STATISTICAL ANALYSIS PLAN

MENTAL TRAINING TECH 24.5

Version 3.0
20/03/2024

Protocol Title: Study of the Effectiveness of Cognitive Stimulation and Neuroplasticity with Mental Training Tech 24.5 (MTT245Cog)
Protocol Version: 17.04.23 (revision 15.01.24)
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SAP Signatures

I give my approval for the SAP entitled “STATISTICAL ANALYSIS PLAN - MENTAL TRAINING TECH 24.5”, version 3.0, dated 20/03/2024.

Principal Investigator

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1. Administrative information

1.1. Document History

Version	Date of Issue	Summary of Change
1.0	08/03/2024	Initial version
2.0	18/03/2024	• 3.3 Primary objective #1: The 6 score dimensions have been specified. • 3.3 Secondary objective: Since information regarding tolerance and adherence is available, assessment of this objective will be conducted. • 3.5 The definition of the MTT24.5 program has been updated. • 3.7 Power calculation has been incorporated. • 3.8 f-BMR and BMR procedure has been described. • 4.2 Outcomes related to adherence have been defined. • 5.3.4 Statistical method for adherence outcome analysis has been outlined.
3.0	20/03/2024	• 5.3.1 and 5.3.2 Standarized effect size calculation has been upddated

1.2. List of Abbreviations and Definitions of Terms

ACE	Addenbrooke's Cognitive Examination
BMRI	Brain magnetic resonance imaging study
CRS	Cognitive Reserve Score
IQR	Inter quartile range
MTT24.5	Mental Training Tech 24.5
SCD	Subjective cognitive decline
SD	Standard deviation
TECH	High-impact cognitive stimulation modalities

2. Introduction

The purpose of this document is to describe the planned analysis and reporting for the neuroplasticity applied program “MENTAL TRAINING TECH 24.5”

This statistical analysis plan is based on the latest version of the protocol dated April 17, 2023 revised on January 15, 2024.

3. Protocol summary

3.1 Background

The human brain has the ability to change and adapt throughout life, challenging the previous belief that the process of brain aging was irreversible. This paradigm shift, driven by scientific discoveries such as those of Eric Kandel, highlights the importance of neuroplasticity, the brain's ability to reorganize, in preserving and recovering cognitive functions. Therefore, it is necessary to incorporate brain training programs into culture and medicine to harness this adaptive capacity of the brain and improve quality of life at all stages.

Based on this knowledge situation, the applied neuroplasticity program "Mental Training Tech 24.5" (MTT24.5) has been developed as a method for inducing brain plasticity to enhance, protect, and preserve cognitive abilities, fostering from a physiological model of brain connection growth and strengthening

3.2 Study hypothesis

- *Hypothesis 1*

  The MTT24.5 cognitive training program is associated with cognitive brain functional changes, as it may increase performance in memory and/or attention and/or verbal fluency and/or language and/or visuospatial skills in adults without clinical cognitive impairment or with subjective cognitive decline (SCD).

- *Hypothesis 2*

  The MTT24.5 cognitive training program is associated with changes in brain neuroplasticity, as it may increase or modify the volume of specific brain areas and/or their level of activation.

- *Hypothesis 3*

  The improvement in performance of the cognitive abilities listed in Hypothesis 1 is associated with changes in brain neuroplasticity.

- *Hypothesis 4*

  Different baseline conditions may modulate responses to the cognitive program, including: a) baseline cognitive reserve, b) history of dementia, c) non-communicable chronic diseases, d) lifestyle habits, and e) medication use.

3.3 Study objectives

- ***Primary objectives***

1. Evaluate the effects of MTT24.5 on cognitive abilities such as memory, attention, orientation, verbal fluency, language and visuospatial skills
2. Assess the effects of MTT24.5 on brain structural characteristics, considering brain volumes in globally standardized areas, as well as those associated with cognitive abilities.
3. Examine the effects of MTT24.5 on brain activation, defined as patterns of metabolism distribution and neuronal perfusion overall, and according to cognitive brain areas with taxonomic mapping.
4. Determine if there are response phenotypes based on the distribution of individual baseline characteristics, such as age, gender, lifestyle factors, medical history, medications, family history of dementia, diet, and baseline cognitive reserve score.

- ***Secondary objective***

Analyze the tolerance and adherence to MTT24.5, and its association with outcomes regarding cognitive variables, as well as structural and/or functional brain changes.

3.4 Study design

It is a prospective, non-randomized controlled clinical study that evaluates the effectiveness and tolerance of a cognitive intervention.

3.5 Interventions description

- ***Control Group***

Participants in this group will not receive any specific cognitive training program during the study period. They will continue with their usual activities and routines.

- ***Intervention Group:***

Participants in this group will receive the cognitive training program MTT24.5.

The MTT24.5, developed as a binomial DATA/TECH, provides the brain with 35 new knowledge (DATA) classified in formal, natural, social, and cultural areas. The TECH consists of 100 high-impact cognitive stimulation modalities designed to enhance memory, attention, verbal fluency, and visuospatial skills. During the program, the brain receives 35 new pieces of knowledge, and the 100 TECHS created to promote changes in brain functionality, improve synaptic efficiency, and achieve permanent plastic changes. The program's duration is approximately 24.5 hours spread across 12 to 16 weeks including in-person training classes of 1.5 hours each week. The

training is complemented with weekly online activities, which entail an additional average dedication of 4 hours.

3.6 Population

- ***Inclusion criteria***
  - Adults aged 21 years and above.
  - Ability to understand the instructions for the training tasks.
- ***Exclusion criteria:***
  - Planned absence that would hinder participation in the program.
  - Hearing, visual, or motor deficits that would impede participation in the program.
  - History of severe degenerative neurological diseases as these conditions may significantly impact brain plasticity and cognitive abilities.
  - History of severe psychiatric disorders as they may influence brain reorganization and complicate interpretation of the results.
  - Unstable treatment or planned changes in medication that may affect brain function and alter results.
  - History of current or recent excessive substance use (within the past 6 months) including alcohol or drugs as these substances may influence brain function and neuroplasticity.

3.7 Sample size

The sample size of 76 subjects (56 cases and 20 controls) will provide a power of at least 80% to detect a minimum difference of 4 points in the score improvement between the two groups, assuming a maximum standard deviation of 5.4 points. This minimum difference of 4 points corresponds to a minimum effect size of 0.8.

3.8 Data collection

Information will be collected on study specific forms/procedure.

- ***Cognitive Reserve Score (CRS):*** It consists of a validated cognitive reserve scale used with authorization from Roldan L. et al. The CRS records the frequency of cognitively stimulating activities carried out throughout life. A total of 24 items are distributed across four aspects: activities of daily living, education/information, hobbies, and social life. The CRS will be obtained for each subject at baseline (pre intervention).

- ***Medical and Lifestyle History Form***. Self-administered medical and lifestyle history form called STEPS 5, adapted from www.who.int/chp/steps, and previously used in the Latin American OPTIMO study. This form will be administrated for each subject at baseline (pre intervention).

- ***The Addenbrooke's Cognitive Examination-III.*** Brief cognitive test that evaluates five cognitive abilities: attention, memory, verbal fluency, language, and visuospatial skills. The ACE score will be obtained for each subject at baseline (pre intervention) and post intervention.

- ***Functional brain magnetic resonance imaging study (f-BMR) and Brain magnetic resonance (BMR).*** The study consisted of the acquisition of two magnetic resonance sequences in a Philips Ingenia 3T resonator as follows:

  a) High resolution 3D T1w sequence, used for measuring volumes of gray and white matter.

  b) EPI sequence: used for functional MRI.

  For functional magnetic resonance imaging, a block paradigm was used, consisting of 1 minute of rest followed by 1 minute of stimulation. The block was repeated 5 times for a total acquisition time of 10 minutes. During the stimulus, the subjects visualized a screen a word representing a color, but painted in a different color, so the same way as in the "interference" section of the Stroop test; The task was to think, silently, the written word, trying to ignore the color information. The task was explained and briefly practiced with the subjects before beginning the acquisition of images to reduced associated stress reaction.

  Prosecution:

  The images obtained were exported in DICOM format and converted to NIFTI format through the dcm2niix tool. T1w images were processed with the tool vol2Brain (https://www.volbrain.net/), which segments the brain into multiple structures, cortical and subcortical structures automatically, and reports the volumes of each structure. Functional magnetic resonance images were processed with SPM12 software to obtain t-statistic maps of the whole brain, registered to the T1 image. Flirt and fnirt tools were used, included in the FSL software package (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki), to register the T1 image to MNI space; the transformation obtained was then applied to the t map to bring it to the MNI space.

  Once the t map was obtained in the MNI space, it was superimposed with the AAL atlas (Automated Anatomical Atlas, (Neuroimage, 15 (2002), pp. 273-289), included in MRIcron software(https://www.nitrc.org/projects/mricron). For each plot of the atlas, the value was calculated average of the parameter t for those voxels with a value of t greater than 3. To those regions without voxels greater than 3 were assigned a value of 0.

  The results were registered as:

a) “Volumes.zip file” which contains a .csv file and a .pdf file for each patient. The .pdf contains a report with the volumes of different structures of the brain, both in absolute terms (cm3) and relative (% of the total), and values reference of normal populations corrected for age and sex. The .csv contains the same values, organized by structure, in tabular format.

b) Activations.csv file: which contains the average value of the thresholded t-statistic for each region of the AAL atlas and for each patient, according to what is explained in the previous section.

In addition, images of all patients are available, both the data raw in DICOM format like NIFTI, as well as t maps.

This study will be conducted on a subset of the subjects at baseline (pre intervention) and post intervention.

3.9 Study outcomes

- ***Primary outcome***
  - Cognitive ability improvement
- ***Secondary outcomes – related to cognitive abilities***
  - Memory ability improvement
  - Attention ability improvement
  - Orientation ability improvement
  - Verbal fluency ability improvement
  - Language ability improvement
  - Visuospatial skills ability improvement
- ***Secondary outcomes – related to brain volume outcomes:***
  - Anterior cingulate gyrus right thickness improvement
  - Anterior cingulate gyrus left thickness improvement
  - Anterior cingulate gyrus right volume improvement
  - Anterior cingulate gyrus left volume improvement
  - Cerebrum grey matter total volume improvement
  - Cortical Grey Matter volume (%) improvement
  - Cortical Grey Matter volume (cm3) improvement

- Middle frontal gyrus right thickness improvement
- Middle frontal gyrus left thickness improvement
- Middle frontal gyrus right volume improvement
- Middle frontal gyrus left volume improvement

- ***Secondary outcomes – related to brain activation:***
  - Left anterior cingulate improvement
  - Right anterior cingulate improvement
  - Left medial cingulate improvement
  - Right medial cingulate improvement
  - Left posterior cingulate improvement
  - Right posterior cingulate improvement

4. Outcomes definitions

4.1 Primary outcome

The primary study outcome, cognitive ability improvement, will be assessed through the difference between the post intervention ACE score and the pre intervention ACE score. This score ranges from 0 to 100.

4.2 Secondary outcomes

- ***Outcomes related to cognitive abilities***

  The ACE score comprises six dimensions, each evaluating a different domain: Memory, attention, orientation, verbal fluency, language, and visuospatial skills abilities.

  Each ability outcome improvement will be assessed using the respective sub-dimensions of the ACE score and computing the difference between post intervention and pre intervention sub-score:

  - Memory ability sub-score improvement
  - Attention ability sub-score improvement
  - Orientation ability sub-score improvement
  - Verbal fluency ability sub-score improvement
  - Language ability sub-score improvement
  - Visuospatial skills ability sub-score improvement

  The memory ability sub-score ranges from 0 to 26, the attention abilities sub-score ranges from 0 to 8, the attention abilities sub-score ranges from 0 to 10, the verbal fluency sub-score ranges from 0 to 14, the language sub-score ranges from 0 to 16, and the visuospatial skills abilities sub-score ranges from 0 to 26.

- ***Outcomes related to brain volume and brain activation:***

  Brain Volume and brain activation improvement outcomes are obtained from the Brain magnetic resonance imaging study (BMRI) and computing the difference between post intervention and pre intervention values. These outcomes are measured for a subset of the intervention group at baseline and post-intervention. The selected subset of measure that are considered in this study are listed in point 3.9

- ***Outcomes related to adherence:***

  Compliance with the study protocol is assessed via two measures:

(1) the number of days that participant completed (“on site days”)

(2) the number of home training complementary exercises that participants completed. As participants were instructed and reminded to complete weekly sessions, the number of active days was used as the primary measure of a participant’s ongoing engagement and compliance with the study protocol.

5. Statistical analyses

5.1 Subject Disposition

A CONSORT flow diagram will be used to summarize the number of subjects who were:

- Enrolled
- Enrolled to each group
- Discontinued in each group
- Included in the Cognitive analysis in each group
- included in the Brain volume and activation analysis

5.2 Distribution of socio demographic and clinical characteristics

All continuous variables will be summarized using the following descriptive statistics: mean, standard deviation (SD) and range if data presented a normal distribution and median, inter quartile range (IQR) and range if data skewed. The frequency and percentages (based on the non-missing sample size) of observed levels will be reported for all categorical measures.

5.3 Statistical analysis methods

5.3.1 Objective 1

The mean baseline ACE score and standard deviation (SD) will be reported for both the intervention and control groups. Additionally, the improvement from baseline to the end of the program will also be presented for each group.

To assess the impact of MTT24.5 on cognitive abilities, a linear model will be utilized for each cognitive domain (global, memory, attention and orientation, verbal fluency, language, and visuospatial skills). Each model will use the improvement score value as the outcome variable and the group (intervention and control) as the independent variable. The group term will evaluate the non-standardized intervention effect, indicating whether the mean improvement in scores (change from baseline) differs between the control and intervention groups. The effect size is defined as the difference between the mean improvement on the intervention group and the mean improvement on the control group.

To compute standardized effect sizes, Cohen's d Statistic will be calculated. Standardized metrics facilitate comparisons across different scores.

The fulfillment of model assumptions will be assessed, and if they are not met, proposals for improvement will be suggested.

5.3.2 Objective 2 and 3

For these objectives the subset of subjects within the intervention group who underwent BMRI will be considered. No control group is available for this assessment.

To evaluate the impact of MTT24.5 on brain volume and brain activation, a t-test will be conducted for each brain improvement outcome to determine whether the mean improvement differs from zero.

For each brain outcome, the non-standardized effect size will be reported as the mean improvement, while the standardized effect size will be calculated using the Cohen's d Statistic for repeated measures.

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5.3.3 Objective 4

To identify potential response phenotypes based on individual baseline characteristics (including age, gender, medical history, medications, family history of dementia, and baseline cognitive reserve score), a linear model will be employed for each covariate of interest. The primary outcome will be modelled with the covariate of interest, the group variable, and their interaction. This interaction term will assess whether the intervention effect varies across covariate groups.

To summarize the results, a forest plot displaying the standardized effect size for each covariate group will be generated.

Covariate / Subgroups of interest:

- Sex (female, male)
- Age (<=65 years, >65 years)
- Years of Education (<=12 years, >12 years)
- Cognitive reserve (low, high)
- ACE score at baseline (<85, >85)
- Diabetes history (yes, no)
- Hypertension history (yes, no)
- Dyslipidemia history (yes, no)
- Dementia family history (yes, no)
- Statins /AAS use (yes, no)

5.3.4 Secondary objective

In order to describe the adherence to MTT24.5 the following metrics will be summarize:

- Mean number of days that participant completed on site program
- Mean number of training complementary exercises that participant completed at home.
- Percentage of subjects who completed the program

5.4 Handling of Missing, Unused, and Spurious Data

Variables with more than 20% of missing data will not be included in the analysis. For variables that have less than 20% of missing values, firstly it will be investigated and tried to complete the missing data. Available data will be included in the data listings and tabulations showing for each report the actual total. No imputation techniques will be used for missing data.

5.5 Confidence intervals and p-values

All applicable statistical tests will be 2-sided and will be performed using a 5% significance level. All confidence intervals presented will be 95% and two-sided.

5.6 Statistical software employed

The statistical software R version 4.3.0 will be used for the analyses.

5.7 Reporting conventions

P-values ≥0.001 will be reported to 3 decimal places; p-values less than 0.001 will be reported as "<0.001". The mean, standard deviation, and any other statistics other than quantiles, will be reported to one decimal place greater than the original data. Quantiles, such as median, or minimum and maximum will use the same number of decimal places as the original data. Estimated parameters, not on the same scale as raw observations will be reported to 3 significant figures.

6. Dummy tables

Figure 1: Consort

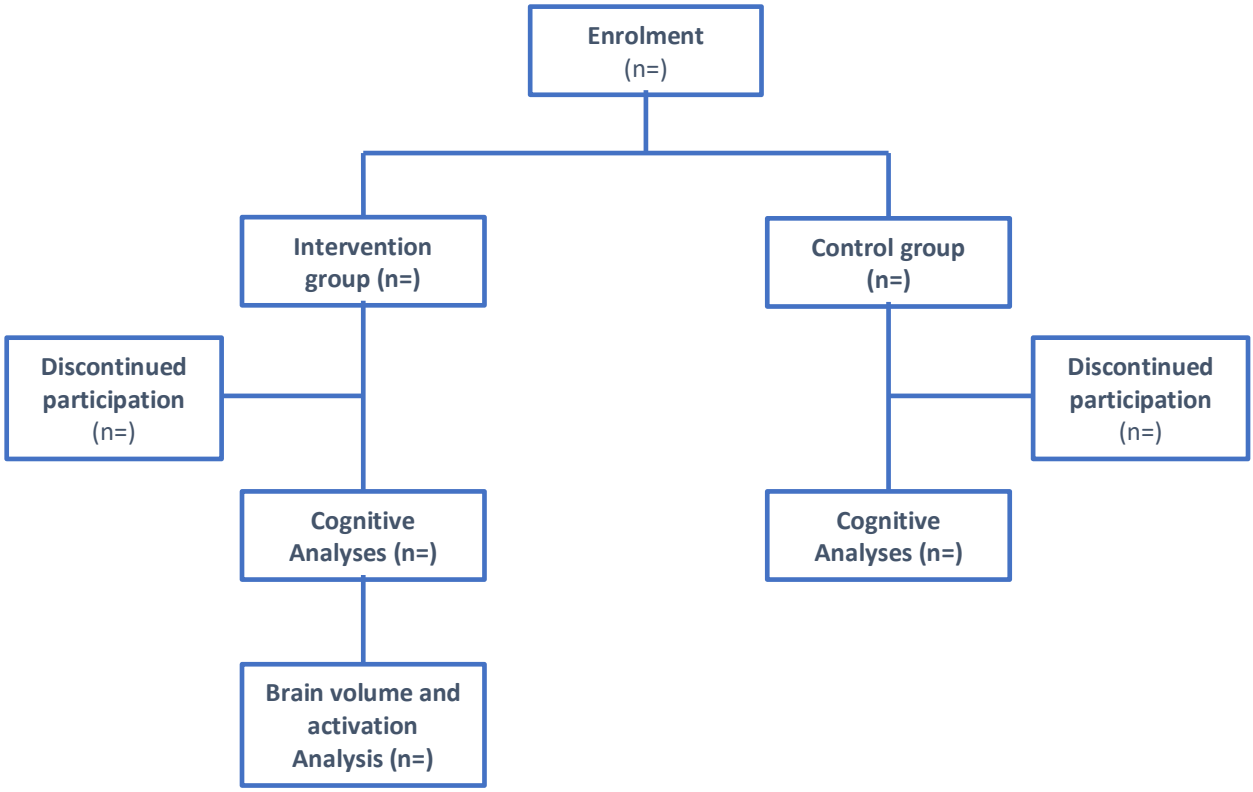

Table 1. Socio-demographic characteristics by group

	Control group	Intervention Group	Intervention Group BMRI subset
	n/N (%)	n/N (%)	n/N (%)
Socio-demographic characteristics			
Sex			
Male			
Female			
Age*			
Age categorized			
<=65 years			
>65 years			
Years of education*			
Mediterranean diet	-		
Fruit consumption			
3 or more per day			
Less than 3 per day			
Vegetal consumption			
2 or more per day			
Less than 2 per day			
Nuts/seeds consumption			
3 or more per day			
Less than 3 per day			
Alcohol consumption			
Physical activity	-		
Clinical characteristics			
Cognitive reserve			
Low			
High			
Diabetes history			
Hypertension history			
Dyslipidemia history			
Myocardial infarction			
Previous cerebrovascular accident			
Sleep apnea syndrome			
Epilepsy			
Parkinson's disease			
Dementia family history			

*Mean and (SD) will be reported

** At least

Table 2. Effect of MTT 24.5 program on cognitive abilities outcomes

	Control group (N=)	Intervention Group (N=)	P-value
Primary Outcome			
Global cognitive ability			
Score at baseline, mean (±SD)			
Improvement from baseline, mean (±SD)			
Non-standardized effect size (95% CI)*			
Standardized effect size (95% CI)**			
Secondary Outcomes			
Attention cognitive ability			
Score at baseline, mean (±SD)			
Improvement from baseline, mean (±SD)			
Non-standardized effect size (95% CI)*			
Standardized effect size (95% CI)**			
Orientation cognitive ability			
Score at baseline, mean (±SD)			
Improvement from baseline, mean (±SD)			
Non-standardized effect size (95% CI)*			
Standardized effect size (95% CI)**			
Memory cognitive ability			
Score at baseline, mean (±SD)			
Improvement from baseline, mean (±SD)			
Non-standardized effect size (95% CI)*			
Standardized effect size (95% CI)**			
Verbal fluency cognitive ability			
Score at baseline, mean (±SD)			
Improvement from baseline, mean (±SD)			
Non-standardized effect size (95% CI)*			
Standardized effect size (95% CI)**			
Language cognitive ability			
Score at baseline, mean (±SD)			
Improvement from baseline, mean (±SD)			
Non-standardized effect size (95% CI)*			
Standardized effect size (95% CI)**			
Visuospatial skills cognitive ability			
Score at baseline, mean (±SD)			
Improvement from baseline, mean (±SD)			
Non-standardized effect size (95% CI)*			
Standardized effect size (95% CI)**			

Abbreviations: CI, confidence interval; SD, standard deviation

**Non-standardized effect size defined as the difference between the mean improvement on the intervention group and the mean improvement on the control group

**The standardized effect size defined as the difference between the mean improvement on the intervention group and the mean improvement on the control group, divided by the pooled standard error (Cohen's d Statistic)

Table 3. Effect of MTT 24.5 program on brain volume outcomes

	Intervention Group (N=)	P-value
Anterior cingulate gyrus right thickness		
Baseline value, mean (±SD)		
Non-standardized effect size (95% CI)*		
Standardized effect size (95% CI)**		
Anterior cingulate gyrus left thickness		
Baseline value, mean (±SD)		
Non-standardized effect size (95% CI)*		
Standardized effect size (95% CI)**		
Anterior cingulate gyrus right volume		
Baseline value, mean (±SD)		
Non-standardized effect size (95% CI)*		
Standardized effect size (95% CI)**		
Anterior cingulate gyrus left volume		
Baseline value, mean (±SD)		
Non-standardized effect size (95% CI)*		
Standardized effect size (95% CI)**		
Cerebrum grey matter total volume		
Baseline value, mean (±SD)		
Non-standardized effect size (95% CI)*		
Standardized effect size (95% CI)**		
Cortical Grey Matter volume (%)		
Baseline value, mean (±SD)		
Non-standardized effect size (95% CI)*		
Standardized effect size (95% CI)**		
Cortical Grey Matter volume (cm3)		
Baseline value, mean (±SD)		
Non-standardized effect size (95% CI)*		
Standardized effect size (95% CI)**		
Middle frontal gyrus right thickness		
Baseline value, mean (±SD)		
Non-standardized effect size (95% CI)*		
Standardized effect size (95% CI)**		
Middle frontal gyrus left thickness		
Baseline value, mean (±SD)		
Non-standardized effect size (95% CI)*		
Standardized effect size (95% CI)**		
Middle frontal gyrus right volume		
Baseline value, mean (±SD)		
Non-standardized effect size (95% CI)*		
Standardized effect size (95% CI)**		
Middle frontal gyrus left volume		
Baseline value, mean (±SD)		
Non-standardized effect size (95% CI)*		
Standardized effect size (95% CI)**		

Abbreviations: CI, confidence interval; SD, standard deviation

**Non-standardized effect size defined as the mean improvement on the intervention group

**The standardized effect size defined as the mean improvement on the intervention group divided by the standard error (Cohen's d Statistic)

Table 4. Effect of MTT 24.5 program on brain activation outcomes

	Intervention Group (N=)	P-value
Left anterior cingulate		
Baseline value, mean (±SD)		
Non-standardized effect size (95% CI)*		
Standardized effect size (95% CI)**		
Right anterior cingulate		
Baseline value, mean (±SD)		
Non-standardized effect size (95% CI)*		
Standardized effect size (95% CI)**		
Left medial cingulate		
Baseline value, mean (±SD)		
Non-standardized effect size (95% CI)*		
Standardized effect size (95% CI)**		
Right medial cingulate		
Baseline value, mean (±SD)		
Non-standardized effect size (95% CI)*		
Standardized effect size (95% CI)**		
Left posterior cingulate		
Baseline value, mean (±SD)		
Non-standardized effect size (95% CI)*		
Standardized effect size (95% CI)**		
Right posterior cingulate		
Baseline value, mean (±SD)		
Non-standardized effect size (95% CI)*		
Standardized effect size (95% CI)**		

Abbreviations: CI, confidence interval; SD, standard deviation

**Non-standardized effect size defined as the mean improvement on the intervention group

**The standardized effect size defined as the mean improvement on the intervention group divided by the standard error (Cohen's d Statistic)

Figure 2. Effect of MTT 24.5 program on cognitive abilities by covariates groups

Forest plot

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