Probabilistic Mapping Reveals Optimal Stimulation Site in Essential Tremor

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Objective: The objective of this study was to obtain individual clinical and neuroimaging data of patients undergoing deep brain stimulation (DBS) for essential tremor (ET) from 5 different European centers to identify predictors of outcome and to identify an optimal stimulation site.

Methods: We analyzed retrospectively baseline covariates, pre- and postoperative clinical tremor scores (for 12 months) as well as individual imaging data from 119 patients to obtain individual electrode positions and stimulation volumes. Individual imaging and clinical data were used to calculate a probabilistic stimulation map in normalized space using voxel-wise statistical analysis. Finally, we used this map to train a classifier to predict tremor improvement.

Results: Probabilistic mapping of stimulation effects yielded a statistically significant cluster that was associated with a tremor improvement >50%. This cluster of optimal stimulation extended from the posterior subthalamic area to the ventralis intermedius nucleus and coincided with a normative structural connectivity-based cerebellothalamic tract (CTT). The combined features “distance between the stimulation volume and the significant cluster” and “CTT activation” were used as a predictor of tremor improvement. This correctly classified a >50% tremor improvement with a sensitivity of 89% and a specificity of 57%.

Interpretation: Our multicenter ET probabilistic stimulation map identified an area of optimal stimulation along the course of the CTT. The results of this study are mainly descriptive until confirmed in independent datasets, ideally through prospective testing. This target will be made openly available and may be used to guide surgical planning and for computer-assisted programming of DBS in the future.

Deep brain stimulation (DBS) is an established therapy for medication-refractory essential tremor (ET) syndrome. The classical target is the ventral intermediate nucleus (Vim) of the thalamus.1 Early after the first published case series of thalamic DBS for ET in 1992, alternative targets inferior to the thalamus have been suggested by various groups, including the caudal zona incerta, the prelemniscal radiations or—more generally—the posterior subthalamic area (PSA).2–4 More recently, anatomic and neuroimaging studies based on diffusion-weighted imaging and tractography have suggested that the cerebellothalamic tract (CTT) might embody a common neuroanatomic...
substrate of stimulation-induced tremor alleviation. These findings could implicate that instead of a confined anatomic (sub)area, stimulation at any point along the CTT network will elicit optimal tremor suppression. Depending on the intended target site of stimulation, reported follow-up, and outcome measure, thalamic and subthalamic DBS have been reported to achieve an on average 48% to 73.8% tremor reduction. Apart from the observed variability of outcomes between studies, there is also a considerable variability across studies about suboptimal and nonresponders that have ranked up to 22%. Furthermore, inconsistent and partly contradictory results were published regarding predictors of outcomes, such as the preoperative tremor severity, age, and stereotactic coordinates of the stimulating electrode.

Hence, after almost 30 years of DBS for ET, it remains unclear if there are solid predictors of outcome and if there is an optimal stimulation site. Previous studies were mostly based on smaller and monocentric series of patients with presumably lower variability in electrode locations. However, a certain degree of variability in electrode placement and measured outcomes is a prerequisite for studies that aim to investigate relationships between stimulation sites and clinical outcomes. To this end, analysis of aggregated data from multiple centers with differing electrode targeting approaches would be appropriate. In this study, we gathered clinical and neuroimaging data of a large cohort of patients that underwent DBS for ET in 5 different centers to identify robust predictors of outcome and to identify an optimal stimulation site by applying probabilistic mapping.

Materials and Methods

Patients

This study retrospectively enrolled datasets of 119 patients with ET syndrome treated chronically with unilateral or bilateral DBS and operated at 5 different European DBS centers. Diagnosis of ET was assessed by specialized movement disorder neurologists according to recommended guidelines and indication for surgery was discussed at multidisciplinary boards. Large parts of the data were recently published (13 patients from a randomized controlled crossover trial and 81 from 4 retrospective studies). Unpublished data from 25 additional patients were included where full datasets were available. Individual datasets were included in the present study if they contained: (1) preoperative tremor scores of validated clinical rating scales, (2) available baseline covariates (sex, age, and disease duration), (3) 12 months of postoperative tremor scores with stimulation on, (4) pre- and postoperative neuroimaging allowing reconstruction of lead location or individual anterior commissure-posterior commissure coordinates of the reconstructed stimulating electrode, and (4) stimulation parameters at 12 months following surgery. Exclusion criteria were a diagnosis of other tremor syndromes (Holmes tremor, dystonic tremor, and Parkinson tremor) or a magnetic resonance imaging (MRI)-verified lesion in the cerebellum, thalamus, or brain-stem. The study was conducted in accordance with the Declaration of Helsinki and approved by the institutional review board of the University Bern (KEK 2018-00841).

Surgical Procedure and Clinical Evaluation

In all patients, electrodes (model 3389 or 3387, Medtronic; Boston Cartesia Vercise directional leads, Boston Scientific, or St. Jude 6148, St. Jude Medical) were implanted stereotactically into the Vim or PSA. The neurostimulation parameters were programmed according to best clinical practice by the local DBS neurologist. Pre-operative “medication-off” and postoperative “stimulation-on, medication-off” tremor severities were assessed “nonblinded” based on the Fahn-Tolosa-Marin rating scale (TRS) in 4 centers and based on the Bain’s TRS in 1 center by a specialized movement disorder neurologists at 12 months postoperatively.

Study Outcomes

The primary outcome measure was the percentage reduction of the part A subscore for upper and lower extremities of the TRS or Bain’s TRS per hemibody at 12 months following DBS lead implantation.

Lead Reconstruction and Estimation of Stimulation Volumes

DBS electrodes were reconstructed using the Lead-DBS toolbox (version 2.3.2) in MATLAB 2019b (The MathWorks, Natick, MA). Postoperative computed tomography (CT) or MRI scans were linearly coregistered to the pre-operative MRI using advanced normalization tools (ANTs) with brain shift correction as implemented in lead-DBS. Multispectral normalization to ICBM 2009b MNI space was carried out by applying the ANTs SyN Diffeomorphic Mapping. This method was shown to segment the subthalamic nucleus (STN) region with high precision comparable to manual expert segmentations in a recent comparative study. DBS electrodes were automatically pre-reconstructed using the phantom-validated and fully automated physical, affective, cognitive, environmental, and relationship (PACER) method. All reconstructed electrodes were individually checked visually for plausibility by examining the postoperative electrode position on the original postoperative image in relation to reliably detectable landmarks by a neurosurgical fellow (author S.B.) and a senior functional neurosurgeon (author A.N.). In a minor number of cases (11 patients and 19 electrodes), lead-DBS failed to detect electrodes automatically or provided an aberrant reconstruction far outside the thalamo-subthalamic region. In these specific cases, we compared the reconstruction result with the electrode position from the postoperative MRI or CT scan and manually corrected the electrode position according to the artifact based on the postoperative images in lead-DBS. For segmented electrodes, the orientation was determined with the directional orientation detection algorithm that was validated in phantom models and clinical studies.
Stimulation Map Construction
We computed a probabilistic stimulation map by aggregating individual stimulation volumes with their associated tremor improvement of the contralateral hemibody at 12 months. All VTAs were estimated in MNI space and left hemispheric VTAs were nonlinearly warped to the right hemisphere.

First, we calculated an N-image of the cohort by aggregating VTAs across the entire cohort. The N-image represents a heatmap and can be thresholded according to the number of times (n) each voxel in the map gets activated. To display the entire stimulation area of the cohort, we calculated the N-image to illustrate all voxels that were at least activated 4 times (representing 10% of small cohorts).

Second, we calculated a mean improvement map. To this end, each voxel was assigned the mean tremor improvement (part A) of all associated stimulation volumes activating this voxel. Each voxel had to be activated by at least 4 different stimulation volumes to be included in the analysis.

Third, we computed a significant improvement map adapted from Reich et al.26 In a first approach and closely akin to the method by Reich et al, the tremor improvement values associated with a given voxel were tested against the remaining improvement values not associated with this voxel by applying a 2-sided t test. With a significance level of 0.05, we yielded a better improvement cluster as well as a worse improvement cluster. However, both clusters were relatively small and did not pass multiple testing correction. Therefore, we applied a right-tailed Wilcoxon signed rank test. With the test data, we calculated the Euclidean distance from the centroid of a stimulation volume to the centroid of the sweet spot. This was repeated 10 times (ie, each stimulation volume was a test volume once). Of note, the sweet spot was spatially stable across the stratified 10-fold cross-validation. A stratified 5-fold cross-validation did not pass multiple testing correction to calculate the significant improvement map. Here, 80% of the data set, or 170 stimulation volumes, were not sufficient.

Outcome Prediction Model
Finally, we validated the significant improvement map. To this end, we applied a stratified 10-fold cross-validation (ie, the data set of 213 stimulation volumes was randomly split into 90% training data [192 stimulation volumes] and 10% test data [21 stimulation volumes]). The significant improvement map or sweet spot was computed with the training data. With the test data, we calculated the Euclidean distance from the centroid of a test stimulation volume to the centroid of the sweet spot. We also calculated the overlap volume between the test stimulation volume and the CTT. This was repeated 10 times (ie, each stimulation volume was a test volume once). Of note, the sweet spot was spatially stable across the stratified 10-fold cross-validation. A stratified 5-fold cross-validation did not pass multiple testing correction to calculate the significant improvement map. Here, 80% of the data set, or 170 stimulation volumes, were not sufficient.

First, we intended to predict continuous tremor improvement. The factors “distance from VTA to the sweet spot” and the “probabilistic overlap volume between VTA and CTT” were fed as predictor variables into a support vector machine regression model. Tremor improvement was the response variable. Our assumption was that both proximity to the sweet spot and activation of the CTT would be meaningful predictors. Of note, a VTA may be close to the sweet spot but not activate the CTT and such a VTA would be assumed to result in less tremor improvement than a VTA with equal distance to the sweet spot but that activates the CTT. With respect to the model, we used a nonlinear regression model with a Gaussian Kernel function. Conceptually, this transforms the 2 predictors “distance” and “overlap volume” to a higher dimensional space. In that space,
the algorithm then computes a linear function that is close to the response variable, while being as flat as possible (MATLAB command `fitsvm`).

Second, we pursued another approach and intended to classify tremor improvement. Again, the combination of factors distance and probabilistic overlap volume were used and fed into a classification ensemble (MATLAB command `fitcensemble` with automatic hyperparameter optimization). The intention was to predict whether stimulation volumes would result in greater or less than 50% tremor improvement.

### Statistical Analysis

Data were analyzed by applying descriptive nonparametric statistics after normality testing and visual inspection of the QQ-plots using SigmaPlot (Systat Software, San Jose, CA) and MATLAB (The MathWorks, Natick, MA) for baseline characteristics, tremor scores, and outcome data. Nested linear mixed-effect models were used to test for the influence of different baseline covariates (sex, age, pre-operative tremor score, center, total electrical energy delivered as fixed factor, and electrodes as random factor) on outcome (percentage change of part A tremor subscores per hemisphere/contralateral hemibody). For statistical testing of the mean improvement map, we applied a one-sided Wilcoxon signed rank test (see above) and multiple testing correction with false discovery rate of 0.05. Data are presented as mean ± 95% confidence interval (CI) if not indicated otherwise. Statistical tests were 2-tailed and a p value <0.05 was considered statistically significant.

### Results

#### Clinical Data

A total number of 119 patients from 5 European centers were included in the study. Table 1 shows the baseline demographic data for each center’s patient cohort. In all patients, the part A subscore was available after 12 months follow-up, which corresponded to 237 hemispheres. The mean tremor reduction (part A) per hemisphere across all centers was 64% (61–68%, 95% CI). Scatter plots of outcomes per center are shown in Figure 1. The results of the nested linear mixed effect model with age, gender, pre-operative tremor intensity, total electrical energy delivered by DBS and center as fixed factors, and patient and lead nested in the patient as a random factor yielded only the 2 factors “pre-operative tremor intensity” and “center” as significantly associated with outcome (Table 2). Individual tremor improvement varied substantially within the entire cohort (range = −25 to 100% tremor improvement). The outcome distribution of the entire cohort was left-skewed. The 33% percentile corresponded to a tremor improvement of 50% and the 67% percentile corresponding to an 80% tremor reduction.

The average (median and range) stimulation parameters of the cohort were 130 Hz (110–220 Hz), 60 μs (20–260 μs), and 2.6 V (0.9–7.4 V).

#### Stimulation Map

Electrodes could be reconstructed in 107 patients (213 hemispheres). Twelve patients (16 hemispheres) had

| TABLE 1. Patient Demographics of Each Included Study Cohort |
|------------------------------------------------------------|
| Variable | Amsterdam | Berlin | Bern | Cologne | Oxford | p    |
|----------|-----------|--------|------|---------|--------|------|
| Included patients | 23        | 35     | 18   | 19      | 24     |      |
| Age at surgery | 73 ± 10   | 74 ± 12 | 67 ± 13 | 61 ± 14 | 62 ± 11 | 0.89 |
| Disease duration | 26 ± 16   | 24 ± 15 | 24 ± 14 | 25 ± 15 | 22 ± 17 | N.S. |
| Sex (% male)    | 56        | 63     | 55   | 58      | 48     | 0.32 |
| Total energy delivered | 56.5 | 67.3   | 72.3 | 70.5    | 92.6   |      |
| Intended target | Vim/PSA   | Vim    | PSA  | Vim     | Vim/PSA | Vim  |

n.s. = not significant; PSA = posterior subthalamic area; Vim = ventralis intermedius nucleus.

### FIGURE 1: Scatterplot of percentage tremor reduction per center and outcome frequency distribution. Twelve months following surgery, the mean tremor reduction based on the part A clinical tremor rating scale subscore was significantly lower in the Bern cohort (48 ± 29%) compared to the other participating centers (ranging between 63% and 69% tremor reduction) (A). The histogram (number of individual hemispheres on the y-axis, % tremor reduction per hemisphere on the x-axis) of postoperative tremor improvement (part A subscale) for all centers reveals a left-skewed distribution (B).
to be excluded from the image analysis as insufficient data quality of the postoperative CT or MRI scan did not allow a sufficiently precise detection of the electrode. Figure 2 displays the spatial distribution of the implanted leads in the Vim and PSA. The stimulation area covers large parts of the motor thalamus (beyond the Vim) and large parts of the PSA lateral to the red nucleus (RN), covering the caudal zona incerta (cZI) and extending into the entire posterior STN. As the results of the linear mixed effect model suggest the factor “center” to be significantly associated with outcome (with patients from the Bern cohort having a significantly worse outcome compared to

| Name                        | Estimate | Standard Error | p       |
|-----------------------------|----------|----------------|---------|
| Intercept                   | 58.60    | 14.50          | 7.48e-05|
| Pre-operative tremor intensity | 2.22     | 0.76           | 0.0039  |
| Age                         | -0.046   | 0.18           | 0.80    |
| Gender                      | -2.64    | 4.42           | 0.55    |
| Center Bern                 | -17.68   | 6.68           | 0.0088  |
| Center Cologne              | 6.30     | 6.96           | 0.37    |
| Center Oxford               | 2.32     | 6.70           | 0.73    |
| Center Amsterdam            | 3.15     | 6.37           | 0.62    |
| TEED                        | -0.035   | 0.024          | 0.15    |

TEED = total electrical energy delivered. Statistically significant results are highlighted in bold characters.

FIGURE 2: Electrode locations and overall stimulation area (N-image). Pseudo 3-dimensional representations of all reconstructed implanted deep brain stimulation leads of the multicenter cohort from a posterior view (A) and view from top (B). The N1-image represents the visualization of all voxels activated at least once from the entire cohort, lateralized on the right hemisphere is shown in a view from top (C), from medial (D), and from posterior (E) and represents the entire area of stimulation across all patients. RN = red nucleus; STN = subthalamic nucleus; Vim = ventralis intermedius nucleus.
the other centers), we compared the electrode implantation sites among centers. Based on both visual inspection and statistical testing, the electrodes from the Bern cohort are on average placed more medial compared to the other centers (2.2 ± 1.45 mm, \( p < 0.001 \) analysis of variance [ANOVA]). For this reason, we decided not to correct for the factor “center” when constructing the stimulation map and outcome prediction models as we assume the difference between centers to result from different electrode placements.

The mean improvement map represents voxels that are associated with a certain clinical outcome. Displaying the whole map is visually not very informative, as areas associated with excellent tremor reduction are covered by areas of suboptimal tremor reduction, we thresholded the map according to our statistical approach by applying a partition into 3 clusters according to the 33%, 67%, and 100% percentiles. We display the suboptimal responder voxels a view from top (D), medial (E), and (F) posterior as well as according to the 0 to 33rd percentile to display “suboptimal” responder voxels a view from top (G), medial (H), and (I) posterior. Voxels that were located anterior and medial in the posterior subthalamic area (PSA) as well as lateral with close relation to the internal capsule were associated with a lower tremor reduction whereas voxels in an intermediate area of the posterior PSA extending through the vertical axis in the central aspect of the Vim were associated with high tremor reduction. RN = red nucleus; STN = subthalamic nucleus; Vim = ventralis intermedius nucleus.

FIGURE 3: Mean clinical improvement map, “excellent” and “suboptimal” responder cluster. Pseudo 3-dimensional views of the mean clinical improvement map is shown in a view from top (A), medial (B), and (C) posterior. The map is color-coded from yellow (low) to red (high) and represents the mean tremor reduction values per voxel (see color bar). For better visualization of the sub-zones of the map, it is further thresholded according to the 67 to 100th percentile to display an “excellent responder cluster” in a view from top (D), medial (E), and (F) posterior as well as according to the 0 to 33rd percentile to display “suboptimal” responder voxels a view from top (G), medial (H), and (I) posterior. Voxels that were located anterior and medial in the posterior subthalamic area (PSA) as well as lateral with close relation to the internal capsule were associated with a lower tremor reduction whereas voxels in an intermediate area of the posterior PSA extending through the vertical axis in the central aspect of the Vim were associated with high tremor reduction. RN = red nucleus; STN = subthalamic nucleus; Vim = ventralis intermedius nucleus.
Suboptimal responder voxels are located in the anterior and medial aspect of the PSA and extend to the ventralis oralis posterior nucleus and the internal capsule laterally. The excellent responder cluster aligns visually well with the tractography-based CTT (see Fig 4). Furthermore, we display the location of the identified significant good responder cluster that projects onto the margin of the posterior aspect of the PSA and Vim. The mean position of implanted DBS electrodes was $X = 12.66$, $Y = -15.02$, and $Z = -3.40$. In comparison, the centroid coordinates of the statistically significant good responder cluster were $X = 13.63$, $Y = -15.13$, and $Z = -2.58$ mm in the MNI space. A projection of the stimulation map onto the stereotactic atlas of Schaltenbrand and Wahren is shown in Figure 5.

**Structural Connectivity**

We displayed the suboptimal and excellent responder clusters as well as the significant good responder cluster in relation to the averaged normative CTT template (see Fig 4). The CTT passed through the significant good responder cluster. This visual impression was confirmed by statistical testing. Voxels within the significant good responder cluster were more likely to contain the CTT as expressed by higher tract probability values compared to the rest of the map ($0.50 \pm 0.13$ vs $0.21 \pm 0.08$; $p < 0.0001$, $t$ test).

**Outcome Prediction Model**

Finally, we evaluated the predictive value of this map by applying a stratified 10-fold cross-validation. The clinical outcome predicted from the significant improvement map by applying the support vector machine was associated with observed clinical outcome (mean $R^2 = 0.14$, $p = 0.02$). Thus, the model estimations explained 14% of the variance of tremor score improvement. Using the distance to the sweet spot alone resulted in an $R^2$ of 0.06, while using the probabilistic overlap volume alone yielded an $R^2$ of $-0.17$ (ie, distance to the sweet spot and probabilistic overlap volume alone would not be a good predictor). In comparison, an ordinary linear regression model with both distance and probabilistic overlap volume would yield an $R^2$ of 0.037, underlining the better performance of the support vector machine. When applying a classification ensemble on the distance and overlap volume, we could correctly classify good and suboptimal responders.
stimulation in the posterior part of the PSA and the inferior aspect of the Vim. The area of this “good responder cluster” coincided with the area of highest likelihood to contain the tractography-based normative CTT. Taken together, these findings suggest that stimulation of the posterior PSA and the Vim along the course of the CTT is associated with the highest likelihood of tremor suppression. The anatomic location of individual stimulation volumes was the overall best predictor of stimulation-induced tremor suppression with a sensitivity of 89% and a specificity of 57% to correctly identify responders with a >50% tremor reduction based on a leave-one-out cross validation.

The 5 included centers placed their electrodes by slightly different targeting areas and approaches. Of note, the identified statistically significant improvement cluster (stimulation sweet spot) is closely related to the mean position of the implanted DBS electrodes (stimulation hotspot). The results can be interpreted in different ways. First, the exact location and extent of the identified significant improvement cluster is too restricted as it depends on the distribution of data and our chosen statistical approach by applying voxel-wise statistical testing and multiple testing correction. Areas of the map that contain more datapoints (which is the case at the stimulation hotspot) will more likely yield significant results and survive multiple testing correction. The fact that the stimulation area, which is associated with a good outcome, is more widespread than the identified significant stimulation sweet spot is in favor of this explanation. On the other hand, the identified significant improvement cluster could indeed reflect the “true” sweet spot and different the different centers just target around this optimal stimulation point based on years of clinical experience and targeting optimization. In line with this view, our findings are based on chronic stimulation settings that reflect empirical stimulation parameter adjustment to optimize the stimulation outcome.

Anatomic and Functional Considerations
The CTT projects from the deep cerebellar nuclei mainly to the contralateral thalamus.29–31 The observation that several previous studies of DBS for ET reported satisfactory tremor reduction upon stimulation of slightly different thalamic and subthalamic targets including the PSA, cZI, or prelemniscal radiations led to the formulation that the CTT might be the underlying neuroanatomic substrate stimulation of which would suppress tremor.5,7,32,33 Since then, multiple studies from different groups studied the relationship among electrode locations, VTAs, and the tractography-based CTT or functional connectivity patterns of good and poor responders but came to different and sometimes
contradictory conclusions. For instance, Akram et al and Al-Fatly et al identified a thalamic sweet spot of optimal connectivity to the contralateral cerebellar dentate nucleus and the ipsilateral primary motor cortex (M1), whereas Middlebrooks identified a thalamic region that was connected to the supplementary motor area (SMA) and premotor area to be correlated with better tremor reduction.\textsuperscript{13,34,35} The results of the present work indicate that the area of stimulation which corresponds to efficient tremor reduction coincides with a high probability to contain the CTT, which is structurally connected to M1 and the contralateral dentate nucleus of the cerebellum (ie, support the view of Akram’s and Al-Fatly’s work as well as the one by Dembek et al).\textsuperscript{6}

The present findings of the location of the probabilistic stimulation sweet spot at the transition between the ventral Vim and the PSA are in line with previous findings from an intra-operative electrophysiological study by Milosevic et al.\textsuperscript{36} In their intra-operative study, the authors investigated the effects of high-frequency microstimulation on both neuronal firing and tremor suppression simultaneously by 2 closely placed microelectrodes. They found that 200 Hz high-frequency stimulation was significantly more effective to reduce tremor reduction and spontaneous cell firing in thalamic neurons compared to 100 Hz stimulation. Notably, they observed that the most ventroposterior stimulation sites had the best effect on tremors. The authors concluded that thalamic neuronal inhibition seems necessary for tremor reduction and may function in effect as a thalamic filter to uncouple thalamocortical from corticospinal reflex loops.

Although there is no question about the existence of cerebellothalamic connections from the deep cerebellar nuclei to the motor thalamus and their relevance in modulating movement, there is some controversy about which deep cerebellar nucleus is predominantly involved in tremor genesis. Based on anatomic tract tracer studies both the dentate nucleus as well as the interposed nuclei were shown to be connected to the motor thalamus and frontal regions including M1 and the SMA depending on the applied methodology and examined species.\textsuperscript{30,31,37–39} The present study results suggest that high-frequency stimulation of cerebellothalamic fibers from the dentate nucleus to the Vim suppress tremors based on probabilistic tractography findings. However, we do not claim that fibers from the interposed nuclei also contribute to this effect. A definitive answer to this controversy can only be provided by well-designed post-mortem anatomic studies in humans.

The presented results point toward a possible causal relationship of CTT stimulation for tremor suppression. However, an involvement of the cZI for tremor genesis and stimulation-induced suppression cannot be ruled out. The cZI is bounded by the medial lemniscus posteriorly, the ascending cerebellothalamic fibers in the prelemniscal radiation anteriorly and the superior part of the STN laterally. As already pointed out by Plaha, the cZI provides a unique GABAergic link between the basal ganglia output nuclei and the cerebellothalamic loop which places it in a key position to transmit synchronized oscillations generating tremor into these loops.\textsuperscript{33} The close anatomic proximity of the CTT and the cZI makes it difficult, if not impossible to segregate DBS induced stimulation effects on these two structures with current neuroimaging and VTA-modeling approaches.

**Limitations**

Limitations of this study are the retrospective nature of the magnitude of used primary outcome data to calculate the probabilistic stimulation map and the two different outcome scales used across centers. However, both clinical TRSs are validated and by using the percentage change of tremor, outcome data become normalized and reflect tremor improvement independent from the scale used. Second, the algorithm for image analysis, including image normalization, lead reconstruction, and electrode orientation is inherently prone to error. Although, most of the applied methods have been validated in phantom models before, without histological confirmation, DBS electrode reconstruction always remains presumptive. These inherent errors are very likely to limit the overall predictive value of the applied model. Third, we used a VTA model that assumes an isotropic environment, although the leads had been implanted in a region with moderate anisotropy. Diffusion weighted imaging and an expansion of the VTA model would be necessary to provide better patient-specific fiber tracking and to accommodate anisotropy. Moreover, each algorithm dedicated to co-registration and normalization involves inherent errors and impact the overall calculation precision. Fourth, we used normative connectome data to estimate the average CTT. Although these normative connectome atlases do not represent patient-specific connectivity, they in turn have the benefit of high signal-to-noise ratios. Furthermore, the results of a recent study by Wang et al put into perspective the limitations of normative versus patient-specific connectivity analysis.\textsuperscript{40} In addition, we did not integrate side effects into our probabilistic modeling approach. One major reason is the retrospective design of the study and missing data. However, it was regular clinical practice across all centers to titrate and optimize the stimulation parameters by balancing the best possible tremor suppression while avoiding limiting stimulation-induced side effects. It is therefore reasonable to assume that the probabilistic
stimulation map in a certain way contains indirect information about side effects. Nevertheless, future research needs to focus on objectively and quantitatively assessed side effects.

Last, we tested different outcome-prediction models based on different assumptions and variables to get to the final results that were not controlled or corrected for these multiple approaches. Still, the optimized algorithm underlying the outcome-prediction model can only explain 14% of the variance of the observed outcome if we seek to predict individual percentage tremor reduction. We suspect that the overall widespread stimulation area and the noisy nature of both clinical outcome and imaging data are the main reason that our applied models fail to predict individual continuous tremor improvement more accurately. Because of the low R-squared values, we further tested a classification with a random forest to predict a binarized outcome instead. The results of the cross-validation and their predictive value need to be interpreted with caution and the presented results are mainly descriptive until confirmed in independent datasets, ideally through prospective testing.

Author Contributions
A.N., T.D., A.H., A.A.K., T.Z.A., T.A.K.N., and C.P. contributed to the conception and design of the study. A.N., S.B., B.A.-F., T.D., M.B., A.L.G., D.K., M.L.L., I.D., A.S.-A., V.V.-V., T.Z.A., R.S., T.A.K.N., and C.P. contributed to the acquisition and analysis of data. A.N., S.B., A.H., M.L.L., I.D., V.V.-V., T.D., T.Z.A., A.L.G., M.B., and C.P. contributed to drafting the text or preparing the figures.

Potential Conflicts of Interest
The authors declared no conflict of interest.

Data Availability
The entire stimulation map as well as the significant improvement cluster will be made publically available as a NIfTI files.

References
1. Lozano AM. Vim thalamic stimulation for tremor. Arch Med Res 2000;31:266–269.
2. Benabid AL, Pollak P, Gervason C, et al. Long-term suppression of tremor by chronic stimulation of the ventral intermediate thalamic nucleus. Lancet 1991;337:403–406.
3. Carrillo-Ruiz JD, Velasco F, Jimenez F, et al. Bilateral electrical stimulation of prelemniscal radiations in the treatment of advanced Parkinson’s disease. Neurosurgery 2008;62:347–357; discussion 357–359.
4. Kitagawa M, Murata J, Kikuchi S, et al. Deep brain stimulation of subthalamic area for severe proximal tremor. Neurology 2000;55:114–116.
5. Coenen VA, Allert N, Paus S, et al. Modulation of the cerebellum-thalamic-cortical network in thalamic deep brain stimulation for tremor: a diffusion tensor imaging study. Neurosurgery 2014;75:657–669; discussion 669–670.
6. Dembek TA, Petry-Schmelzer JN, Reker P, et al. PSA and VIM DBS efficiency in essential tremor depends on distance to the dentatorubrothalamic tract. Neuroimage Clin 2020;26:102235.
7. Fiechter M, Nowacki A, Oertel MF, et al. Deep brain stimulation for tremor: is there a common structure? Stereotact Funct Neurosurg 2017;95:243–250.
8. Cury RG, Fraix V, Castrioto A, et al. Thalamic deep brain stimulation for tremor in Parkinson disease, essential tremor, and dystonia. Neurology 2017;89:1416–1423.
9. Sandoe C, Krishna V, Basha D, et al. Predictors of deep brain stimulation outcome in tremor patients. Brain Stimul 2018;11:592–599.
10. Papavassiliou E, Rau G, Heath S, et al. Thalamic deep brain stimulation for essential tremor: relation of lead location to outcome. Neurosurgery 2004;54:1120–1129; discussion 1129–1130.
11. Bhatia KP, Bain P, Bajaj N, et al. Consensus Statement on the classification of tremors from the task force on tremor of the International Parkinson and Movement Disorder Society. Mov Disord 2018;33:75–87.
12. Barbe MT, Reker P, Hamacher S, et al. DBS of the PSA and the VIM in essential tremor: a randomized, double-blind, crossover trial. Neurology 2018;91:e543–e550.
13. Al-Fatly B, Ewert S, Kubler D, et al. Connectivity profile of thalamic deep brain stimulation to effectively treat essential tremor. Brain 2019;142:3086–3098.
14. Bot M, van Rootsvelaar F, Contarino MF, et al. Deep brain stimulation for essential tremor: aligning thalamic and posterior subthalamic targets in 1 surgical trajectory. Oper Neurosur 2018;15:144–152.
15. Nowacki A, Bogdanovic M, Sarangmat N, et al. Revisiting the rules for anatomical targeting of ventralis intermediate nucleus. J Clin Neurosci 2019;68:97–100.
16. Nowacki A, Debove I, Rossi F, et al. Targeting the posterior subthalamic area for essential tremor: proposal for MRI-based anatomical landmarks. J Neurosurg 2018;131:820–827.
17. Bain PG, Findley LJ, Atchison P, et al. Assessing tremor severity. J Neurol Neurosurg Psychiatry 1993;56:868–873.
18. Stacy MA, Elble RJ, Ondo WG, et al. Assessment of interrater and intrarater reliability of the Fahn-Tolosa-Marin tremor rating scale in essential tremor. Mov Disord 2007;22:833–838.
19. Horn A, Li N, Dembek TA, et al. Lead-DBS v2: towards a comprehensive pipeline for deep brain stimulation imaging. Neuroimage 2019;184:293–316.
20. Avants BB, Epstein CL, Grossman M, Gee JC. Symmetric diffeomorphic image registration with cross-correlation: evaluating automated labeling of elderly and neurodegenerative brain. Med Image Anal 2008;12:26–41.
21. Ewert S, Plettig P, Li N, et al. Toward defining deep brain stimulation targets in MNI space: a subcortical atlas based on multimodal MRI, histology and structural connectivity. Neuroimage 2018;170:271–282.
22. Husch A, Petersen MV, Gemmar P, et al. PaCER—a fully automated method for electrode trajectory and contact reconstruction in deep brain stimulation. Neuroimage Clin 2018;17:80–89.
23. Dembek TA, Hoevels M, Hellerbach A, et al. Directional DBS leads show large deviations from their intended implantation orientation. Parkinsonism Relat Disord 2019;67:117–121.
24. Hellerbach A, Dembek TA, Hoevels M, et al. DiODe: directional orientation detection of segmented deep brain stimulation leads: a sequential algorithm based on CT imaging. Stereotact Funct Neurosurg 2018;96:335–341.

25. Oostenveld R, Fries P, Maris E, Schoffelen JM. FieldTrip: open source software for advanced analysis of MEG, EEG, and invasive electrophysiological data. Comput Intell Neurosci 2011;2011:156869.

26. Reich MM, Horn A, Lange F, et al. Probabilistic mapping of the antidystonic effect of pallidal neurostimulation: a multicentre imaging study. Brain 2019;142:1386–1398.

27. Schaltenbrand G, Wahren W. Atlas for stereotaxy of the human brain. 2nd ed. Stuttgart: Thieme, 1977.

28. Behrens TE, Berg HJ, Jbabdi S, et al. Probabilistic diffusion tractography with multiple fibre orientations: what can we gain? Neuroimage 2007;34:144–155.

29. Gallay MN, Jeanmonod D, Liu J, Morel A. Human pallidothalamic and cerebellothalamic tracts: anatomical basis for functional stereotactic neurosurgery. Brain Struct Funct 2008;212:443–463.

30. Hoover JE, Strick PL. The organization of cerebellar and basal ganglia outputs to primary motor cortex as revealed by retrograde transneuronal transport of herpes simplex virus type 1. J Neurosci 1999;19:1446–1463.

31. Orioli PJ, Strick PL. Cerebellar connections with the motor cortex and the arcuate premotor area: an analysis employing retrograde transneuronal transport of WGA-HRP. J Comp Neurol 1989;288:612–626.

32. Blomstedt P, Sandvik U, Linder J, et al. Deep brain stimulation of the subthalamic nucleus versus the zona incerta in the treatment of essential tremor. Acta Neurochir 2011;153:2329–2335.

33. Plaha P, Khan S, Gill SS. Bilateral stimulation of the caudal zona incerta nucleus for tremor control. J Neurol Neurosurg Psychiatry 2008;79:504–513.

34. Akram H, Dayal V, Mahlknecht P, et al. Connectivity derived thalamic segmentation in deep brain stimulation for tremor. Neuroimage Clin 2018;18:130–142.

35. Middlebrooks EH, Tuna IS, Almeida L, et al. Structural connectivity-based segmentation of the thalamus and prediction of tremor improvement following thalamic deep brain stimulation of the ventral intermediate nucleus. Neuroimage Clin 2018;20:1266–1273.

36. Milosevic L, Kalia SK, Hodaie M, et al. Physiological mechanisms of thalamic ventral intermediate nucleus stimulation for tremor suppression. Brain 2018;141:2142–2155.

37. Aumann TD, Rawson JA, Finkelstein DJ, Home MK. Projections from the lateral and interposed cerebellar nuclei to the thalamus of the rat: a light and electron microscopic study using single and double anterograde labelling. J Comp Neurol 1994;349:165–181.

38. Jomtellt H, Ekerot CF. Topographical organization of projections to cat motor cortex from nucleus interpositus anterior and forelimb skin. J Physiol 1999;514:551–566.

39. Rouiller EM, Liang F, Babalian A, et al. Cerebellothalamocortical and pallidothalamocortical projections to the primary and supplementary motor cortical areas: a multiple tracing study in macaque monkeys. J Comp Neurol 1994;345:185–213.

40. Wang Q, Akram H, Muthuraman M, et al. Normative vs. patient-specific brain connectivity in deep brain stimulation. Neuroimage 2021;224:117307.