area of flare are capsaicin dose-dependent,[13]

TABLE 3 Results of analyses of variance for repeated measures (rm-ANOVA) of the hyperalgesia-related parameters assigned to ABC set "A" during the feature selection step of data processing (Figure 3). F and p-values are shown for main effects and their interaction, with significant results marked in bold letters. For the direction of the effects, see Figure 3

	ANOVA factor "treatment"		ANOVA factor "measurement"		ANOVA interaction "treatment" by "measurement"	
Parameter	***F***	***P***	***F***	***P***	***F***	***P***
"VasAll"	6.206	**.0249**	56.98	**$<2.10^{-16}$**	2.151	.0685
"VasPin"	2.484	.136	52.3	**$<2.10^{-16}$**	2.269	.0561

Note: Degrees of freedom, df = 1,15 for "medication," 5,75 for "measurement" except for "SponPain," df = 8,120, and df = 5,75 for the interaction term except for "SponPain," df = 8,120.

this might explain the more significant results in the previous study of[13] in which 250 μg capsaicin had been injected.

The present analysis used techniques of feature selection[35] to explore the parameter space of measurements related to the effects of intradermally injected capsaicin. A predefined hypothesis about the parameters shown drug effects was not pursued considering the heterogeneous observations of parameter specific drug effects in studies using the same pain model (Table 1). While data exploration produced a wide range of effect sizes quantified as Cohen's *d*,[31] ABC analysis allowed a mathematically precise definition of a subset of parameters in which antihyperalgesic or anti-allodynic effects were sufficiently pronounced to merit further analysis. This finally pointed at "VasAll," pain intensity in the area of allodynia, as the model parameter displaying significant analgesic drug effects in the present study. Possibly, an additional parameter suitable for the assessments of pregabalin effects on capsaicin-induced hyperalgesia was "VasPin," pain intensity in the area of hyperalgesia. The only negative effect of pregabalin was observed in blood flow around the side of capsaicin injection (Figure 3); however, this parameter did not show a significant drug effect (additional analysis of variance, effect of medication or interaction medication by session: $P > .2$).

The presently observed increase in the subjects' tiredness following administration of pregabalin agrees with its known side effects profile.[46] In human experimental studies, this can be a confounder of specific analgesic effects, including in studies using the capsaicin pain model.[47] Such side effects are routinely met with opioids or cannabinoids.[48,49] Several additions to the study design have been proposed to control for placebo effects, including the use of surrogate markers of pain such as nociceptive event-related cortical potentials[50] or the inclusion of non-nociceptive stimuli such as acoustic event-related cortical potentials.[51] Due to the complex design of the present study with pregabalin treatment and placebo sessions plus training and a dense data acquisition, additional recording of non-nociceptive bioresponses had been dismissed. Another design modification to reduce placebo effects is the introduction of an active placebo, such as benzodiazepine administration in studies assessing analgesic effects of opioids[52] or cannabinoids.[53] This has also been included in studies of pregabalin effects, where diazepam[54] or diphenhydramine[17] was occasionally used as an active placebo. However, this was not regularly implemented,[19] and moreover, midazolam serving as active placebo had been observed to produce inconclusive effects that could have been interpreted as analgesia.[55]

4.2 | Limitations

In the present assessments, the capsaicin-based pain model was chosen owing to its good record of providing results about analgesic drug effects that agree with the clinical effects in the pain settings where the respective drugs are mainly used.[9,10] Pregabalin was chosen since it was among the drugs most frequently assessed for analgesic actions with this model and it is among the first-line analgesics advised for neuropathic pain, which is currently the most problematic clinical settings for which most novel analgesics are being developed. Alternatives were not tested, and this would require much more complex or multiple studies and the difficult assessment of equianalgesic doses.

From prior knowledge (Table 1), it is difficult to judge whether pregabalin is indeed the best reference compound to be used in the present pain model, or whether alternatives should be seriously considered. A single study[56] reported the model as being sensitive also for gabapentin, which is an alternative first-line drug in the treatment of neuropathic pain.[57] Six studies[17,55,58-60] showed that the model is sensitive to opioids (alfentanil, fentanyl, morphine), whereas it failed in one study.[60] However, doubts may be raised about the use of opioids as standard references in this model as they are third-line drugs for the treatment of neuropathic pain due to safety concerns.[57]

A further limitation is the use of only a single dose of pregabalin. The dose of 300 mg was carefully selected based on prior evidence of its efficacy in the present pain model and its vicinity to the clinically advised dose. Too low doses might have spoiled the positive outcome of the study while too high doses might have challenged the data quality due to side effects interfering with the acquisition and validity of the pain-related data. Nevertheless, more doses provide better information about analgesic effects that are usually non-linearly related to the dose, reaching asymptotically a maximum.

Finally, when testing the capsaicin-based experimental pain model with the development of novel analgesic drugs in mind, it should be kept in mind that there are alternatives to this model with a comparable record of agreement of the results with relevant clinical settings. The best prediction of clinical analgesia seems to be reached with human pain models in which chemical hyperalgesia was induced by capsaicin, but also with human pain models which used UV-B hyperalgesia + contact heat, UV-B hyperalgesia + punctate pressure and chemical pain induced using intranasal gaseous CO_2 stimulation.[61]

5 | CONCLUSIONS

Based on the statistical significance of treatment effects on pain intensity in the areas of hyperalgesia and allodynia, present data indicate small antihyperalgesic and anti-allodynic effects of an oral single dose of 300 mg pregabalin on experimental pain induced by intradermal capsaicin injection. A novelty of the present analysis was the use of a precise item categorization technique, implemented as computed ABC analysis, in a human experimental pharmacological

context. By using this data science-based approach including the definition of a numerical criterion of feature ranking and the calculation of a limit for parameter selection, the most suitable hyperalgesia-related parameter to quantify the effects of pregabalin in this model was identified to be the pain intensity in the area of allodynia, that is a parameter obtained using Q-tip stimulation at the mid-point of along eight linear paths, arranged vertically, horizontally and diagonally around the capsaicin injection site, where a change in sensation (burning, tenderness, more intense prickling) was indicated by the individual and the site of capsaicin injection. Results of this study provide further support for the ability of the intradermal capsaicin pain model to show analgesic effects of pregabalin. This can serve as a basis for the design of studies where the inclusion of this particular pain model and pregabalin is planned.

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CONFLICT OF INTEREST

The authors have declared no further competing interests.

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