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Human defensive freezing: associations with hair cortisol and trait anxiety.

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Abstract

The anticipation of threat facilitates innate defensive behaviours including freezing reactions. Freezing in humans is characterized by reductions in body sway and heart rate. Limited evidence suggests that individual differences in freezing reactions are associated with predictors of anxiety-related psychopathology including trait anxiety and hypothalamic-pituitary-adrenal (HPA) axis activity. However, previous human studies focused on acutely circulating cortisol levels, leaving the link between freezing and more stable, individual trait markers of HPA axis activity unclear. We investigated whether individual differences in anticipatory freezing reactions are predicted by accumulated hair cortisol concentrations (HCC) and trait anxiety, in a well-powered mixed sample of police recruits at the start of the police training, and age, sex and education matched controls (total N=419, mean age = 24, N_{women}=106, N_{police recruits}=337). Freezing-related reactions were assessed with posturographic and heart rate measurements during an active shooting task under threat of shock. The anticipation of threat of shock elicited the expected reductions in body sway and heart rate, indicative of human freezing. Individual differences in threat-related reductions in body sway, but not heart rate, were related to lower HCC and higher trait anxiety. The observed links

between postural freezing and predictors of anxiety-related psychopathology suggest the potential value of defensive freezing as a somatic marker for individual differences in stress-vulnerability and resilience.

Keywords: freezing, heart rate, body sway, cortisol, anxiety, defensive reactions

1. Introduction

The detection of threat elicits a range of defensive behaviours varying from hard-wired, automatic freezing reactions to subsequent learned instrumental actions (Ledoux, Daw, & Emerson, 2018). In this cascade of defensive behaviours lies a vast potential for individual variation (McNaughton & Corr, 2004; Niermann, Figner, & Roelofs, 2017b). While some individuals immediately switch into active fight-or-flight, or goal-directed actions, others freeze with variable duration and strength (Kozłowska, Walker, McLean, & Carrive, 2015; Niermann et al., 2019). It is important to understand this variability for more than just theoretical purposes. Maladaptive threat processing is key to understanding anxiety disorders, which burden many individuals in our society (Lewis-Fernandez et al., 2010).

Across many species, freezing is an innate defensive reaction that is characterized by movement cessation (Fanselow, 1994; Eilam, 2005). While it is one of the most important readout measures of anxiety in non-human animals, freezing also occurs in humans (Hagenaars, Oitzl, & Roelofs, 2014; Hashemi et al., 2019; Löw, Weymar, & Hamm, 2015). Upon distal threat detection and in anticipation of potential threat, human postural freezing is typically accompanied by heart rate deceleration (or: bradycardia) (Azevedo, Volchan, Imbiriba, & Rodrigues, 2005; for a review see Roelofs, 2017) and it may help optimizing the detection of threatening information in the environment (Campbell, Wood, & McBride, 1997; Lojowska, Gladwin, Hermans, & Roelofs, 2015). At this threat-anticipatory stage, freezing reactions are thought to facilitate risk assessment and prepare subsequent defensive actions (Blanchard, Griebel, Pobbe, & Blanchard, 2011; Gladwin, Hashemi, van Ast, & Roelofs, 2016; Klaassen et al., 2021).

Although it has been suggested that freezing responses may serve as an important predictor of individual differences in anxiety sensitivity (Koch et al., 2017; Roelofs, 2017), relatively little is known about what might drive individual differences in human freezing. Freezing is regulated by various neurochemical pathways, which may explain individual variability. One of these pathways comprises the hypothalamic-pituitary-adrenal (HPA) axis, where the release of corticotropin-releasing hormone (CRH) leads to the production of glucocorticoids. The main glucocorticoid in primates is cortisol, whereas in rodents and some other species it is corticosterone. These glucocorticoids facilitate energy mobilization, recovery and normalization to homeostasis, as well as the adaptation of physiological

responses to future threat (Joëls, 2017). Non-human animal studies indicate that CRH interacts with the autonomic nervous system to optimize defensive responses including freezing (Sherman & Kalin, 1988; Stiedl, Meyer, Jahn, Oegren, & Spiess, 2005; Takahashi & Rubin, 1993; Fox, Shelton, Oakes, Davidson, & Kalin, 2008; Kalin, Shelton, Rickman, & Davidson, 1998). Most of the relevant evidence for this study comes from recent work on freezing in non-human primates focusing on long-term accumulated hair cortisol concentrations (HCC). Those showed that monkeys with low HCC responded with increased freezing to intruder threat (stare phases), whereas monkeys with high HCC reacted with less freezing (Hamel et al., 2017). Also, in rodents, high freezers have been reported to have relatively low corticosterone levels (Frank et al., 2006).

Limited evidence suggests that individual differences in freezing reactions are associated with cortisol levels in humans as well, which may also explain individual differences in reported anxiety (Niermann, Figner, Tyborowska, van Peer, Cillessen, & Roelofs, 2017a; Roelofs, Hagenars, & Stins, 2010, respectively). Stronger reported state anxiety, for example, has been associated with stronger postural immobility as well as bradycardia in response to aversive pictures (Roelofs et al., 2010). Another example comes from Niermann et al. (2017a) who studied the relation between internalizing symptoms, basal cortisol and immediate and delayed freezing one hour later. Path analyses indicated that participants with low basal salivary cortisol levels showed relatively reduced recovery from freezing responses one hour after a formal stress-induction and hence, relatively prolonged freezing reactions. Those prolonged freezing reactions stemming from the delayed recovery were in turn linked to increased levels of internalizing anxiety and depression symptoms (Niermann et al., 2017a). Note that, although the same study also showed that initial increases in freezing (immediately after stress-induction) were positively associated with basal cortisol levels, it was only the delayed recovery from freezing combined with lower basal cortisol levels that predicted internalizing symptoms.

Together the findings reported above suggest that stronger freezing is associated with more self-reported anxiety and low basal cortisol levels. However, positive associations of high cortisol in strong freezers as well as high cortisol in anxiety have also been reported (Buss, Davidson, Kalin, & Goldsmith, 2004; Kalin et al., 1998; Mantella et al., 2008). These seemingly conflicting studies all focused on acutely circulating peripheral cortisol levels (as assessed in saliva, blood, and urine). While it is difficult to pinpoint with certainty the reason for these contrasting findings, it is important to consider that the HPA axis is highly reactive to a wide array of external events. Arguably, the large diurnal fluctuations in cortisol, along with many other external influences that impact cortisol signaling, make acute cortisol measurements less reliable as predictors of *trait-like* differences in HPA axis function. Methodological advancements have enabled the assessment of chronic HPA axis activity by

measuring accumulated cortisol in hair (Kirschbaum, Tietze, Skoluda & Dettenborn, 2009) which is less influenced by such fluctuations (for review see Stalder & Kirschbaum, 2012). Indeed, Kirschbaum et al. (2009) showed that cortisol measured in hair can retrospectively assess cortisol secretion for a period up to approximately six months. This makes HCC potentially a reliable candidate to robustly measure individual differences in stress-related vulnerability.

The main aim of the current study is therefore to assess whether individual differences in threat-anticipatory freezing reactions are predicted by HCC and reported (trait) anxiety. As research on individual differences typically requires large samples in order to detect reliable relationships, we investigated our research question in an adequately powered sample of participants at the baseline measurement of an ongoing longitudinal study in police recruits (total N=419). We tested freezing during an active shooting task that was previously shown to allow detection of individual differences in human freezing in a more ecologically-valid, dynamic task context (Gladwin et al., 2016, Hashemi et al., 2019). During this task, participants had to make timely shooting decisions under threat of shock while they were standing on a stabilometric force platform, and body sway and heart rate were recorded as indices of anticipatory freezing reactions. We hypothesized that enhanced freezing reactions under threat of shock were associated with stable, long-term markers of anxiety-related vulnerability including lower HCC and higher trait anxiety.

2. Methods

2.1. Participants

This cohort is part of a prospective study on the role of defensive reactions in trauma resilience in police recruits. Details on the design and methods of the full study can be found in the Netherlands Trial Registry (NTR6355) and in our protocol article (Koch et al., 2017). The project was approved by the Independent Review Board Nijmegen (IRBN registration number NL48861.072.14) and was conducted in accordance with these guidelines. All participants gave written informed consent before the start of the experiments.

We tested 427 participants including 337 students from the Dutch Police Academy and 82 age, gender, and education matched healthy participants. Eight participants were excluded from the analysis because of discontinuation of the task (n=4), hardware problems (n=1), non-compliance to task instructions (n=2), or data loss (n=1), leaving a total sample of 419 participants (313 men and 106 women, mean age 24, ranging from 18 to 45 years). At the time of the baseline measurement, the police recruits were in the first year of their education, had not yet received specific shooting-related training, and had performed very little active police duty. The police recruits and matched civilians were therefore not treated as different groups within this study because our hypotheses were similar for both at the

baseline measurement. Exclusion criteria were: average daily use of more than three alcoholic beverages, average use of psychotropic medication or recreational drugs weekly or within 72 hours prior to testing, alcohol use within 24 hours prior to testing, current or recent diagnosis (within the last three months) of psychiatric or neurological disorders, regular use of systemic corticosteroids, metal objects or fragments in or around the body, medical plaster that could not be taken off, history or current neurological or endocrine treatment, history of head surgery, current periodontitis, claustrophobia, epilepsy or pregnancy. Additional exclusion criteria for the control participants were: experience in law enforcement or the military, and being involved in training or an occupation involving potential trauma exposure. This resulted in the recruitment of mostly young male participants that were in general and higher vocational education including the sports academy. During the test day, 12 of the 419 participants reported a violation of our substance use criteria (N=1 drank more than 3 alcoholic beverages daily, N=6 drank alcohol within 24 hours before study participation, and N=5 reported weekly or more frequent drug use). We therefore checked whether the significance of all results remained the same whether including or excluding those participants from the analyses. As this was the case, we report the results that included all participants.

2.2. Freezing-eliciting active shooting task

The shooting task (see figure 1) is a speeded decision-making task under threat of shock designed to elicit anticipatory freezing reactions prior to action (Gladwin et al., 2016). Participants performed the task holding a button box in their right hand while standing on the stabilometric platform that was placed in front of a computer screen (see figure 1c). The participants were instructed to detect whether a virtual opponent was drawing a gun or a phone, and to only shoot the virtual opponent, as fast and accurately as possible, if they drew a gun.

Trials began with one of two visually distinguishable opponents standing in a parking garage. These opponents served as cues that signaled the level of threat. One of the opponents signalled threat of an aversive electrical shock (high threat cue) whereas the other signalled shock safety and never led to any shock (low threat cue). The opponent associated with threat of shock was counterbalanced across participants, and the order of high and low threat trials was randomized. After a short (500-1500ms; 10% of trials), medium (1500-6000ms; 10%), or long (6000-6500ms; 80 %) preparation period, the opponent either drew a gun or a mobile phone (The draw – see figure 1). Long intervals were usually presented to allow for a sufficient number of trials for which the time course of anticipatory body sway reductions and bradycardia could be analysed. Short and medium intervals were presented to make the moment of attack unpredictable. During the following

response window, the participant had to fire (in case of a gun draw) or withhold (in case of a phone draw). Firing involved pressing a button as soon as possible, as participants had approximately 500ms (individually titrated - see below) to respond. If participants responded to a gun-draw too late (miss) or if they fired in response to a phone-draw (false hit), participants were punished by a visual representation of being shot. This feedback was accompanied by an electric shock in the high threat condition, but not in the low threat condition. If the participant responded too late (a miss), the punishment was performed by the opponent. However, if the participant shot a phone-drawing opponent (a false hit), the punishment was carried out by a police officer who was standing in the back of the garage. The trial ended with an inter-trial interval that varied between 3.0 and 4.5s ($M = 3.4s$, $SD = 0.36s$).

In line with other threat paradigms investigating defensive reactions, the frequency of electric shocks was kept constant during the task and between subjects (Löw et al., 2015; Terburg et al., 2018). The participant's response window (time between the opponent drawing the gun and firing) was titrated in such a way that participants would be shot on +/- 50% of the trials. An algorithm dynamically adjusted the duration of the response window iteratively (within the bounds of 100ms to 2000ms) throughout the task (across low and high threat conditions). This was accomplished by either increasing or decreasing the reaction time of the opponents' shooting response by 10%, depending on the participants' reaction times in the previous trial. Participants viewed their own "in-task" hands holding a gun during the entire trial, and could fire at any time. Before the start of the task, electric shocks were set to an unpleasant but not painful level with a standardized work-up procedure, which consisted of five electrical shocks of variable intensities that were rated on their unpleasantness (adapted from Klumpers et al., 2010).

Participants received explicit instructions on which opponent was associated with threat of shock, and were verbally checked whether they understood the threat contingencies before the experiment started. To get acquainted with the task and threat contingencies, participants first underwent three blocks each of 52 fast-paced training trials (80% short preparation intervals and shortened intertrial intervals). The final measurement phase consisted of three blocks of 28 trials each.

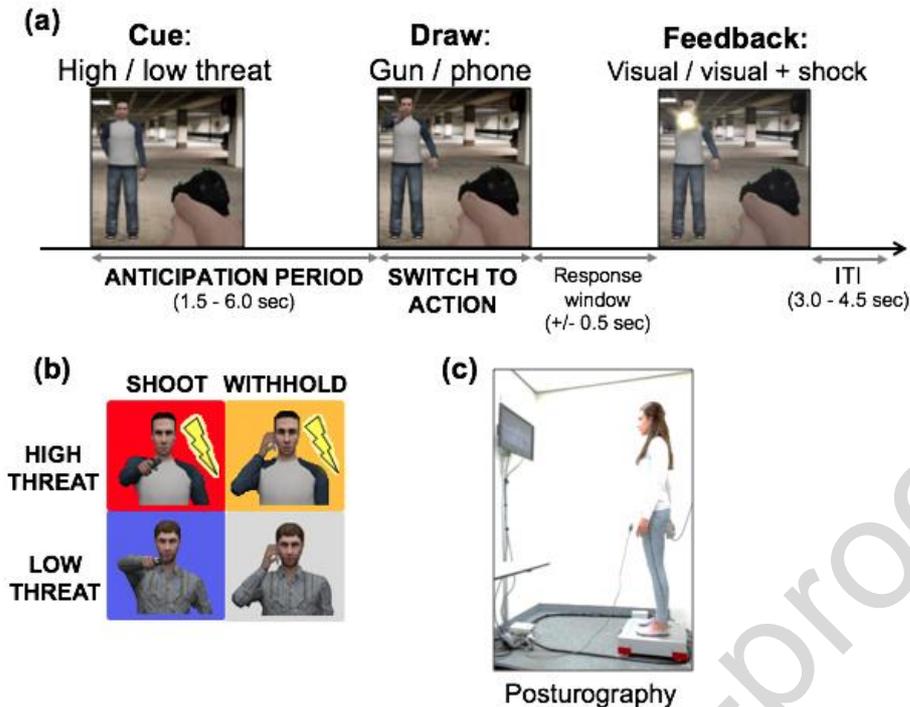


Figure 1. a) Schematic representation of the shooting task. During the anticipation period, a cue (one of two visually distinguishable opponents – see b) is shown indexing either threat of shock (high threat cue) or shock safety (low threat cue). The cue indicates the level of threat for a variable anticipation period of 1.5 to 6.0 seconds. This is the time window in which we measure threat anticipatory freezing in terms of body sway and heart rate reductions (see panel c). After this, the opponent either draws a gun or a phone (the draw). A gun signals the participant to shoot (by button press) whereas a phone requires the participant to withhold responding. Incorrect shooting decisions (too slow, too fast, or false alarms) are indicated by visual feedback of being shot. b) Overview of the 4 conditions, including high and low threat as well as shoot and withhold conditions. On high threat trials only, the visual feedback of being shot is accompanied by an electric shock. c) Participants performed the task while standing on a stabilometric force platform enabling body sway and concurrent heart rate measurements as indices of postural freezing and bradycardia, respectively. [Informed consent was obtained to publish the photograph which allows the identification of the individual in the picture].

2.3. Assessment of HCC

Hair strands with average lengths of 2.78 cm were cut scalp-near from a posterior vertex position. The aim was to obtain 3 cm segments for estimating the cortisol secretion over approximately the last 3 months, given that the average hair growth rate is one cm per month (Pragst & Balikova, 2006). Obtained hair strands ranged between 1 and 3 cm, with full length hair strands (3cm) obtained for the majority of participants (N = 271) and 6 participants with hair lengths of 1 cm. Importantly, control analyses indicated that variation in hair length did not influence any of our key findings (see supplementary material for details). HCC were determined via a LC-MS/MS-based method (Gao et al., 2013), which is a selective and reliable procedure for the assessment of cortisol concentrations in hair samples. As hair sampling was restricted to participants with sufficient scalp hair length,

HCC could be assessed for 343 participants. Six participants showed relatively high HCC values (above 3 standard deviations from the mean) and were therefore excluded from the analyses (Final sample 337; $M = 9.74$ pg/mg, $SD = 16.44$ pg/mg).

2.4. Assessment of trait anxiety

Before the shooting task, participants completed several questionnaires (Koch et al., 2017) including the Dutch version of the Spielberger Trait Anxiety Inventory (STAI) (Van der Ploeg, 1984; Spielberger 1983), which was analysed for the current study. The STAI is a widely used 20 item self-report instrument to assess general trait levels of anxiety. Internal consistency of trait anxiety scores (STAI-trait) was high, as indicated by a Cronbach's alpha of 0.89. Mean levels of trait anxiety were slightly lower compared to normative values in previous samples (34.9 for male working adults, and 38.3 for male college students), but showed considerable variance ($M = 31.3$, $SD = 7.4$) (see also supplementary table 1, Spielberger, 1983). Note that the larger part of the sample consists of a relatively resilient group of police recruits.

2.5. Assessment of psychophysiology

Stabilometric platform. The shooting task was performed on a custom-made stabilometric force platform (dimensions 50 cm x 50 cm) located in front of a monitor displaying the task (see figure 1c). The force plate consisted of four sensors measuring the displacement of the centre of pressure, or body sway, both in the anterior-posterior (AP) and medial-lateral (ML) directions. Participants stood in a relatively stable position with feet approximately 30 cm apart, and were instructed to stand as still as possible.

Heart rate. Electrocardiograms (ECG) were collected using three Ag/AgCl electrodes with adhesive patches and amplified with the BrainAMP EXG MR system and EXG AUX apparatus. One electrode was placed below the right clavicle and one on the left side of the chest, just below the sixth rib. The ground electrode was attached under the left clavicle.

2.6. Data preprocessing

All preprocessing was performed with Matlab 2015a (Mathworks, Natick, MA, US). Raw electrocardiogram (ECG) and body sway data were downsampled to 125 Hz (with an initial sampling frequency of 2500Hz). The raw signal was filtered with a Butterworth band-pass filter (body sway: 0.01-10Hz, heart rate: 0.5-10 Hz). ECG data were subsequently assessed via an in-house automatized R-peak detection algorithm and visually inspected. Full details of this can be found in the supporting information. For body sway, we calculated the standard deviation of the AP direction within a moving time window of 1 second, which was then visually checked for spikes or other noise (see detailed preprocessing steps on body

sway in the supporting information). Reductions in body sway in the AP direction were taken as an index of postural freezing, consistent with previous analyses. Due to the spaced feet position (30 cm apart) on the balance board, the AP direction has a larger movement range and therefore a greater sensitivity to affective modulations compared to the ML direction (Hagenaars, Stins, & Roelofs, 2012).

Analysis of body sway and heart rate only included trials with a duration of at least 6 seconds, which is required for detectable freezing to evolve within this task context (Gladwin et al., 2016). Additional details of data preprocessing can be found in the supporting information. During the anticipation interval, event-related changes were calculated between 2.5 to 6.0 seconds for body sway and heart rate (inter-beat interval, IBI), relative to a baseline period of 1 second before cue onset. The time window of the analysis was chosen to exclude non-specific orienting effects from threat-related prolonged bradycardia and body sway (Hagenaars et al., 2012; Hermans, Henckens, Roelofs, & Fernández, 2013). To obtain a freezing index per individual, we averaged body sway as well as heart rate (IBIs) responses within the anticipation period (2.5s - 6.0s) for high and low threat trials separately, and additionally computed the high-low threat contrasts on these averages.

To assess the internal reliability of freezing measures including body sway and heart rate, we performed non-parametric Spearman's rho correlations between odd and even trials. Analyses indicated a moderate to high reliability for body sway (low threat $R_s = 0.62$, $p < 0.001$; high threat $R_s = 0.78$, $p < 0.001$) as well as for heart rate data (low threat $R_s = 0.74$, $p < 0.001$; high threat $R_s = 0.75$, $p < 0.001$).

2.7. Statistical analyses

We used Bayesian linear mixed models (BLMM) implemented through the brms package in R (version 3.3.3; R Core Team, 2015) which interfaces to Stan (Carpenter et al., 2016). Mixed models were used because of the advantages for modelling random effects, the ability to model non-normal distributions and the opportunity to use data from subjects with missing data. A Bayesian implementation was used for enhanced model convergence. For all models we used the generic, default priors of the brms package to give fast and accurate model convergence, without making the results idiosyncratic for study-specific assumptions on the data. These are improper flat priors for population-level (i.e., fixed) effects, weakly informative Student-t priors for group-level effects (i.e., random intercepts and slopes), and LKJ-Correlation priors for random correlations (Bürkner, 2016). Coefficients were reported as statistically significant when the associated 95% posterior credible intervals did not overlap with zero. All continuous predictors were standardized and categorical factors (cue: high/threat) were coded using sum-to-zero contrasts. To facilitate the interpretation of our results, to allow comparisons to other studies using other statistical methods, and to check

our results for robustness we followed up our BLMMs with analogous non-Bayesian bivariate correlation analyses.

2.7.1. Anticipatory freezing reactions

The analyses of main interest consisted of two separate BLMMs, with the dependent variable of body sway for the first model, and heart rate for the second model. BLMMs typically model two types of effects. Fixed effects model the average effects of interest, and are comparable to intercepts and slopes in simple regressions. Random effects account for the non-independency of multiple datapoints from one participant and therefore prevent type 1 errors. For both models, we therefore included a per-participant random intercept and modelled cue (high, low threat) as within-subject fixed effect (testing for expected threat-level effects) with random slopes varying across participants. All other factors included in the model consisted of scaled and centred between-subject fixed factors including HCC and trait anxiety as main predictors of individual variability, as well as shooting reaction times and accuracy on withhold as well as shooting trials. The last two factors were included based on associations previously found between freezing and defensive action, suggesting that while freezing has often been interpreted as a passive coping mechanism, in fact, it plays a role in action preparation (Gladwin et. al 2016; Hashemi et al. 2019). Based on this we expected that stronger freezing under threat of shock would also be followed by faster and more accurate shooting actions. These latter results are described in the supplementary material.

We also modelled the interaction effects of the cue (high/low threat) with between-subject factors separately. This approach follows a maximal random effects structure that properly deals with within- and between-subjects variances and avoids inflated type-1 errors (as recommended in Barr, Levy, Scheepers, & Tily, 2013). Body sway was converted to an ordinal variable to minimize the influence of outliers. For heart rate, we added a constant to circumvent negative values. Models were fitted using 6 chains with 8000 iterations each (3000 warm-up) or more when necessary for convergence. Both models included a skew normal distribution to appropriately accommodate deviations from normality.

To exclude some potentially confounding effects on HCC and heart rate, we added two complementary control analyses all details of which can be found in the supplementary material.

To corroborate the BLMM findings on individual differences, and to explore whether the observed BLMM results were model-specific or could also be captured in simple bivariate relations, we report additional, complementary Spearman (rank) correlations as follow-up for the predictors that showed a significant effect in the BLMM analyses. Given recent concerns about reproducibility of scientific findings (Jasny, Chin, Chong, & Vignieri, 2011) and the small effect sizes that can be expected for individual differences analyses

(Schönbrodt & Perugini, 2013), we performed a split-half validation of all significant bivariate relations. Demonstrating the robustness of our results, seven out of eight correlations remained significant in each of our randomly-generated splits of the dataset that contained 50% of the data. Only one association did not reach significance in both subsamples, yet it reached significance in the other half of the sample with a similar effect size (see supporting information for details).

Although we integrated all predictors in two BLMMs for body sway and heart rate to minimize multiple testing, we report the results in separate sections aligned with our hypotheses for reading fluency. We first mention the expected threat-level effects on anticipatory freezing reactions, followed by individual differences explained by their associations with HCC and trait anxiety. Lastly, we report replication results relating freezing responses to action preparation.

2.7.2. Behavioural performance

Although the task was primarily designed to elicit threat-anticipatory freezing in an active task context, we also verified the typical behavioural effects in terms of reaction times (RT) and accuracy in two separate additional analyses. All details on behavioural performance can be found in the supporting material.

3. Results

3.1. Anticipatory freezing reactions

We first verified that the task produced the intended threat effects on freezing-related reactions. We found strong reductions in sway and heart rate during the anticipation period, and as expected (Gladwin et al., 2016, Hashemi et al., 2019), the threat of shock (high threat condition) induced stronger reductions in body sway as well as heart rate compared to shock safety (low threat condition) (main effect of cue: [body sway:] $B = 10.82$, 95% CI [2.12, 19.49]; [heart rate:] $B = 0.31$, 95% CI [0.19, 0.42], see figure 2). The magnitude of reductions in body sway and heart rate were positively correlated and together were indicative of human freezing reactions (high threat: $R_s = 0.26$ $p < 0.001$, low threat: $R_s = 0.24$ $p < 0.001$; see supplementary figure 2).

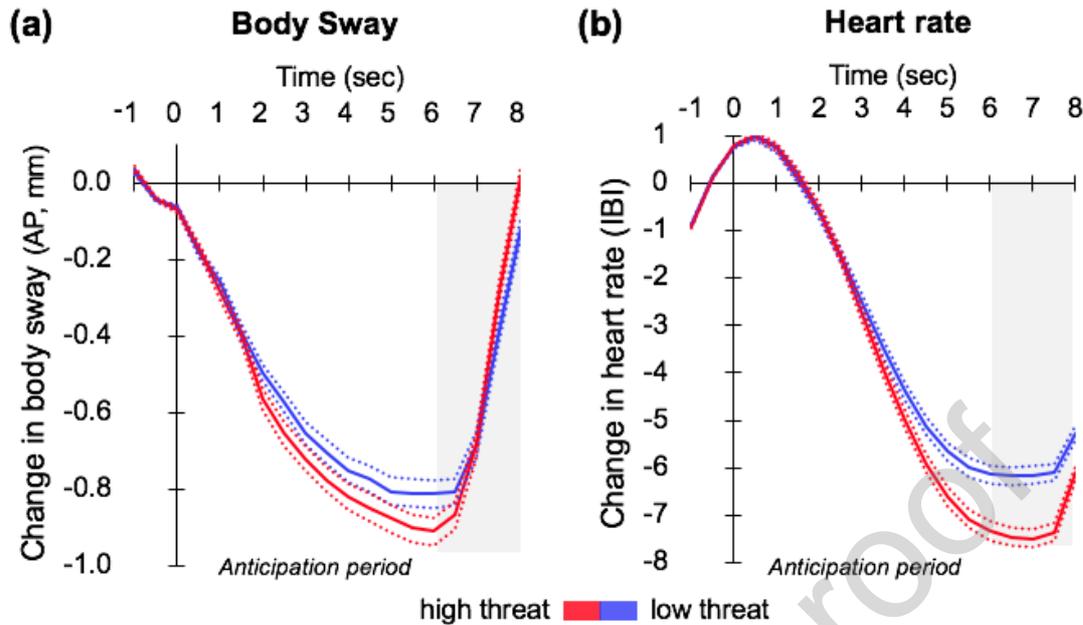


Figure 2. Mean freezing responses as a function of time and threat. Anticipation of shock (high threat, as compared to low threat) was associated with stronger reductions in body sway, as assessed in anterior-posterior (AP) deviations of centre of pressure (2a) as well as bradycardia, observed in enhanced mean heart rate decelerations reflected in changes in inter-beat intervals (IBI) (2b). Standard errors of the mean are illustrated in dotted lines. The shaded area signals the response window, which was excluded from the analysis.

3.2. Individual differences in freezing and associations with HCC and trait anxiety

Next, we report our analyses of main interest: the BLMMs testing whether individual differences in freezing-related reactions are predicted by HCC and trait anxiety. For significant predictors, we additionally checked whether these relations were model-specific or could also be captured in simple bivariate correlations.

Body sway. Stronger body sway reductions were associated with lower HCC independent of threat magnitude ($B = 32.15$, 95% CI [7.11, 57.17]). Figure 3 also suggests an interaction between cue (high/low threat) and trait anxiety with more freezing particularly under threat (see figure 3) but the interaction fell short of reaching significance ($B = 7.24$, 95% CI [-0.54, 15.21]).

Follow-up bivariate correlational analyses (see Supplementary figure 3) confirmed that subjects with more body sway reductions showed lower HCC ($R_s = 0.13$, $p = 0.01$ $N = 341$, see Supplementary figure 3a). Once more, there was a relation between body sway reduction and higher trait anxiety (for high vs. low threat task conditions) ($R_s = -0.10$, $p = 0.04$ $N = 417$, see Supplementary figure 3b). Subsequent follow-up analyses indicated that the associations for body sway with HCC and trait anxiety were specific for the high threat condition ([HCC:] $R = 0.11$ $p = 0.05$; [anxiety:] $R_s = -0.10$, $p = 0.04$). Thus, increased threat-

induced bodily freezing was associated with blunted hair cortisol and increased trait anxiety. There was no association between HCC and trait anxiety ($R_s = -0.06$ $p = 0.26$ $N = 343$).

Heart rate. BLMM for threat-anticipatory heart rate reductions showed no significant relations with HCC or trait anxiety ([HCC:] $B = 0.08$ $CI [-0.24\ 0.41]$); [trait anxiety:] $B = 0.002$ $CI [-0.32\ 0.33]$, see figure 3).

To conclude, individual differences in freezing-related reductions in body sway -but not in heart rate- were predicted by long-term HCC and trait anxiety, factors that were previously linked to anxiety-related vulnerability. All results remained unchanged after including potential confounding factors (see supplementary tables 2 and 3 for all details).

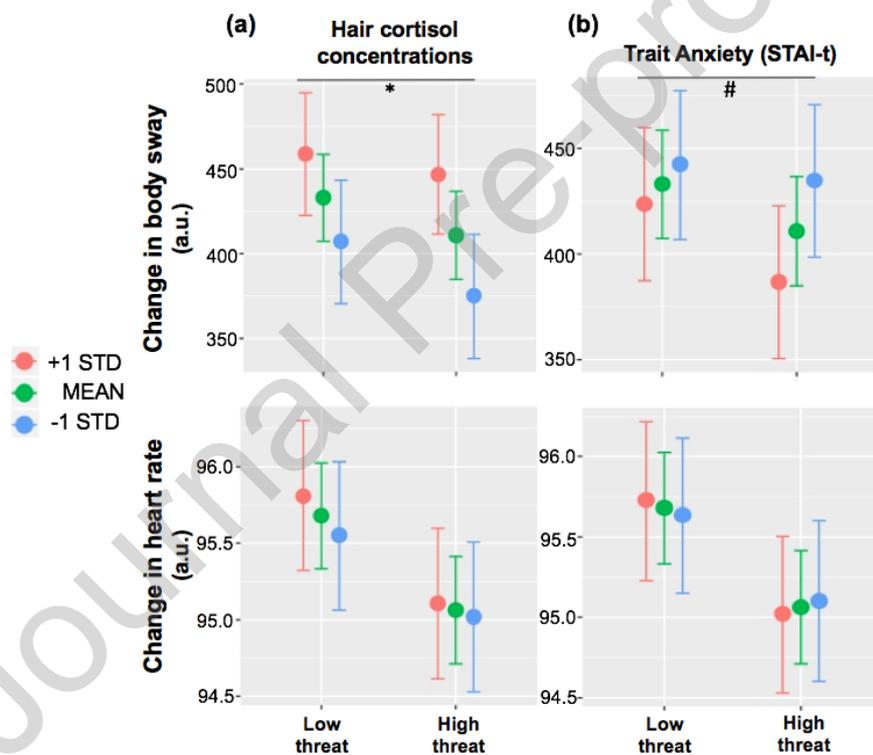


Figure 3. BLMM results showing the relations between each predictor (a: hair cortisol concentrations, b: trait anxiety) and our indicators of freezing: body sway (upper panel) and heart rate (lower panel). For each association, the mean predictor value across subjects is shown in green, relatively high predictor values in red [1 SD above mean] and relatively low predictor values in blue [1 SD below mean]. All between-subject fixed effects illustrated here were scaled and centered for accurate modelling results. Consequently, the scale of the axes is in relative (arbitrary) units (a.u.). Low values on the Y-axis reflect stronger freezing. (a) Individuals with lower hair cortisol concentrations showed stronger reductions in body sway (upper panel) but not stronger reductions in heart rate (lower panel) independent of the level of threat. (b) Subjects with higher trait anxiety showed (at trend) stronger reductions in body sway for high (compared to low) threat conditions. There were no such relations for heart rate (lower panel).

4. Discussion

The aim of this study was to reveal whether individual differences in human freezing reactions relate to two important predictors of anxiety-related psychopathology: HCC and trait anxiety. After verifying that our threat manipulation produced the expected freezing effects, our findings demonstrate that stronger threat-induced body sway reductions were robustly related to lower accumulated HCC and higher trait anxiety. Together, these results show for the first time in a well-powered human sample that postural freezing in humans may be related to stable long-term markers of stress-relevant coping.

Our findings replicate previous findings on long-term accumulating cortisol levels in primates, demonstrating that lower HCC levels are associated with stronger postural freezing (Hamel et al., 2017). In contrast to our current findings and the findings on HCC by Hamel et al. (2017), some previous studies on the relation between freezing and acutely circulating basal cortisol levels indicated that stronger freezing was associated with higher cortisol levels (Kalin et al., 1998; Niermann et al., 2017a). Two factors may account for these differences. Firstly, Niermann et al. (2017a) distinguished acute and delayed freezing reactions after stress-induction and found that only the *delayed recovery* of freezing -one hour after stress-induction- (and not the magnitude of the acute freezing reaction), was related to internalizing symptoms. Interestingly, this prolonged freezing due to delayed recovery combined with lower basal cortisol levels was related to internalizing (anxious and depressive) symptoms. Secondly, the contrasting findings for acutely circulating cortisol and HCC could be due to the different time scales of the cortisol assessments. Acutely circulating cortisol levels from saliva, plasma, or urine stand in clear contrast with the accumulated cortisol concentrations of several weeks or even months in hair. As such, HCC can be viewed as a long-term, more state-independent marker of HPA axis functioning. Apparently, when considering this long-term marker, we see a picture emerging from our data and the previous primate data (Hamel et al., 2017) that low basal cortisol levels are associated with stronger freezing.

The aetiology of having lower cortisol levels as we observed in individuals with stronger bodily freezing is unclear. Currently, it is generally acknowledged that acute stressors increase cortisol levels acutely, whereas chronic stress may also lead to a general decrease in HPA axis activity (Miller, Chen, & Zhou, 2007; Steudte-schmiedgen, Kirschbaum, Alexander, & Stalder, 2016). This lowered HPA axis functioning, or hypocortisolism, has been suggested to result from two potential pathways. On the one hand, hypocortisolism may signal a stress-vulnerable phenotype (Fries, Hesse, Hellhammer, & Hellhammer, 2005). On the other hand, hypocortisolism may be the result of compensatory mechanisms in which an enhanced negative feedback loop due to acute high levels of cortisol prevents overshooting of chronic high cortisol levels (Kanter et al., 2001;

Yehuda, Halligan, Grossman, Golier, & Wong, 2002). Interestingly, a study by Frank et al. (2006) found that a high anxiety genetic strain of mice that showed strong freezing responses had rather low levels of ACTH and corticosterone compared to a low anxiety genetic strain with more active coping styles and high HPA axis activity. Further investigation is needed on whether low HPA axis activity and strong freezing signal an inability to adequately cope with acute threat, e.g. by preventing a flexible shift to more active coping behaviours like fight-or-flight. In our healthy sample we did not find any relation between trait anxiety and HCC levels either.

In addition to the association with HCC, we found a modest relation between stronger threat-related postural freezing and higher trait anxiety (at trend in the BLMM and significant in the simple correlation). This result is in line with previous work indicating more pronounced freezing reactions in individuals with higher anxiety (Frank et al., 2006; Niermann et al., 2017a; Roelofs et al., 2010). Previous associations were based on relatively small samples and passive tasks where active threat coping was not an option. We therefore present the first evidence that individual differences of anticipatory freezing reactions are linked to trait anxiety, also in more dynamic and ecologically-valid environments where active responding enabled preventing threat. While our study tested a healthy sample, trait anxiety is one of the strongest dimensional predictors of anxiety and depression symptoms (Knowles & Olatunji, 2020).

The link between stronger postural freezing and trait markers of stress vulnerability may suggest that excessive (in this case, postural) freezing itself, more than bradycardia, constitutes a somatic marker of stress vulnerability. Altered patterns of freezing in vulnerable individuals with stress-related symptoms were reported in previous studies. In non-clinical healthy samples, experiencing aversive life-events as well as anxiety were associated with *increased* freezing reactions (including both reductions in body sway and heart rate, Hageraars et al., 2012; Roelofs et al., 2010). In contrast, clinical samples of PTSD patients were shown to express *reduced* freezing reactions (reduced bradycardia in Adenauer, Catani, Keil, Aichinger, & Neuner, 2010; reduced body sway and bradycardia in Fragkaki, Roelofs, Stins, Jongedijk, & Hageraars, 2017; Orr & Roth, 2000). This is thought to result from increased hyperarousal and excessive sympathetic reactivity that suppress the adaptive, preparatory parasympathetically-dominated freezing reaction. By this, the hierarchically organized defence cascade may be dysregulated as it does not shift from initial preparatory freezing to fight-or-flight actions (Lang, Davis, & Öhman, 2000; Mobbs, Hagan, Dalgleish, Silston, & Prévost, 2015). Accordingly, one could argue that increased freezing reactions in our healthy sample may signal heightened responsivity to threat that is still adaptive. However, due to the relation between postural freezing and predictors of stress vulnerability (low HPA axis activity and trait anxiety), increased postural freezing may reflect a

heightened vulnerability to future challenges or traumatic experiences. As such, we speculate that traumatic life-experiences would turn this heightened threat responsivity into immediate sympathetic activations, which may lead to reduced freezing-related reactions as described in PTSD patients (Adenauer et al., 2010). Increased sympathetic arousal may heighten the threshold for obtaining a parasympathetically-dominated state that is typical for the freezing response. Future longitudinal research is needed to address this issue within a prospective framework, as well as in clinical samples, to explore whether threat-enhanced freezing and low HPA axis activity are linked to resilient or maladaptive stress responding.

We note some limitations of our study. Firstly, the effect size of the observed correlations is small by traditional standards (i.e., coefficients between 0.1-0.15). Recent meta-analyses however show that traditional guidelines for interpreting correlation coefficients may have been too stringent as most of the associations reported are small (Gignac & Szodorai, 2016). A potential reason for this is that observed correlations between experimental measures are always attenuated by the imperfect measurement reliability of the measures used, resulting in a lower bound of the true association (Hedge, Powell, & Sumner, 2017; Vul, Harris, Winkielman, & Pashler, 2009). Furthermore, evidence from simulations show that increasing sample size may be associated with decreasing correlation coefficients, and that a large sample size (e.g. $N > 250$) may therefore lead to effects that are perceived as small but in fact capture the true effect size more accurately (Schönbrodt & Perugini, 2013). Since we were able to show that all but one of the correlations remain significant even when we perform a split-half cross validation (see supporting information), the correlations found within our large sample should be considered robust. While the small effect size makes these associations of limited immediate clinical use, they still expand theoretical insights into the potential mechanisms of freezing and anxiety.

A second limitation of our findings is that all associations found in relation to hair cortisol and anxiety were specific to body sway reductions. Thus, results did not generalize to concomitant bradycardia responses, which in some studies are taken as a proxy for freezing when measurements of body sway are not possible (e.g. neuroimaging environments, Hashemi et al., 2019; Wendt, Löw, Weymar, Lotze, & Hamm, 2017). Our results appear to point to the specificity as well as sensitivity of body sway reductions when it comes to predicting stress vulnerability. However, previous associations between individual differences in stress vulnerability and freezing reactions were not consistently associated with only body sway or heart rate (Hagenaars et al., 2012; Niermann et al., 2017a). This variability may be due to task demands (active vs. passive tasks) or sample-specific factors. For example, the previous investigations were done in samples predominantly consisting of female university students and assessed freezing in passive picture processing paradigms. Given the consistent relationship found between heart rate

and body sway reductions during threat-anticipatory freezing reactions, and the fact that both measures were found to have a comparable measurement reliability in our study (as described in the methods section), future research should further explore the specific contributions of body sway and bradycardia during anticipatory freezing reactions (Niermann et al., 2017a; Roelofs et al., 2010).

Lastly, our study is part of a larger study among police recruits (see preregistration Koch et al., 2017), and therefore our sample consisted predominantly of a group of young, male police recruits, who were selected on various resilience characteristics including relatively low trait anxiety (as compared to normative values, Spielberger et al., 1983). Although we controlled for gender effects in the analysis and there is, at least to our knowledge, no evidence for gender differences in freezing reactions, future studies need to confirm the generalizability of our results to females and non-selected civilians with greater variability in anxiety and stress symptoms.

5. Conclusion

Individual differences in human postural freezing as assessed by body sway reductions were related to potential stress vulnerability factors indexed by lower HCC and higher trait anxiety. This implies that basic defensive reactions such as postural freezing may mechanistically relate to HPA axis changes and maladaptive threat processing that are implicated in anxiety disorders.

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Author information

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Data availability. The datasets analysed during the current study are available from the corresponding authors upon reasonable request.

Code availability. The code that has resulted in the reported findings is available from the corresponding authors upon reasonable request.

Competing interests. All authors declared no competing interests.

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Highlights

- Cohort study (N=419) on individual differences in defensive freezing in relation to markers of anxiety-related psychopathology including accumulated hair cortisol concentrations (HCC) and trait anxiety.
- Freezing-related reactions were assessed with posturographic and heart rate measurements during an active shooting task under threat of shock.
- Individual differences in postural freezing, but not heart rate, in response to acute threat were related to lower HCC and higher trait anxiety.
- Defensive freezing may constitute a promising somatic marker for stress- and anxiety vulnerability.

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