Antisaccade performance in spider phobia and its association with multimodal correlates of fear

Statistical Analysis Plan (SAP)

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SAP responsibilities

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1. Introduction

1.1 Study Background and rationale

The antisaccade task is a widely used paradigm to study inhibitory control[1–3]. Studies employing the antisaccade task indicate that inhibitory control is impaired in individuals with high levels of anxiety[4–6]. However, studies investigating patients with anxiety disorders are sparse.

The identification of aberrant inhibitory control functioning in patients suffering from anxiety disorders could help to understand the neurocognitive basis of key symptoms (e.g., altered physiological responses to feared stimuli[7,8] and avoidance behavior) and pave the way for specifically targeted interventions. Therefore, it is highly relevant to understand how putative alterations in inhibitory control are related to multimodal measures indexing the experience of fear.

The cross-sectional study part presented in this SAP is embedded in an overarching clinical study, which is registered with Current Controlled Trials. (Study ID: ISRCTN12918583; Registered on 28th February 2022). While the full study also includes an interventional part that tries to manipulate antisaccade performance via a fear-specific antisaccade training, this cross-sectional study part aims to investigate antisaccade performance in individuals with spider phobia (SP) compared to healthy controls (HC) and its relation to correlates of fear.

1.2 Study objectives

One key aim of this cross-sectional study-part is to compare inhibitory control performance between spider phobic and healthy control individuals, as well as the impact of phobia-related stimulus material on inhibitory control performance. Inhibitory control performance will be indexed by antisaccade latencies and error rates (which will serve as primary and secondary outcome, respectively; see 5.1 and 5.2) in an emotional antisaccade task. Furthermore, we aim to analyze associations between antisaccade performance and multimodal measures indexing fear, which are described below (see 5.3), including psychophysiological measures (see. 5.3.1). Lastly, we aim to replicate findings regarding psychophysiological hyper-arousal in spider phobic patients in response to phobia-related stimuli[7,8] to ensure validity of our paradigm (see 2.1).

2. Methods

2.1 Study Design

To investigate inhibitory control performance, participants complete an emotional antisaccade task which employs schematic pictures of spiders and flowers. Antisaccades will be retrieved across three blocks with 20 pictures of spiders (phobia-related) and 20 pictures of flowers (neutral) presented randomized within each block. To retrieve multimodal measures indexing fear, and to replicate findings regarding psychophysiological hyper-arousal in spider phobic patients in response to phobia-related stimuli, participants will also complete a free-viewing task. Here, eight pictures of spiders (phobia-related), as well as eight pictures of negative valence and eight pictures of neutral valence from the Geneva Affective Picture System (GAPED)[9] are presented. Pictures will be presented twice across two blocks with randomized presentation of stimuli within each block. Afterwards, participants will rate the pictures regarding valence and arousal on a visual analog scale. Furthermore participants will complete the Spider Phobia Questionnaire (SPQ)[10] as well as a Behavioral Avoidance Test, indexing self-reported spider-phobic symptoms and avoidance behavior, respectively.

To summarize, in the antisaccade task, antisaccade performance (latencies, error rates, see 5.1 and 5.2) will be studied via a 2x2 design with the between-subject factor group (SP vs. HC) and the within-subject factor stimulus condition (phobia-related vs. neutral).

The free-viewing task will employ a 2x3 design with the between-subject factor group (SP vs. HC) and the within-subject factor stimulus condition (phobia-related vs. negative vs. neutral).

2.2 Randomization

As this cross-sectional study part does not include an intervention, no randomization of participants is needed. Participants will be assigned to their respective group based on diagnosis (SP vs. HC). In the antisaccade-task and in the free-viewing task, stimuli will be presented in a fully randomized order within each block to prevent effects of order of stimulus presentation (see 2.1).

For the interventional study part, which is not subject to this SAP, the randomization scheme will be found in the registered report[11].

2.3 Sample size

Based on a study using the antisaccade task in individuals with post-traumatic-stress disorder[12], which indicated large effect sizes regarding differences in antisaccade latencies between groups, we calculated an a-priori power analysis using G*Power[13] to detect a large effect

(Cohen´s f = .40, α = .05, power = .85) in our primary outcome (i.e. antisaccade latencies). Results indicated a required total sample size of 59 participants (30 per group).

2.4 Timing of final analysis

Analyses described in this SAP will be performed after completion of data collection and data cleansing.

3. Statistical principles

3.1 p-values

Significance levels for statistical analyses (see below) will be set to $p = .05$.

3.2 Missing values and outliers

If less than 50% of all trials in the antisaccade task (see 5.1 and 5.2) are valid, the respective participant will be excluded from analyses. For a trial to be classified as valid, onset latency of the first saccadic eye movement after stimulus onset must be greater than 70 milliseconds (ms). Starting coordinates of the eye movement must fall within a square of 5° x 5° around the fixation stimulus. Additionally, blinks must be absent. Trials with artifacts or with no recorded eye movements after stimulus onset are also classified as invalid. In regard to psychophysiological data, trials with artifacts will be excluded from analyses. In case of SCR and startle response, trials on which participants exhibit a zero response will be included in the analysis to calculate mean magnitude values[14,15] (see 5.3.1). The number of participants characterized as non-responders in terms of skin conductance or startle response will be reported in the final manuscript. Cut-off criteria for non-responders will be set after first cleansing of data to find an optimal signal-to-noise ratio and will be reported in the manuscript.

For all outcome measures (see 5.3.1–5.3.3) statistical outliers will be identified by visual inspection using plot functions. Outliers will be defined as values that exceed three standard deviations from the mean. Identified outliers will be investigated regarding plausibility of their values. If values seem plausible and not attributable to measurement error or lack of task comprehension, they will be included in the analyses. Possible distortions of results due to the respective outlier will be assessed and reported in the manuscript.

4. Trial population

4.1 Eligibility criteria

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

4.2 Recruitment

A CONSORT-Flow diagram will be presented in the manuscript.

4.3 Sample characteristics

Sociodemographics, SPQ- and BAT-values, as well as additional measures (see 5.4) for each group (SP vs. HC) will be presented using mean and standard deviations as well as counts and percentages, respectively. Group differences will be investigated using t- and chi-square tests. Additionally, a table will be provided depicting mean psychophysiological responses (see 5.3.1) to all three conditions of the free-viewing task (phobia-related vs. negative vs. neutral) for each group (SP vs. HC) using means and standard deviations. In the same way participants ratings of arousal and valence of the stimuli employed will be described.

5. Outcome measures

5.1 Primary outcome measure

The primary outcome will be antisaccade latency, measured in milliseconds. 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

To analyze associations of antisaccade performance and multimodal measures indexing fear (see 6.3), multimodal psychophysiological responses towards phobia-related stimuli will be obtained. Towards this aim, we will calculate contrast values between the mean physiological responses during the free-viewing task in the phobia-related and neutral condition for each participant[16].

To test the validity of our design and replicate previous studies revealing hyper-arousal towards fear specific stimuli, we will additionally calculate and compare mean responses per stimulus category (phobia-related, negative, neutral) and group (SP, HC). For heart rate data we will calculate mean change scores from baseline in 0.5-second-bins for each stimulus category (see

5.3.3.1). Data for skin conductance and startle response will be standardized, applying z-transformation. In case of skin conductance and startle response, mean responses will be calculated as mean magnitude values, including zero responses[14,15].

5.3.1.1 Heart rate

Heart rate will be defined as beats per minute (bpm) measured via electrocardiography (ECG). ECG data will be preprocessed using the PhysioData Toolbox[17]. The signal will be filtered using a band pass filter with a low cut-off at 1 Hz and a high cut-off at 50 HZ. From the ECG signal R-peaks will be retrieved, which serve to calculate the heart rate measured in bpm. The ECG signal will be visually inspected for artifacts, as well as erroneous or missed R-peaks. Missed R-peaks will be added manually, erroneous R-peaks will be deleted. Trials with artifacts in the ECG signal will be discarded.

To retrieve contrast values that will be associated with antisaccade performance (see 6.3) the mean heart rate in bpm across the full trial period of 6 seconds will be used to calculate mean heart rate responses per stimulus category for each participant. Mean heart rate values in the neutral condition will then be subtracted from mean heart rate values in the phobia-related condition[16].

To replicate previous studies revealing hyper-arousal towards fear specific stimuli, heart rate will be converted to 0.5-second-bins. Then, change scores will be calculated by subtracting the 1-s prestimulus baseline heart rate from each bin. Change scores will then be averaged for all trials of a stimulus category[7,8].

5.3.1.2 Skin conductance response

In line with publication guidelines[14], skin conductance response (SCR) will be defined as the largest increase in conductance, occurring between 1 to 4 seconds after stimulus onset. This also impedes unwanted interactions with the acoustic startle stimulus, which occurs between 4 and 5.5 seconds after stimulus onset. Data will be preprocessed using the PhysioData Toolbox[17]. The raw signal will be filtered and resampled to a 20 Hz time-vector spanning the length of the filtered signal, using spline interpolation. By first applying a moving minimum filter, followed by smoothing the result applying a Gaussian kernel, the tonic component is calculated. The phasic component will be calculated by applying a 1st order high pass Butterworth filter of 0.5 Hz. To detect small responses the minimum amplitude will be set to 0.1 µS[14]. The signal will be visually inspected for artifacts. Trials with artifacts in the signal will be discarded.

5.3.1.3 Startle response

In line with publication guidelines for human startle eye blink measures[15], startle response will be defined as the difference between peak and baseline amplitude of the activity of the musculus

orbicularis oculi (unilateral) in a 200 ms response window after startle probe onset. Activity will be measured via electromyography (EMG). The raw EMG signal will be filtered using a band-pass filter ranging from 10 to 500 Hz, as well as rectified and smoothed, using a low-pass resistor-capacitor filter, utilizing a time constant of 10 ms. A C++-based software tool will be used to identify peak responses in the rectified and integrated EMG signal. Additionally, the signal will be visually inspected for artifacts (e.g., preceding blinks immediately before the startle probe), as well as erroneously detected response peaks. Trials with artifacts in the EMG signal will be discarded, erroneously detected response peaks will be corrected manually.

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[18]).

5.3.3 Psychometrics

Self-reported spider-phobic symptoms will be defined as a sum value, obtained from the SPQ[10].

5.4 Additional measures

Further measures will be retrieved, including prosaccadic performance, as well as different questionnaire measures. While these measures are not subject to our analyses (see 6) they will be reported to further characterize the sample (see 2.1).

A full list of questionnaire measures will be found in the registered report[11].

6. Analyses methods

All analyses will be conducted as indicated. In case of significant group differences regarding relevant sample characteristics (see 2.1 and 5.4) the respective variables will be included in the statistical models. For all models, required statistical assumptions will be checked before conducting the respective analyses.

6.1 Analysis of primary outcome

To investigate differences in inhibitory control efficiency (indexed by antisaccade latencies) between spider phobic and healthy control individuals as well as the influence of phobia-related stimulus material on inhibitory control efficiency a 2x2 mixed ANOVA, employing group (SP vs. HC) as a between-subject factor and stimulus condition (phobia-related vs. neutral pictures) as a within-subject factor will be conducted.

6.2 Analysis of secondary outcome

To investigate inhibitory control effectiveness (indexed by antisaccade error rates) between spider phobic and healthy control individuals, as well as the influence of phobia-related stimulus material on inhibitory control effectiveness, a 2x2 mixed ANOVA employing group (SP vs. HC) as a between-subject factor and stimulus condition (phobia-related vs. phobia-unrelated pictures) as a within-subject factor will be conducted.

6.3 Associations of primary/secondary outcome with multimodal measures indexing fear

Furthermore, we aim to investigate the relationship between the primary/secondary outcome and multimodal measures indexing fear (see 5.3) in spider phobic individuals. Therefore, multiple regression analyses will be conducted with the primary/secondary outcome as dependent variable respectively. Outcomes will index overall antisaccade performance independent of stimulus condition (phobia-related vs. neutral). If significant effects of stimulus condition (see 6.1 and 6.2) are found, analysis will also be conducted for contrast values of outcomes (phobia-related - neutral). Contrast values (phobia-related-condition - neutral condition, see 5.3.1) for heart rate, SCR and startle response, as well as final BAT distance in cm and SPQ-sum score will be entered as independent variables, using the forced entry method. Tolerance and variance inflation factor (VIF) will be investigated to account for possible multicollinearity.

6.4 Replicating findings of psychophysiological hyper-arousal in patients with spider phobia in response to pictures of spiders

To replicate findings of psychophysiological hyper-arousal in spider phobic patients in response to pictures of spiders and to ensure the validity of our free-viewing task, an ANOVA will be calculated employing a 2x3 design with the between-subject factor group (SP vs. HC) and the within-subject factor stimulus category (phobia-related vs. negative vs. neutral) for each of the three psychophysiological measures (i.e. heart rate, SCR and startle response, see 5.3.1). For heart rate measures an additional within-subject factor (half seconds, 12 factor levels) will be added. In case of significant results, post-hoc t-tests will be conducted, applying Bonferroni correction to account for multiple testing. The contrast-values (phobia-related condition - neutral condition, see 5.3.1), calculated to investigate associations between antisaccade performance and multimodal measures indexing fear, will also be compared between groups, using t-tests.

6.5 Statistical software

Analyses will be performed using SPSS version 28.0.

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