Statistical Analysis Plan (SAP)

Cognitive Training Combined With Transcranial Direct Current Stimulation in Older Adults in a Home-based Context" Acronym: **TrainStim-Home**
Version 1
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Intervention therapy: six-session cognitive training over three weeks with anodal tDCS over the left dorsolateral prefrontal cortex (DLPFC)

Control therapy: six-session cognitive training over two weeks with sham stimulation

Study population: healthy older individuals

Clinical Phase: mono-centric randomized placebo-controlled trial

Clinicaltrials.gov Identifier: NCT04817124

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Content

1 Study Background

1.1 Study Objective

Developing interventions against age-associated cognitive decline is especially important, given the increase of aging populations around the world. Implementing combined cognitive training and transcranial direct current stimulation (tDCS) interventions has been beneficial in the past, supporting training effects and enabling transfer to other cognitive domains (Jones et al., 2015; Perceval et al., 2016; Berryhill, 2017; Antonenko et al., 2018; Antonenko et al., 2022). However, access to clinical centers offering multisession tDCS-accompanied cognitive training is limited. In order to increase accessibility, we conducted a clinical trial regarding the home-based and remote supervised application of tDCS in healthy older adults. We aim to provide evidence for the feasibility and the effects of a multisession home-based cognitive training in combination with tDCS on cognitive functions in healthy older adults (see Thams et al., 2022 for study protocol).The analyses described in this statistical analysis plan (SAP) will demonstrate the feasibility and efficacy of a two-week cognitive training intervention with concurrent tDCS in healthy older adults.

This SAP was prepared in accordance with the *Guidelines for the Content of Statistical Analysis Plans in Clinical Trials* (Gamble et al., 2017).

1.2 Primary hypothesis

The primary hypothesis of the project is that the self-application of tDCS combined with cognitive training by participants themselves in an ecologically valid environment (i.e., at participant's home) is feasible. Feasibility will operationalized by two-thirds of successfully performed interventional sessions per participant for at least 60 % of all participants. A session is considered successful when it is registered as fully completed in the cloud and the participant has not initiated contact concerning a problem or rescheduling.

1.3 Secondary hypotheses

Secondary hypotheses state that the combination of cognitive training and tDCS is superior compared to cognitive training alone with regard to cognitive training tasks, transfer tasks, at all follow up measures as defined by the secondary outcomes in healthy older adults.

1.4 Study Design

The TrainStim-Home trial is a randomized, double-blind, placebo-controlled monocenter study. The experimental group receives a six-session cognitive training intervention over two weeks, accompanied by tDCS over the left dorsolateral prefrontal cortex (DLPFC). The intervention of the control group consists of the same six-session cognitive training combined with sham stimulation.

Random allocation to experimental and control groups, respectively, will be performed with a 1:1 ratio. Stratified block randomization will be used. Strata will be chosen according to age (60-70 and 71-80 years) and initial performance in the letter updating task (≤ 5, ≥ 5/25 correctly recalled lists). After successful completion of telephone screening and baseline assessment (defined as meeting all eligibility criteria) and giving written informed consent, participants will be divided into four groups according to the age and performance strata. For the randomization we will use the blockrand package

in R[1].

1.5 Sample Size Calculation

As the primary goal of this study will be to assess feasibility, and as it is recommended to employ results of feasibility trials for sample size calculation of a planned subsequent trial (Hagen et al., 2011), we chose a sample size of N = 30 (Rahbek et al., 2017). To infer feasibility, the lower bound of the 95 % confidence interval of the proportion of participants who fulfilled the feasibility criterion needs to be at 60 %. Thus, 76 %, i.e., n = 23 participants will have to meet the feasibility criterion.
With 15 participants per stimulation group (anodal vs. sham stimulation), we will be able to scope the general feasibility of this home-based intervention and explore descriptively the benefit of anodal tDCS over sham with regard to performance after the training on the trained and untrained working memory tasks to obtain estimates of effect sizes for power calculations of future randomized controlled trials (Elmasry et al., 2015; Antonenko et al., 2018). Using an independent t-test with a two-sided significance level of 0.05 and a power of 80 % we will be able to demonstrate an effect of Cohen's d = 1.06 or higher on behavioral performance. Since this effect size is high and might be unrealistic, we use the secondary analyses on efficacy measures solely exploratory to inform future trials.

2 Analysis sets

2.1 Definitions

The **full analysis set** will consist of all participants who received at least one day of intervention. In case participants withdraw informed consent after baseline assessment, they will be considered as screening failures and therefore will not be included in the full analysis set. The **per protocol analysis set** comprises all subjects who successfully completed two-thirds of the home-based sessions (thus fulfilling the criterion for feasibility). Safety measures will be assessed during tDCS intervention and all participants who received at least one day of intervention will be included according to their actual treatment in **the safety analysis set**.

2.2 Application

The efficacy analysis regarding the effect of tDCS-accompanied training on working memory performance will be done using the full analysis set. For the safety analysis, analysis will be done in the safety analysis set, which is the same as the full analysis set.

3 Trial centres

Participants will be recruited in one centre: Greifswald.

3.1 Recruitment

Participants will be recruited through advertisements in the local newspapers and distribution of flyers in local senior citizen clubs. Telephone screenings will be conducted with all potential participants and

[1] http://www.R-project.org, http://www.rstudio.com, https://CRAN.R-project.org/package=blockrand

study information will be provided. Eligible candidates will be invited for baseline assessment. Following baseline assessment (V0) participants will be included if neuropsychological testing is unobtrusive.

Information on recruitment flow can be found in the CONSORT flow diagram (Figure 1).

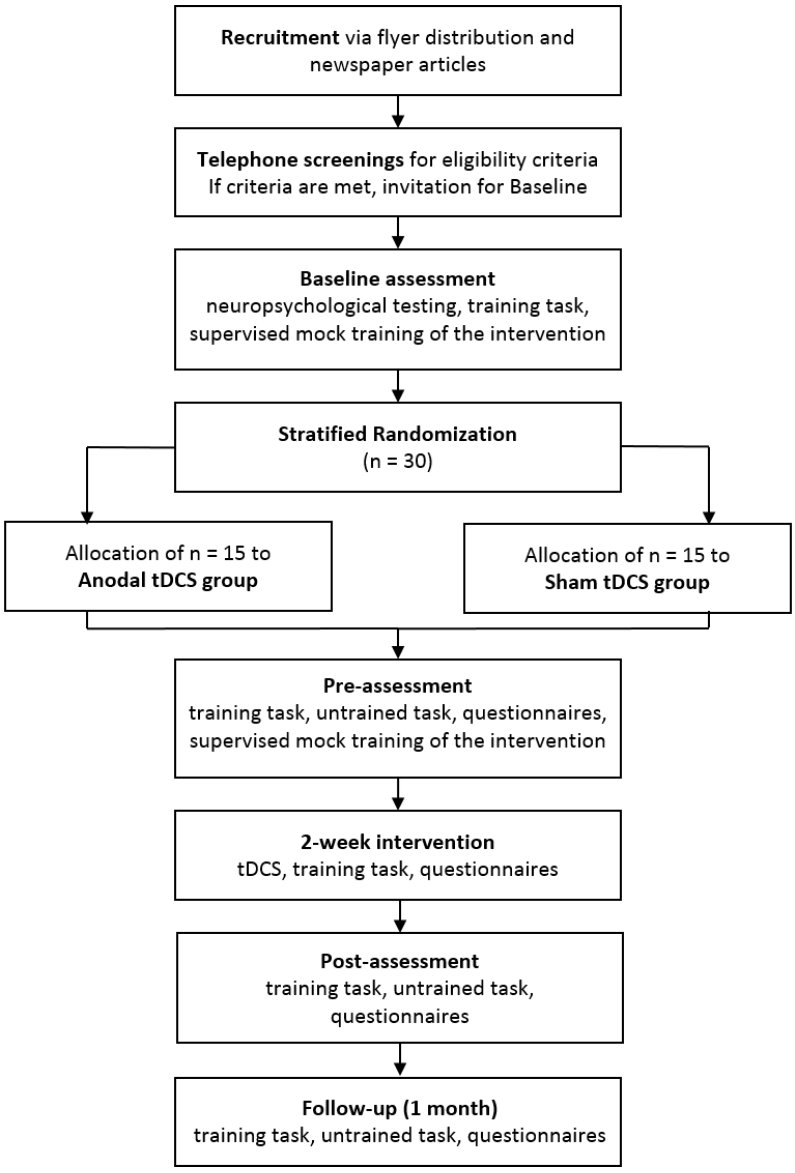

Figure 1. TrainStim-Cog study flowchart. tDCS, transcranial direct current stimulation. Obtained from TrainStim-Home study protocol (Thams et al., 2022).

4 Analysis variables

Table 1. TrainStim-Home outcome measures.
Adapted from TrainStim-Home study protocol (Thams et al., 2022)

			Baseline	**Pre**	**T1-T6**	**Post**	**FU**
					(2 weeks)	**(3 days)**	**(1 month)**
			~3h	**~1,5h**	**~1h**	**~1,5h**	**~1,5h**
Time point	**Measurement**	**Mode**	**V0**	**V1**	**V2-V7**	**V8**	**V9**
Enrollment							
Eligibility screening		Paper	x				
Informed consent		Paper	x				
Neuropsychological Screening	Demographic data	Paper	x				
	Geriatric depression scale(Brink et al., 2013)	Paper	x				
	Oldfield handedness inventory(Oldfield, 1971)	Paper	x				
	CERAD Plus(Morris et al., 1989)	Paper	x				
	Digit Span(Lezak et al., 2004)	Paper	x				
Intervention					↔		
Training task	Letter updating(Dahlin et al., 2008; Passow et al., 2017)	Tablet computer	x	x	x	x	x
Brain stimulation	tDCS (anodal vs. sham)	Device			x		
Questionnaires	Self-reported well-being questionnaire	Paper		x	x	x	x
	PANAS(Watson et al., 1988)	Paper			x		
	Adverse events questionnaire(Antal et al., 2017)*				x		

Additional assessments						
Untrained task	n-back(Lezak et al., 2012)	Computer	x		x	x
Feasibility	Sessions completed (primary outcome)	Cloud system		x	x	
	Feasibility questionnaire	Paper			x	

Abbreviations: T1-T6, training 1-6; FU, follow-up-assessment; V0-V9, visits 0-9; CERAD Plus, The Consortium to Establish a Registry for Alzheimer's Disease, neuropsychological test battery, German version, extended to CERAD Plus with the Trail Making Test A + B and Phonematic Fluency (S-Words); tDCS, transcranial direct current stimulation; PANAS, positive and negative affect schedule. All measures were acquired on site or at the respective participants home, except for screening which was done via telephone. *assessed only at the end of each training week (V4 and V7).

4.2 Primary Outcome

Primary outcome measure will be the feasibility of home-based, remotely-controlled and independently self-applied tDCS combined with cognitive training. Feasibility will inferred if 60% of the participants will have successfully performed two thirds (n= 4) of the planned sessions. A session is defined as successful when it is registered as fully completed in the cloud and the participant has not initiated contact concerning a problem or rescheduling.

4.3 Secondary Outcomes

Feasibility

- feasibility will be inferred additionally, if the questionnaire item assessing overall satisfaction with tDCS and training equipment is rated by a participant with 'agree' or 'strongly agree' in 60 % of all participants

At post- and follow-up assessments (V8, V9 the following secondary outcome measures will be analyzed:

Training task

- working memory performance in the trained task (operationalized by number of correctly recalled lists at each session)

Transfer task

- Performance on numeric n-back task (% correct, d-prime)

4.4 Safety Outcomes

Safety parameters are assessed via self-report questionnaire every third day of training (V4, V7). The questionnaire was adapted from Antal et al. (2017) and includes intensity ratings with regard to itching, pain, burning, warmth/heat, metallic/iron taste, fatigue/decreased alertness and other sensations.

5 Handling of missing values

Primary outcome of this trial is the feasibility of the intervention. For the primary outcome no missing value imputation will be done, since missing information is related to non-adherence of the participants. Secondary efficacy outcome analyses will be done using complete data without any imputation, due to the small sample size and the exploratory framework of the study.

6 Statistical analyses

For all analyses (including the analysis of the primary outcome) appropriate descriptive statistics (absolute score, mean, standard deviation, median, interquartile range, absolute and relative frequencies) depending on the scale and distribution of the outcome variable will be presented.

Statistical analyses will be divided to analyze

1. feasibility
2. training effects on letter updating task (at post and 1 month follow-up)
3. transfer effects on n-back task (at post and 1 month follow-up)

Training and transfer measures will be corrected for performance at pre-assessment.

6.1 Primary analysis

Feasibility data (primary outcome) will be analyzed using descriptive statistics. Feasibility will be inferred when 60 % of the participants (n =18) complete at least two-thirds (n=4) of the home-based sessions successfully. The point estimate of the primary feasibility criterion and the corresponding 95% CI will be calculated and presented. The 95%CI will be calculated using the formula of Agresti & Coull (Agresti A., Coull B.A. Approximate is better than 'exact' for interval estimation of binomial proportions. Am. Stat. 1998;52:119–126. doi: 10.2307/2685469).

6.2 Secondary analyses

Secondary feasibility outcome, as measured by questionnaire (percentage of participants with high satisfaction tDCS and training equipment), will be analyzed similarly to the primary outcome. The point estimate and the 95%CI of the feasibility criterion (according to the Agresti & Coull formula) will be presented.

Data distributions of the questionnaire items will be visually assessed for normality using q–q plots.

Data on behavioral tasks from all participants (included at randomization and completed post-assessment) will be analyzed using linear mixed models. (See details below)

Additionally, a subgroup analysis will include only those participants who successfully completed two-thirds of the home-based sessions (thus fulfilling the criterion for feasibility; per protocol analyses).

<u>Training task effects</u>

Using linear mixed models (random intercept models accounting for clustering of measures within individuals), the performance of the letter updating task over the study period including all measures after start of intervention V2-V9 will be used as dependent variable, stimulation group (tDCS, sham) as factor, and letter updating performance at pre-assessment as well as age and sex as covariates. The time point of measure will also be included as variable and will be modelled as categorical variable or as continuous variable that reflects the changes over time. Additionally, an interaction term for group X time point will be included to model differential changes over time in both groups. The letter updating task score at post assessment will be evaluated between treatment groups based on this regression model via marginal means to examine immediate training effects. The letter updating task

score at fu assessment will be evaluated between treatment groups based on this regression model via marginal means to examine sustained training effects.

Transfer task effects

Effects on the n-back task will be evaluated using similar linear mixed models (random intercept models accounting for clustering of measures within individuals). The performance of the n-back task will include measures after start of intervention V8, V9 (post and fu assessment), will be used as dependent variable, stimulation group (tDCS, sham) as factor, and letter updating performance and n-back-performance at pre-assessment as well as age and sex as covariates. Additionally, the time point of the measure and the interaction between group and time point will be included as fixed effects. The n-back score at post assessment will be evaluated between treatment groups based on this regression model via marginal means to examine immediate transfer effects. The n-back score at fu assessment will be evaluated between treatment groups based on this regression model via marginal means to examine sustained transfer effects. We will assess percent correct and d-prime in two different linear mixed models.

Type of link function (logistic, linear, ordinal) will depend on the scaling of the dependent variable. In case of skewed continuous data, variables will be transformed before analysis.

All secondary analyses will be done using the full analysis. All secondary analyses will be done in an exploratory framework.

Effect estimates and corresponding 95%CI will be reported for each outcome for time points post and follow-up (V8, V9).

6.3 Safety/Tolerability

Safety outcomes will be reported separately as incidences (n, incidence rate with 95%CI) in total and by intervention group based on the safety analysis set. Incidence rates and 95%CI will be based on poisson regression models that account for the different observation periods for each participant. Group comparisons will be done using incidence rate ratios and 95%CI. Results of safety analysis will be interpreted and discussed thoroughly also for minor group differences, since statistically significance is not of importance here.

6.4 Planned subgroup analyses

We will conduct subgroup analyses regarding participant's cognitive reserve, operationalized by their education years and their commitment to the intervention, operationalized by the number of successful conducted training sessions. Subgroups will be achieved through median splits.

All subgroup analyses will be done within an exploratory framework, using descriptive analyses.

6.5 Example table for the description of baseline characteristics

Table 2. Baseline characteristics of the study sample.

	All n =	TDCS group n =	Sham group n =
Age (years)			
Gender (n, % female)			
Education (years)			
GDS			
Semantic fluency			
BNT (max. 15)			
MMSE (max. 30)			
Word list learning			
Total (max. 30)			
Trial 1 (max. 10)			
Trial 2 (max. 10)			
Trial 3 (max. 10)			
Word list retrieval (max. 10)			
Word list intrusions			
Figure copying (max. 11)			
Figure retrieval (max. 11)			
Phonematic fluency			
Trail-making test			
Part A (sec)			
Part B (sec)			
Digit-span			
Forward			
Backward			

Data are shown as the mean (SD) or n(%). GDS, Geriatric Depression Scale. BNT, Boston Naming Test. MMSE, Mini Mental Status Examination. RT, reaction time.

7. References

Antal A et al. (2017) Low intensity transcranial electric stimulation: Safety, ethical, legal regulatory and application guidelines. Clin Neurophysiol 128:1774-1809.

Antonenko D, Kulzow N, Sousa A, Prehn K, Grittner U, Floel A (2018) Neuronal and behavioral effects of multi-day brain stimulation and memory training. Neurobiol Aging 61:245-254.

Antonenko D, Thams F, Grittner U, Uhrich J, Glockner F, Li SC, Flöel A (2022) Randomized trial of cognitive training and brain stimulation in non-demented older adults. Alzheimers Dement (N Y) 8:e12262.

Berryhill ME (2017) Longitudinal tDCS: Consistency across Working Memory Training Studies. AIMS Neuroscience 4:71-86.

Brink TL, Yesavage J, Lum O (2013) Geriatric depression scale. Evidence-Based Diagnosis: A Handbook of Clinical Prediction Rules:297.

Dahlin E, Neely AS, Larsson A, Bäckman L, Nyberg L (2008) Transfer of learning after updating training mediated by the striatum. Science 320:1510-1512.

Elmasry J, Loo C, Martin D (2015) A systematic review of transcranial electrical stimulation combined with cognitive training. Restor Neurol Neurosci 33:263-278.

Gamble C, Krishan A, Stocken D, Lewis S, Juszczak E, Doré C, Williamson PR, Altman DG, Montgomery A, Lim P (2017) Guidelines for the content of statistical analysis plans in clinical trials. Jama 318:2337-2343.

Hagen NA, Biondo PD, Brasher PMA, Stiles CR (2011) Formal Feasibility Studies in Palliative Care: Why They Are Important and How to Conduct Them. Journal of Pain and Symptom Management 42:278-289.

Jones KT, Stephens JA, Alam M, Bikson M, Berryhill ME (2015) Longitudinal Neurostimulation in Older Adults Improves Working Memory. PLOS ONE 10:e0121904.

Lezak MD, Howieson DB, Loring DW, Fischer JS (2004) Neuropsychological assessment: Oxford University Press, USA.

Lezak MD, Howieson DB, Bigler ED, Tranel D (2012) Neuropsychological Assessment, 5th edition Edition. Oxford ; New York: Oxford University Press.

Morris JC, Heyman A, Mohs RC, Hughes JP, van Belle G, Fillenbaum G, Mellits ED, Clark C (1989) The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part I. Clinical and neuropsychological assessment of Alzheimer's disease. Neurology 39:1159-1165.

Oldfield RC (1971) The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 9:97-113.

Passow S, Thurm F, Li S-C (2017) Activating Developmental Reserve Capacity Via Cognitive Training or Non-invasive Brain Stimulation: Potentials for Promoting Fronto-Parietal and Hippocampal-Striatal Network Functions in Old Age. Frontiers in Aging Neuroscience 9:33.

Perceval G, Floel A, Meinzer M (2016) Can transcranial direct current stimulation counteract age-associated functional impairment? Neurosci Biobehav Rev 65:157-172.

Rahbek MA, Mikkelsen EE, Overgaard K, Vinge L, Andersen H, Dalgas U (2017) Exercise in myasthenia gravis: A feasibility study of aerobic and resistance training. Muscle & Nerve 56:700-709.

Thams F, Rocke M, Malinowski R, Nowak R, Grittner U, Antonenko D, Floel A (2022) Feasibility of Cognitive Training in Combination With Transcranial Direct Current Stimulation in a Home-Based Context (TrainStim-Home): study protocol for a randomised controlled trial. BMJ Open 12:e059943.

Watson D, Clark LA, Tellegen A (1988) Development and validation of brief measures of positive and negative affect: the PANAS scales. Journal of personality and social psychology 54:1063.