Statistical analysis plan

Official Title of the study: The effect of noninvasive brain stimulation on symptoms of cervical dystonia

NCT number: NCT07681284

Document date: 10th September, 2026

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Section 1: Administrative Information

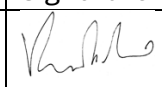
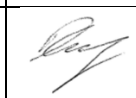
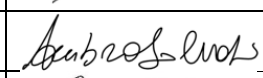
Index 1-6. (Sections/indices based on Gamble et al., 2017, JAMA. doi:10.1001/jama.2017.18556)

Trial full name	The effect of noninvasive brain stimulation on symptoms of cervical dystonia
Trial acronym	NetworkStim
Clinical trial number	NCT07681284
SAP version	v1.1. 10th September, 2026
Protocol version	This plan has been written based on information contained in the corresponding study protocol, v1.1 (10/9/26).
Roles and responsibility	Dr. Daniel T. Corp (contributor) (Fondazione Policlinico Universitario Campus Bio-Medico, Italy, and Deakin University, Australia), Dr. Ambra Salvati (contributor), Prof. Vincenzo Di Lazzaro (principal investigator) (Fondazione Policlinico Universitario Campus Bio-Medico), Dr. Paul A. Agius (statistician) (Deakin University)

SAP revision history

Version	*Key change/s*	*Reason(s) for change*	*Date*
1.0			7/7/2026
1.1	Coversheet added		10/9/2026

SAP signatures

Name	*Signature*	*Date*
Prof. Vincenzo Di Lazzaro		15/5/2026
Dr. Daniel Corp		15/5/2026
Ambra Salvati		15/5/2026
Paul A. Agius		18/5/2026

Abbreviations

cTBS	Continuous theta-burst stimulation
TMS	Transcranial magnetic stimulation

fMRI	Functional magnetic resonance imaging
CD	Cervical dystonia
TWSTRS	Toronto Western spasmodic torticollis rating scale
RMT	Resting motor threshold
BoNT	Botulinum toxin
MEP	Motor evoked potential
QoL	Quality of life
GDS	Global dystonia severity rating scale
FTM	Fahn Tolosa Marin modified tremor scale

Section 2: Introduction

Index 7. Background and rationale

See Protocol and registration documents for details. Briefly, cervical dystonia (CD) is a common movement disorder, yet at present, we do not have adequate therapies targeting the biological substrates of the disorder. Transcranial magnetic stimulation (TMS) has shown efficacy in other disorders, such as major depression, yet the evidence for TMS in CD has been mixed, due to methodological factors such as the brain region targeted, and dose.

Index 8. Objectives

See Protocol and registration documents. Briefly, the primary aim of this study is to test the efficacy of 8 days of continuous theta-burst stimulation (cTBS, a form of TMS) to the somatosensory cortex (S1) in patients with CD.

Section 3: Study Methods

Index 9. Trial design

Parallel-group, randomised, sham-controlled, double blind. Investigators measuring patient symptoms will be blinded from allocation group, investigators delivering the intervention will not be blinded, as this is not feasible. 1:1 treatment allocation ratio.

Those who are allocated to the sham cTBS intervention will be offered the opportunity to return after their final session is completed to undergo the real cTBS intervention. In this case, they will be unblinded. These patients will receive the Toronto Western spasmodic torticollis rating scale (TWSTRS), Quality of Life (QoL) scale, Global dystonia severity rating scale (GDS), and Fahn Tolosa Marin (FTM) modified tremor scale at T1 and T2, but not any other assessments or measurements (e.g., MRI, MEP collection) at any other timepoints. See Index 26 for outcome definitions.

Index 10. Randomisation

A computerised adaptive randomisation procedure will be performed, adjusting for baseline characteristics (TWSTRS score, age, and sex). See Protocol document for further detail.

Index 11. Sample size

An a priori power analysis of the study was conducted using G*Power to determine the sample size required to detect a time x condition interaction (repeated measures,

within-between interaction), with 3 levels for the time factor (pre, post, and follow-up 1. i.e., T1, T2, T3) and 2 levels for the condition factor (active and sham continuous theta-burst stimulation - cTBS). Follow-up 2 (T4) is not a part of the primary model, and this effect 4-5 weeks post will be assessed in a separate analysis (see below). An effect size of Cohen's f = 0.3 (medium effect) was assumed for the primary time x condition interaction, informed by preliminary data obtained in a study of 13 patients with CD conducted at Deakin University and a second study of patients with neglect, on which the present 4x day continuous theta-burst stimulation (cTBS) protocol is based (Nyffeler et al., 2019, Brain). Other parameters included a significance level of $\alpha = 0.05$, a statistical power of 0.80, an assumed correlation between repeated measures = 0.5, and a correction for non-sphericity of $\varepsilon = 1$.

The analysis indicated that a total sample size of 20 participants (10 per group) would be sufficient to detect the interaction effect with 80% power. Allowing for ~20% attrition, the target recruitment was increased to 26 participants (13 per group).

Index 12. Framework
The protocol tests for superiority of real cTBS compared to sham cTBS on the primary outcome of the Toronto Western Spasmodic Torticollis Rating Scale (TWSTRS) total score. Superiority will be tested via the time by condition interaction using a linear mixed-effects model (see section 6, index 27).

Index 13. Statistical interim analyses and stopping guidance
One interim analysis will be performed to evaluate safety and efficacy.

Index 14. Timing of final analysis
Immediately after the final patient's final data point has been collected.

Index 15. Timing of outcome assessments
The primary outcome (and other symptom scales) will be measured on day 1 before the cTBS intervention (T1), day 10 after the cTBS intervention (T2), two weeks post the final session (T3) and 4-5 weeks post the final session (T4). MRI outcomes and MEPs will be measured at T1 and T2. See Protocol and registration documents for further details.

Section 4: Statistical Principles
Index 16-18. Confidence intervals and P values
Data will be expressed as means and 95% confidence intervals and significance will be set at $p<0.05$.

Index 19. Adherence and protocol deviations
Patients will be considered to have adhered to the protocol if they complete 6/8 cTBS days (75%). As per Good Clinical Practice (GCP) guidelines, a protocol deviation is an unplanned, usually minor departure from the Institutional Review Board-approved protocol, Standard Operating Procedures (SOPs), or GCP requirements. For example, a visit outside a study visit window, or an incomplete non-safety related examination or assessment. All deviations will be recorded in the source documents.

Index 20. Analysis populations
Data will be analysed using an intention-to-treat (ITT) framework, with all randomised participants analysed in their originally assigned treatment groups using all available outcome data. A per-protocol analysis will also be reported, including participants who received the cTBS on ≥6/8 (≥75%) days and had no major protocol deviations.

Section 5: Trial Population
Index 21. Screening data
The number of patients screened, the number of patients enrolled, the number of patients included in analyses, will be reported in the final paper, including the reasons for non-enrolment or dropout.

Index 22. Eligibility
See Protocol and registration documents.

Index 23. Recruitment
Recruitment information will be summarised in a participant flow diagram consistent with CONSORT standards.

Index 24. Withdrawal/follow-up
Levels of withdrawal from intervention will be reported in the paper, including timing and reasons.

Index 25. Baseline patient characteristics
Patient characteristics, such as age, sex, symptom severity, time since diagnosis, resting motor threshold, BoNT treatment regime, will be presented descriptively in the manuscript.

Section 6: Analysis
Index 26. Outcome definitions
The primary outcome will be the TWSTRS total score. Secondary outcomes will be the TWSTRS subscales (severity, disability, pain), the QoL scale, GDS, FTM, kinematic movement pattern data, motor evoked potentials (MEPs), and resting-state fMRI data (rs-fMRI). The FTM is a modified version of the full FTM scale, relevant only to questions measuring tremor in head and neck regions. Data will not be transformed or presented as change statistics from baseline; scores as they are recorded for each subject and timepoint will be included in the linear mixed-effects models (see index 27).

Index 27. Analysis methods
Primary outcome
Total TWSTRS score will be analysed using a linear mixed-effects model fitted using restricted maximum likelihood estimation, with fixed effects represented by time (T1, T2, T3) and experimental condition (real vs sham cTBS). The random intercept will be represented by participants. Age and sex will be included as covariates in the model to improve precision, irrespective of baseline group differences.

The model's assumptions will be tested using graphical analysis of the residuals to identify any outliers and assess response normality and homoskedasticity. If statistical assumptions are violated (e.g., outliers, non-normality, heteroskedasticity), the same mixed model will be applied using robust standard errors (Huber-White estimator). If the response is non-normal, a generalized linear mixed model will be explored.

The primary objective is to determine whether total TWSTRS scores differ over time (T1, T2, T3) between the real and sham cTBS conditions. This will be assessed via the time by condition interaction, which will be the primary endpoint/inferential test condition determining whether the active cTBS is superior to the sham cTBS in ameliorating CD symptoms. Degrees of freedom for fixed effects will be estimated using the Satterthwaite approximation. If this interaction is significant, post-hoc comparisons will examine between-group differences at each post-treatment timepoint (simple effects, 2 comparisons), and test within-group changes from baseline (Pre) to each post-treatment timepoint separately within the real and sham conditions (simple effects, 4 comparisons). Multiple comparisons will be controlled using Bonferroni correction within each family of contrasts.

A separate analysis will assess whether the effects of the cTBS are still present at the follow-up timepoint 4-5 weeks after the intervention (T4). The same analytical approach using a linear mixed-effects model will be applied. This will be an additional endpoint to assess the durability of the effect.

Unblinded patients who return to receive the real intervention, after receiving sham in the main portion of the study (described in index 9) will be assessed using the same statistical methods as above (on the TWSTRS). Because these subjects will be unblinded, these data will not contribute to the main analyses of the clinical trial nor toward the assessment of the effectiveness of the intervention. However, results of these analyses will be presented to provide additional data as to the changes in symptoms due to the real cTBS intervention, within the context of unblinding, either in the supplementary material or the main manuscript.

Secondary outcomes

Clinical scales: The same analytical approach will be applied to the other clinical scales (TWSTRS subscales, QoL, GDS, FTM), as above for the primary outcome.

Motor evoked potentials: MEPs (recorded via an input-output curve) will be collected from electromyography over two separate muscles at T1 and T2 and analysed using a linear mixed-effects model, investigating an overall three-way interaction (time x condition x TMS intensity), within each muscle separately. As above, age and sex will be included as covariates. Should there be a significant three-way or two-way interaction, simple effects will be analysed using the same procedures as described above.

Resting-state fMRI data: will be collected at T1 and T2 and the primary analyses will use a seed-to-voxel approach. The primary seeds of interest (#1) will be located within the sensorimotor cortex - 4mm spheres at the xyz coordinates of cTBS in the S1 cortex (unilaterally and bilaterally). Additional seeds of interest will be run from: 2) the

sensitive and specific cluster (surviving 4/4 comparisons) in the sensorimotor cortex from Corp, et al. [1]; 3) 4mm spheres in the globus pallidus interna derived from the optimal site for deep brain stimulation in individuals with CD, as per Reich et al. [2], x = ±19.4, y = -10.1, z = -5.9 mm (unilaterally and bilaterally); 4) the sensitive and specific cluster (surviving 4/4 comparisons) in the cerebellum from Corp, et al. [1]. These will each be run within the whole brain, and additionally, the primary seeds of interest will be analysed constrained to the CD network as defined by Corp, et al. [1]. Analyses will measure the interaction between time (T1 vs. T2) and condition (active, sham), with age and sex included as covariates. Family-wise error (FWE) rate adjusted p-values less than 0.05 will be considered significant.

Kinematic Data: will be collected at T1 and T2 and the same mixed model analyses will be conducted as described above for the primary outcome.

Index 28. Missing data
All available data will be included following an intention to treat framework. Data will be assumed to be missing at random. No data will be imputed.

Index 29. Additional analyses
Associations between symptom scores and neurophysiological measures will also be analysed. Significance will be set at $p<0.05$ uncorrected. Additional post-hoc exploratory analyses may arise which will be described as such in the paper. The number of analyses performed will be reported.

Index 30. Harms
All adverse events will be documented in terms of severity, seriousness, and causality, and reported as per the principles of Good Clinical Practice. Safety of the intervention will be analysed by exploring descriptive statistics arising from the structured safety questionnaires.

Index 31. Statistical software
The primary outcome, secondary outcome clinical scales, and MEPs, will be analysed using Stata software or R software if this proprietary software is unavailable. fMRI data will be analysed using standard neuroimaging software and pre-processing pipelines.

Index 32. References
See Protocol and registration documents for references to Data Management Plan, Trial Master File, and Standard Operating Procedures.

References
[1] Corp DT, Kungshamn J, Ellis EG, Friedrich MU, Niemi KJ, Greenwood CJ, et al. Mapping the Neuroanatomy of Dystonia Using Causal Brain Lesions. medRxiv 2025:2025.12. 07.25341373.
[2] Reich MM, Horn A, Lange F, Roothans J, Paschen S, Runge J, et al. Probabilistic mapping of the antidystonic effect of pallidal neurostimulation: a multicentre imaging study. Brain 2019;142(5):1386–98.