Association of changes in antisaccade performance and multimodal measures of fear in spider phobic patients after an antisaccade training.

Statistical Analysis Plan (SAP)

Anne S. Hildebrand[a], Fabian Breuer[b], Julia Vogelsang[c], Johannes B. Finke[a], Leandra Bucher[a], Tim Klucken[a], Elisabeth J. Leehr[b], Kati Roesmann [c]

[a] Department of Psychology, Clinical Psychology and Psychotherapy of the University of Siegen, 57072, Siegen, Germany

[b] Institute for Translational Psychiatry, University of Münster, 48149, Münster, Germany.

[c] Institute of Psychology, Clinical Psychology and Psychotherapy in Childhood and Adolescence, University of Osnabrück

[d] Corresponding Author

SAP responsibilities

Role in SAP development	**Name**
SAP author	Anne S. Hildebrand
SAP reviewer	Fabian Breuer
SAP author	Julia Vogelsang
SAP reviewer	Johannes B. Finke
SAP reviewer	Leandra Bucher
SAP reviewer	Tim Klucken
SAP author	Elisabeth J. Leehr
SAP author	Kati Roesmann

1. Introduction

1.1 Background

According to the Attentional Control Theory, a fear-evoking stimulus causes an attentional shift towards the fear-evoking stimulus. Thus, the fear-evoking stimulus may tip the balance in favor of stimulus-driven bottom-up processing rather than cognitive top-down processing, which is evolutionarily functional regarding survival (Eysenck et al., 2007). *Inhibitory control*, the cognitive ability to regulate attention and suppress irrelevant or unwanted responses, plays a crucial role in managing this attentional shift (Diamond, 2013). Deficits in inhibitory control can exacerbate the difficulty in diverting attention away from fear-evoking stimuli, thereby perpetuating anxiety symptoms (Derakshan et al., 2009). To investigate inhibitory control, previous research has used the antisaccade paradigm. This paradigm examines the ability to suppress reflexive eye movements (saccades) towards a sudden visual stimulus in the visual periphery and instead make a voluntary eye movement in the opposite direction (antisaccades) (Hutton & Ettinger, 2006). Though inhibitory control is considered a relevant factor in anxiety (Ansari & Derakshan, 2010; Shi et al., 2019), the potential of antisaccade training as an intervention to increase inhibitory control for anxiety disorders has not yet been explored. Further, it is unclear, whether potential effects of an antisaccade training employing fear-evoking stimulus material are specific to changes in antisaccade performance in response to disorder-related stimuli or influence antisaccade performance irrespectively of employed stimuli (Giel et al., 2017). Lastly, the time course of potential training effects, as well as putative effects on psychophysiological fear responses and avoidance behavior are largely unknown.

The present SAP refers to the third part of a multi-parted clinical study addressing these research gaps (Study ID: ISRCTN12918583; Registered on 28th February 2022; please also consult the study protocol which highlights the rationale for the three aims addressed (one aim for each part; Hildebrand et al., 2023). In part 1 and 2, we have investigated the baseline antisaccade performance in patients with spider phobia compared to healthy controls (Aim 1, see SAP on planned analyses of data obtained during the baseline assessment) as well as the effects of antisaccade training on inhibitory control in patients with spider phobia (Aim 2: see SAP on planned analyses of data obtained during the intervention). In the third part, we will investigate factors potentially influencing antisaccade performance and their associations with multimodal measures of fear, as well as the effect of time between the antisaccade training and the antisaccade-task on antisaccade performance. Additionally, associations of putative

changes in AS performance and changes in psychophysiological fear responses and avoidance behavior are largely unknown. These aspects are addressed in this SAP on Aim 3, which - in contrast to Aim 1 and 2 - focuses on the patients with spider phobia only. Participants in this longitudinal study design might vary in regards to training effects, reflecting interindividual differences. This would suggest a hierarchically structured data with time (i.e. different time points of assessments, level-1-predictor) nested in participants (level-2-predictor).

1.2 Objectives

,The primary objective of Aim 3 is 1.) to explore factors potentially influencing antisaccade performance and their associations with multimodal measures of fear. In the group of patients with spider phobia only, we here test in a cross-over-design manipulating training order (antisaccade training first vs. prosaccade training first), whether training order - and thereby time between the antisaccade training and the antisaccade-task - would affect antisaccade performance in the antisaccade task and avoidance behavior in the BAT, while also examining if these time effects are specific to the type of stimulus used.

2.) We further aim to conduct exploratory analyses on patients with spider phobia to understand potential associations between antisaccade performance and multimodal behavioral and psychophysiological parameters reflecting fear, which were assessed at different time points. Specifically, we want to examine how antisaccade efficiency (indexed by latencies, see SAP 1, section 5.1) and antisaccade effectiveness (indexed by error rates, see SAP 1, section 5.2) before versus after an antisaccade training, relate to putative changes in contrast values (phobia-related - neutral) reflecting enhanced psychophysiological responses to phobia-related stimuli in heart rate, startle reflex, skin conductance, self-reported fear (see SAP 1, section 5.3) and avoidance behavior indexed by BAT.

3.) Finally, we will also check for hierarchical data structure, with the assumption that time (level-1-predictor) might be nested in participants (level-2-predictor). Therefore, interindividual differences may contribute to the prediction of training effects on antisaccade performance and avoidance behavior. In case of indication for hierarchical data structure, MLMs will be employed.

2. Methods

2.1 Study Design

In this longitudinal study, patients with spider phobia, all aged between 18 and 65 years, were assessed regarding training-induced changes in inhibitory control functions via an emotional antisaccade task, using phobia-related and neutral stimulus materials (schematic pictures of spiders and flowers). Additionally, psychophysiological measures (heart rate, skin conductance response and startle response) to phobia-related, negative and neutral stimuli and avoidance behavior to a real spider (behavioral avoidance test) were assessed in a passive viewing task and behavioral avoidance test, respectively.

We employed a 2 (training order: antisaccade training first vs. prosaccade training first) × 3 (time: baseline vs. post-1-assessment vs. post-2-assessment) × 2 (stimulus material: phobia-related vs. neutral) design, with training order as a between-subject factor and time and stimulus material as within-subject factors.

Outcome measures (primary: antisaccade latencies, secondary: antisaccade error rates; and avoidance behavior, see 5.3.2) were obtained before (baseline assessment), after a first training period (post-1-assessment), and after a second training period with switched training conditions (post-2-assessment).

2.1.1 Training

Information on training and the control condition can be found in the SAP on intervention data (see SAP 2, section 2.1.1).

2.2 Randomization

Patients were randomly assigned to the two training orders, using a block randomization scheme.

2.3 Sample Size

Based on a study using antisaccade training and the antisaccade task in clinical samples [10], we calculated an a-priori power analysis using G*Power 3.1 [14] for a mixed measures ANOVA (Analysis of Variance) to detect large effect sizes (Cohen´s f = .25, α = .05, power = .8) in our primary outcome (i.e. antisaccade latencies) for the training effect. Results indicated a required total sample size of 22 participants (12 per group). Please note, that sample size calculation was based on an ANOVA and is very small for the application of an MLM (Hoyle & Gottfredson, 2015; Snijders, 2005).

2.4 Timing of final analysis

Data will be analyzed after completion of final baseline and intervention analyses (see SAP 1 and SAP 2).

3. Statistical principles

3.1 p-Values and Effect Size

For all analyses, significance levels will be set to $p <= .05$ (Bonferroni corrected). Required assumptions will be tested for all statistical tests. As an effect size for ANOVAs, partial-Eta^2 (η_p^2) will be used. Pearsons (for normally distributed data) or Spearman's (for non-normally distributed data) correlation coefficients will be reported.

In case of a hierarchical data structure, MLMs will be used. Effect sizes for fixed effects will be estimated with semi-partial R^2 (or η^2). Model fit will be estimated using Pseudo-R^2, reflecting the variance explained by the fixed effects, and the variance explained by the entire model, including both fixed and random effects.

3.2 Missing Data and Outliers

Information on missing data and outliers in primary, secondary, and exploratory outcome measures can be found in the SAP on baseline data (see SAP 1, section 3.2). The same criteria were applied to data retrieved at post-1-assessment and post-2-assessment.

For ANOVAs and correlation analyses, patients will be excluded, if less than 50% of all trials at any time point (baseline, post-1-assessment, post-2-assessment) in the antisaccade task are valid. For more details see SAP 1, section 3.2.

For multimodal indexes of fear (see correlation analyses, section 6.2), patients will be excluded for one index, if one of the two relevant time points (baseline, post-2-assessment) is missing. Note, that a participant that is excluded for the correlations regarding one index might not be excluded for a second index, where no data is missing.

As MLMs are very robust towards missing data, sample size for MLM (only in case of hierarchical data structure) will also include participants with partially missing data (e.g. missing data in the antisaccade task at post-1-assessment, but not baseline and post-2-assessment).

4. Trial population

4.1 Eligibility criteria

Patients with spider phobia had to fulfill the criteria of specific phobia according to the Diagnostic and Statistical Manual of Mental Disorders-IV (SCID-I, Section F (Lobbestael et al., 2011)). Note that the study also included healthy control participants (not fulfilling the criteria of any psychiatric disorder and a score < 19 in the Spider Phobia Questionnaire (SPQ, Olatunji et al., 2009), which are not included in the following analyses. For analyses including healthy control participants see SAP 1 and SAP 2.

A full list of eligibility criteria can be found in the study registration (Study ID: ISRCTN12918583).

4.2 Recruitment

Please refer to our study protocol (Hildebrand et al., 2023). A CONSORT-Flow diagram will be presented in the manuscript.

4.3 Sample characteristics

Sociodemographic sample characteristics will be presented for the spider phobic sample .

5. Outcome measures

5.1 Primary outcome measure

The primary outcome will be antisaccade latency, measured in milliseconds (ms). Antisaccade latency is defined as the time between stimulus onset and the initiation of a correct antisaccade. Antisaccade latency reflects inhibitory control efficiency.

5.2 Secondary outcome measure

The secondary outcome will be antisaccade error rate in percent (%). Antisaccade error rate is defined as the proportion of trials, in which an individual performs an erroneous prosaccade towards the presented stimulus. Antisaccade error rate reflects inhibitory control effectiveness.

5.3 Multimodal measures indexing fear

5.3.1 Psychophysiological measures

Multimodal psychophysiological responses towards phobia-related, neutral and negative stimuli were obtained at baseline and post-2-assessment. Contrast values (phobia-related - neutral) are employed as multimodal measures indexing fear. For more detailed information on the preprocessing of psychophysiological measures see SAP 1 section 5.3.1.

5.3.1.1 Heart rate

Heart rate will be defined as beats per minute (bpm) measured via electrocardiography (ECG). The mean heart rate in bpm across the full trial period of 6 seconds will be used as a contrast value (phobia-related - neutral) indexing fear.

5.3.1.2 Skin conductance response

Skin conductance response (SCR) will be defined as the largest increase in conductance, measured via electrodermal activity (EDA), occurring between 1 to 4 seconds after stimulus onset. Again, contrast values (phobia-related - neutral) of the mean magnitude will be calculated.

5.3.1.3 Startle response

Startle response will be defined as the difference between peak and baseline amplitude of the activity of the musculus orbicularis oculi (unilateral), measured via electromyography (EMG) in a 200 ms response window after startle probe onset. Again, contrast values (phobia-related - neutral) of the mean magnitude will be calculated.

5.3.2 Avoidance behavior

Avoidance behavior in response to a real-life spider will be defined as the final distance between the participant and the spider in centimeters (cm) during the BAT (for a detailed description of the BAT please refer to the work of Schwarzmeier and colleagues (Schwarzmeier et al., 2020)), obtained at baseline, post-1-, and post-2-assessment.

5.3.3 Psychometrics

Self-reported spider-phobic symptoms will be defined as a sum value, obtained from the Spider Phobia Questionnaire (SPQ; Olatunji et al., 2009), obtained at baseline and post-2-assessment.

6. Analyses

All analyses will be conducted as indicated and required statistical assumptions will be checked before conducting the respective analyses. In case of (unexpected) baseline differences of the factor training in relevant sample characteristics (see 4.3), we will control for the respective variables in the statistical analyses.

6.1 ANOVA

6.1.1 Analysis of primary outcome

To investigate potential effects of training order on inhibitory control efficiency in spider phobic patients (indexed by antisaccade latencies) a 2x3x2 mixed measures ANOVA, employing training order (antisaccade training first vs. prosaccade training first) as a between-subject factor, and time (baseline vs. post-1-assessment vs. post-2-assessment) and stimulus material (phobia-related vs. neutral) as within-subject factors, will be conducted.

6.1.2 Analysis of secondary outcome

The analyses described in 6.1.1 will be repeated with the secondary outcome (antisaccade error rates) as the dependent variable.

6.1.3 Analysis of avoidance behavior

The analyses described in 6.1.1 will be repeated with the final distance in the BAT as the dependent variable.

6.2 Correlation analysis: associations between changes in antisaccade performance and changes in multimodal measures indexing fear

Bivariate correlations will be calculated to analyze associations between antisaccade performance across time and changes in the multimodal contrast values indexing fear to phobia-related stimuli (phobia-related - neutral). Toward this aim, the following variables will be correlated:

Preregistered outcomes

1. antisaccade latencies (baseline, post-1, post-2)
2. antisaccade error rate (baseline, post-1, post-2)

Change Scores: multimodal measures of fear

3. avoidance behavior (post-2 – baseline)
4. heart rate (post-2 – baseline)
5. startle reflex (post-2 – baseline)
6. skin conductance (post-2 – baseline)
7. self-reported fear (post-2 – baseline)

In case of significant correlations of any change score in multimodal measures of fear with either antisaccade latencies (primary outcome) or error rates (secondary outcome), the respective change score will be included in the respective MLM.

6.3 Multilevel Modelling

Additionally, due to the longitudinal design, the data structure will be tested for a hierarchical structure. In case of a hierarchical data structure, the primary (section 6.1.1) and secondary outcome (section 6.1.2) will be explored utilizing multilevel models.

6.4 Statistical software

Analyses will be performed using RStudio (Version 2023.03.0+386; R-4.2.2).

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