



Research paper

Ready and waiting: Freezing as active action preparation under threat

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HIGHLIGHTS

- Freezing is an essential defensive response often studied using passive viewing tasks.
- We developed a shooting task manipulating threat and action preparation.
- Freezing was operationalized using heart rate and body sway measurements.
- Freezing was found to be strongly related to the ability to respond.
- Freezing is a state of active preparation for a possible fight/flight response.

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ABSTRACT

Freezing is a defensive response characterized by rigidity and bradycardia, but it is unclear whether it is a passive versus active preparatory state. We developed a shooting task in which preparation and threat were manipulated independently: Participants were either helpless or able to respond to a possible upcoming attack, and attacks were either associated with an electric shock or not. Essentially, a purely anticipatory preparatory period was used during which no stimuli occurred. Freezing was assessed during this period. In addition to heart rate, body sway was measured, using a stabilometric force platform. The efficacy of the threat manipulation was confirmed via self-report. The ability to prepare led to decreases in heart rate and postural sway, while threat led to decreased heart rate. Further, exploratory analyses suggested that aggressive participants showed reduced initial freezing for threatening opponents, but increased postural freezing when armed. The results suggest that freezing may involve active preparation. Relations to results in passive viewing tasks are discussed.

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Freezing is a defensive response that occurs on the detection of relatively distant threat, in which an animal is immobile, shows reduced heart rate, and is highly vigilant towards the threatening stimulus [1–3]. In contrast to passive tonic immobilization (“playing dead”), freezing in the sense of “attentive immobility” may actively prepare the animal for further defensive responses [4–6], as suggested by increased rather than decreased startle responses during freezing [7,8]. Freezing is preserved in humans as shown, first, by self-report in simulated [9] and actual threatening or traumatic situations [10–12]. Second, freezing has been experimentally studied using physiological measures: Aversive stimuli evoking fear of physical injury [13–16] as well as social threat [17] are associated with freezing as measured via reduced heart rate and

body sway. Freezing has recently been shown to evoke a shift in the perception of stimuli with low versus high spatial frequency [18], which was interpreted in terms of freezing being a preparatory state aimed at optimally countering threat.

Despite this possible functional role of freezing as active and attentive preparation, studies of freezing in humans have as yet focused on passive viewing tasks. Therefore, the current study was designed to study freezing in an active context. We developed a shooting task with trials in which participants were confronted with either a safe or a threatening opponent, who performed an attack (drawing a gun and subsequently shooting) or a non-attacking action (holding up a phone) after a preparation interval. Threat was manipulated by having a successful attack by the threatening opponent be followed by an electric shock. Active versus passive preparation was manipulated by providing the participant with a gun or leaving them unarmed. Being armed allowed the participant to shoot the opponent before his attack was completed,

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if the response was made quickly enough after the initiation of the enemy's attack. This enabled us to study the time course of freezing during the preparation interval as a function of threat and response availability. This is somewhat similar to an interesting study published recently, in which participants could or could not actively avoid an approaching threat [19]. Freezing was found to increase when subjects could not actively avoid threat. However, while in that study task-relevant stimulus changes occurred during the period in which freezing was assessed, in the current study the preparation interval does not involve any dynamic changes in stimuli: Subjects are in a "pure" state of anticipation, and only at the end of the preparation interval does any further stimulus event occur. This enables us to focus on anticipatory activity unrelated to the occurrence of salient stimulus changes. In the Löw et al. study, a possible interpretation of the threat-related physiological changes is that they occurred due to stimulus changes. Thus, an essential difference between the studies is that the current study assessed the specific psychophysiological and bodily effects during the anticipation of a threat stimulus, rather than in response to the changing stimulus properties.

The current study is aimed at the study of freezing in the context of an anticipatory period, during which no stimulus changes occurred but subjects could prepare to respond to an upcoming possible attack. Freezing was assessed while participants were performing the shooting task, under four conditions defined by two factors: Action preparation (Active versus Passive conditions) and Threat (Threat versus Safe conditions). In addition to heart rate, body sway was measured, using a stabilometric force platform. A secondary aim of the study was to extend the literature on relationships between freezing and individual differences from the passive to the active context; to this aim we explored correlations between measures of freezing and anxiety and aggression. Individual differences in freezing have been shown to be related to individual differences in human research on freezing. More trait anxious participants show increased freezing when confronted with angry faces [17], and participants with experience of traumatic life events exhibit more freezing in response to aversive stimuli [14]. We aim to extend these results in the current study. However, it is not certain that freezing is necessarily related to anxiety specifically. As described above, freezing is a functional defensive response that may marshal resources to respond to threat in general, not just "cower in fear". To further typify what freezing is and how it should be conceptualized, we therefore also tested relationships between measures of freezing and aggression. Taken together, the current study thus allows a first comparison of two different views of freezing in the context of preparation under threat: freezing as a passive state more likely to be shown by anxious participants in helpless conditions, versus freezing as active preparation more likely to be seen in aggressive participants when they are able to fight back.

1. Method

1.1. Participants

30 students at the Radboud University Nijmegen performed the study for course credit or financial compensation. The group consisted of 16 females and 14 males, mean age 24.6 (SD = 7.8). All participants had normal or corrected to normal vision. Inclusion criteria as assessed by self-report were no past or present psychiatric or neurological condition. The study was approved by the local ethics committee and all participants gave written informed consent. The sample size was based on the range found in similar previous studies and on power analyses which indicated sufficient power for the primary within-subject comparisons and exploratory

between-subject correlational analyses. There was no stopping-rule that allowed data collection to be stopped before completion.

1.2. Materials and procedure

At the start of the experiment, participants completed a number of questionnaires, of which a Dutch translation of the STAI-T measure of trait anxiety [20,21] and a Dutch version of the STAXI measure of trait aggression [22,23] are reported in the current paper. The State-Trait Anxiety Inventory (STAI; [20]) is used for the assessment of current ('state') and general ('trait') levels of anxiety, of which the trait level was used in the current study. The Dutch aggression questionnaire provides an overall measure of an individual's tendency to lose his or her temper or describe themselves in terms of irritable or hot-headed. This was assessed using the Spielberger Trait-Anger expression inventory, a 10-item subscale of the State-Trait Anger Expression Inventory [22]. Respondents rate the degree to which they react in an angry fashion from 1 (almost never) to 4 (almost always), and responses are summed for a global score. The scale has been shown to have good psychometric properties and there is good support for the measure's construct validity [20].

Following the questionnaires, heart rate and shock electrodes were attached to the participants. The electrocardiogram was measured using a BioPac MP150 system sampling at 200 Hz. Shocks were administered to the second and third fingers of the left hand using a Digitimer Constant Current Stimulator DS7A (www.digitimer.com) and standard Ag/AgCl electrodes. The shock consisted of 1 ms positive current followed immediately by 1 ms negative current. Shock intensity was adjusted to be uncomfortable but not painful per participant.

Participants then removed their shoes and stepped onto a stabilometric balance board to perform the shooting task, after a baseline measurement of the sensor values for the empty board. The board was a custom-made 1 m × 1 m stabilometric platform, of which the pressure at each of its corners was sampled at 200 Hz. The baseline measurement allowed sensor values to be converted to Center of Pressure position, by, per sensor, subtracting the baseline and dividing by the total effect of the subject's weight on the board. Participants were given a joystick to be used as a response device (of which only the button pressed via the index finger was used). Participants held a joystick in their right hand, with the right arm bent and pointing forward and with the left hand supporting the joystick. Responses were given by pressing the trigger fire-button on the stick with their right index finger.

1.3. Shooting task

The shooting task (illustrated in Fig. 1; see Supplementary materials for the visual stimuli of all possible trial sequences) consisted of an introduction, training, and measurement phase. In all phases, the screen showed a view of a parking garage, with an opponent character in the center of the screen, an armed policeman in the background (alternatively on the left or right of the screen per block), and a view of the participant's own "in-task" hands, holding a gun or not. Essentially: There were two opponents, who could be easily visually distinguished. As explained in more detail below, both opponents behaved identically: They both sometimes drew a gun to shoot the participant. However, for one of the opponents, the participant would receive an electric shock when being shot by that opponent. For the other opponents, the participant would not receive the electric shock when being shot, but would see the same visual stimulus of the opponent shooting and was still instructed to try to avoid being shot when possible.

In the *introduction* phase, participants were exposed to the meaning of Safe and Threat opponents and the Armed and Unarmed conditions. First, four trials were presented in which the partici-

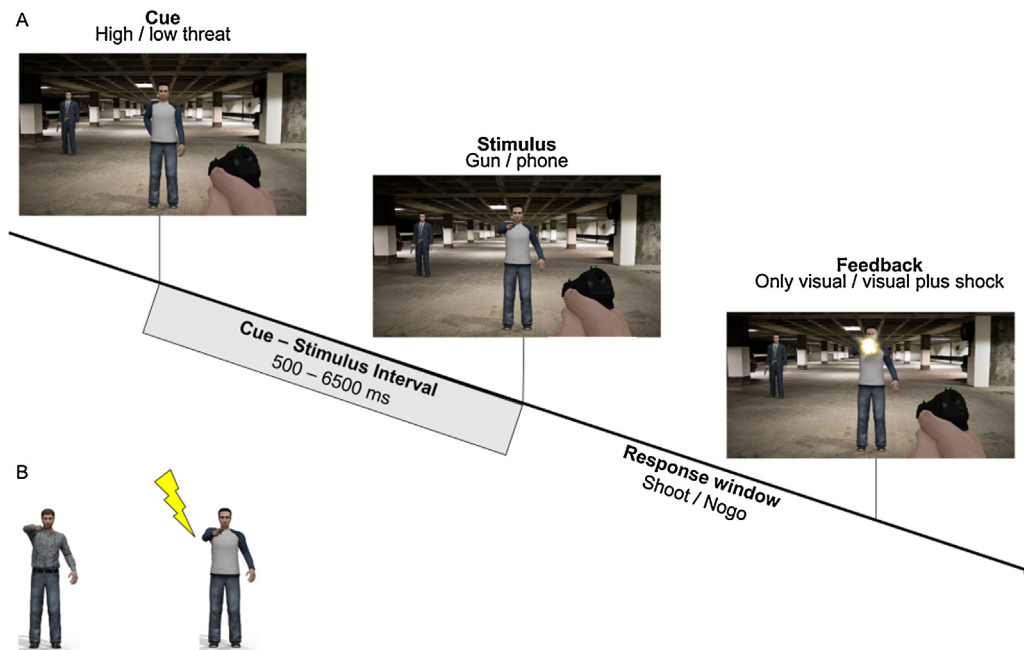


Fig. 1. Illustration of the task.

Note A. Trials began with the appearance of the cue, i.e., one of two opponents. This was followed by a variable cue-stimulus interval of up to 6.5 s. After this interval, the opponent took one of two actions: he either drew a gun (the Go stimulus) or held up a mobile phone (the NoGo stimulus). When the gun was drawn, the opponent would shoot after a brief delay, seen as a gun-flash. For one of the two opponents (the Threat opponent), being shot was associated with the participant receiving an electric shock. For the other opponent (the Safe opponent), only the onscreen visual feedback of the opponent firing was presented. This is illustrated in sub-figure B; which opponent was associated with shocks was randomly varied over subjects. On each trial participants could be armed or unarmed themselves. If they were armed, they had the ability to shoot an opponent as soon as he drew the gun, thereby avoiding being shot if they were fast enough. If the participant shot at any other time (i.e., after the opponent took action but pulled out a phone, or before the opponent did anything), the policeman in the background would always shoot the participant. A figure showing the graphics for all events in the task is provided as Supplementary material.

participant's hands were empty and held up to each side: this represented the Unarmed condition, in which no response could be made. Trials began with the appearance of one of two opponents (the cue). After a variable interval, the opponent drew a gun and, after a brief shooting delay, fired at the participant. For one of the two opponents (the Threat cue), this firing action was associated with a simultaneous electric shock; for the other opponent (the Safe cue), the same visual feedback of the gunshot was shown but without a shock. The difference between the Threat and Safe opponent was verbally explained to participants and after the introduction phase it was checked whether participants understood the shock contingency.

In the *training* phase, participants performed two blocks of 120 training trials in which the full task was performed (pilot work had shown that such training was important for differential physiological effects of the Safe versus Threat opponent). On each trial, the Safe or Threat opponent appeared, and the participant was shown to be either Armed or Unarmed; these four conditions were presented in randomized order in sequences of four trials. Following the appearance of the opponent (cue), a short (60%), middle (20%), or long (20%) cue-stimulus interval (CSI; see Fig. 1A) was presented, until the opponent either drew a gun or a phone (the stimulus). Short intervals were between 500 and 1500 ms, middle intervals were between 1500 and 6000 ms, and long intervals were between 6000 and 6500 ms. The distribution of CSIs was chosen to allow relatively many training trials in a relatively brief period. After the interval, the opponent performed one of two actions: he was either shown to hold up a mobile phone (the NoGo stimulus), or to pull out a gun aimed towards the participant (the Go stimulus). In the latter case the opponent would fire the gun after a brief delay. In trials in which the participant was armed, this delay provided an opportunity to shoot the opponent and avoid being fired upon. Incorrect responses (either on the opponent holding up a phone, or prior to any action by the opponent), were punished by the policeman

depicted in the background immediately firing on the participant, in order to avoid strategic false-positive responding. This attack was associated with a shock if and only if the opponent's attack would also have been associated with a shock. Thus, participants had to fire back if and only if the opponent drew a gun. The time interval between the opponent drawing a gun and firing was titrated (over both opponent types) so that participants would be able to avoid the shot 50% of the time (note, to clarify: This interval, between drawing the gun and firing it, is independent of the Cue-Stimulus Interval between the appearance of the opponent and the opponent taking an action).

The final *measurement* phase consisted of 5 blocks of 30 trials each in which the full task was performed at a slower pace: The cue-stimulus interval was now distributed as 20% short, 20% middle, and 60% long, in order to acquire a sufficient number of long-interval trials for the analysis of the time course of freezing measures. The short and middle intervals were still presented so that attacks could occur throughout the interval. The inter-trial intervals were set to vary in between 5–8 s.

After completing the shooting task participants filled in post-experimental questionnaire about their subjective responses to the task using the nine-point Likert scales of the self-assessment manikins [24] as follows. To assess awareness of the experimental contingencies, participants were asked which of the two aggressors in the task was associated with shock and which was not, and how confident they were about that. For each of the aggressors, participants scored their motivation to shoot that character and rate its valence, arousal, and dominance.

1.4. Analyses

All preprocessing and analyses were performed using Matlab [25]. Differences in self-report measures between the Safe and

Threat cues were tested using paired *t*-tests, in order to check whether the effect of the manipulations was as expected. Although the current study was not specifically designed to assess behavioral effects, we report reaction times and accuracy for the trials included in the freeze analyses ($CSI > 6s$). The mean accuracy was calculated for Threat versus Safe trials, for Phone and Gun trials separately, for trials on which the participant was armed. Median reaction times were calculated for Safe & Gun and Threat & Gun trials, for trials on which the participant was armed and responded before being shot. Effects of threat were tested using a paired *t*-test.

Raw body sway was calculated as the standard deviation of the center of pressure in the anterior-posterior direction in overlapping 2000 ms windows covering the cue-stimulus interval from 0 to 6000 ms post-cue (i.e., the first appearance of the opponent in that trial). Successive windows were shifted by 100 ms, providing information on the time course of physiological effects and allowing time-dependent effects to be detected. The median sway per time window was calculated over trials for each subject, to avoid effects of trials with outlying values and the need to select arbitrary outlier cut-offs. Results using the mean and outlier-trial rejection resulted in similar but more noisy results.

We also analyzed body sway after band-pass filtering in the 1–7 Hz frequency band (using a 3rd order Butterworth bandpass filter implemented in the *butter* function of the Mathworks Signal Processing Toolbox). This was done due to the possibility that the preprocessing step of filtering may improve the ratio of relevant signal to noise: slow drifts versus faster oscillations could well capture different physiological effects. Previous literature on body sway has also studied effects in roughly this range [26], although the specific range presented in the current paper must be considered exploratory. However, we note that the results were not highly sensitive to the precise range limits, as using a 1–10 Hz range resulted in highly similar results.

Heart rate was calculated as the inter-beat interval of the ECG. Identically to the procedure for body sway, each subject's median heart rate over trials was calculated per 2000 ms time window of the cue-stimulus interval.

Heart rate and body sway data were analyzed using a sliding windows analysis, following a repeated measures ANOVA for the average of the signal over the whole preparation interval (0–6 s), with factors Armed (armed versus unarmed) and Threat (threat versus safe). The sliding windows analyses were performed as follows. For each 2000 ms time window of the cue-stimulus interval for trials with long (6000–6500 ms) intervals, paired *t*-tests were performed for main effects of threat and armed and their interaction, as well as for effects of threat for the armed and unarmed condition separately. Each time window was measured relative to a baseline period from 1000 ms to 0 ms before the trial's cue. Multiple comparison over time windows was corrected as follows, analogously to cluster analysis in fMRI [27–29]: Using permutation tests of the data in which condition was randomized, we found that the length of contiguous time windows with a nominally significant effect was above nine for heart rate and four for balance board in less than 5% of the iterations. Effects are therefore only reported if they were significant in more than nine consecutive time windows for heart rate and four consecutive time windows for body sway. We acknowledge that the use of paired *t*-tests to test vectors of contrast scores does not provide the protection of an omnibus *F*-test over those contrasts that represent main effects and interactions. However, in the current analyses the time course of effects is of primary importance, and the time course of the omnibus test does not have a clear relationship to the time course of the contrasts of interest; the different contrast scores must therefore be treated as conceptually independent tests, similarly to tests involving different physiological variables.

Individual differences were non-parametrically tested using Spearman's correlations between body sway and heart rate, and scores on the STAI and STAXI questionnaires. Individual differences were calculated as the sum score of the items of the questionnaire, recoding the score for items with a reversed meaning such that high scores reflect higher levels of trait anxiety and aggression on the respective questionnaires. Scores were analyzed as continuous variables.

2. Results

2.1. Subjective measures

Two participants did not fully complete the post-experimental questionnaire, leaving 28 participants for analyses. All participants correctly reported which enemy was the Safe and which was the Threat cue. The threat manipulation was effective in terms of self-reported subjective effects: Participants reported that their emotional response to the Threat versus the Safe enemy was an increased motivation to shoot ($t(27)=6.679$, $p<0.001$), more negative valence ($t(27)=4.54$, $p<0.001$), and increased arousal ($t(27)=6.256$, $p<0.001$); there was no difference in Dominance ratings for the Threat versus the Safe enemy. Participants reported a high level of being aware of the armed – unarmed manipulation (8.5, $SD=0.17$, for being armed; 8.6, $SD=0.13$, for being unarmed), indicating that the visual feedback indicating the armed/unarmed factor was effective.

2.2. Heart rate

Fig. 2A shows heart rate during the cue-stimulus interval. Repeated measures analysis of mean heart rate over the whole preparation interval showed a significant effect of being Armed ($F(1, 29)=33.36$, $p<0.001$).

In the sliding windows analyses, a main effect of Threat was found in the later part of the preparation interval ($p<0.05$ over consecutive time windows covering 2.1–4.3 s and 4–6 s). A main effect of Armed versus Unarmed was found over all tested time windows from 0.1 s post-cue onwards ($p<0.05$), due to a decrease in heart rate when subjects were armed and hence able to prepare a response. The interaction between Armed and Threat was not significant. However, under the hypothesis that freezing is related to action preparation, threat effects within the Armed condition are of particular interest, even though the interaction did not reach significance. We therefore performed additional follow-up analyses, in which effects of threat were tested within the armed and unarmed conditions separately. Indeed, threat had a significant effect (in terms of the length of the sequence of nominally significant time points) within the armed condition only, not in the unarmed condition. This conditional threat effect for armed trials was more robust than the main effect of threat in terms of coverage of time windows (involving almost all windows covering 2.1–6 s). Clearly, any interpretation of this result must be tentative. No correlations with trait aggression or trait anxiety were found for heart rate effects.

Please note that, when visualized together with the very strong preparatory effect, the absolute size of the significant effect of threat may seem relatively small; however, it is in the same range as usual threat-related bradycardia effects.

2.3. Body sway

Raw body sway decreased during the later phase of the cue-stimulus interval, but no significant effects of Threat versus Safe or Armed versus Unarmed were found, nor interactions of contrasts with individual difference variables.

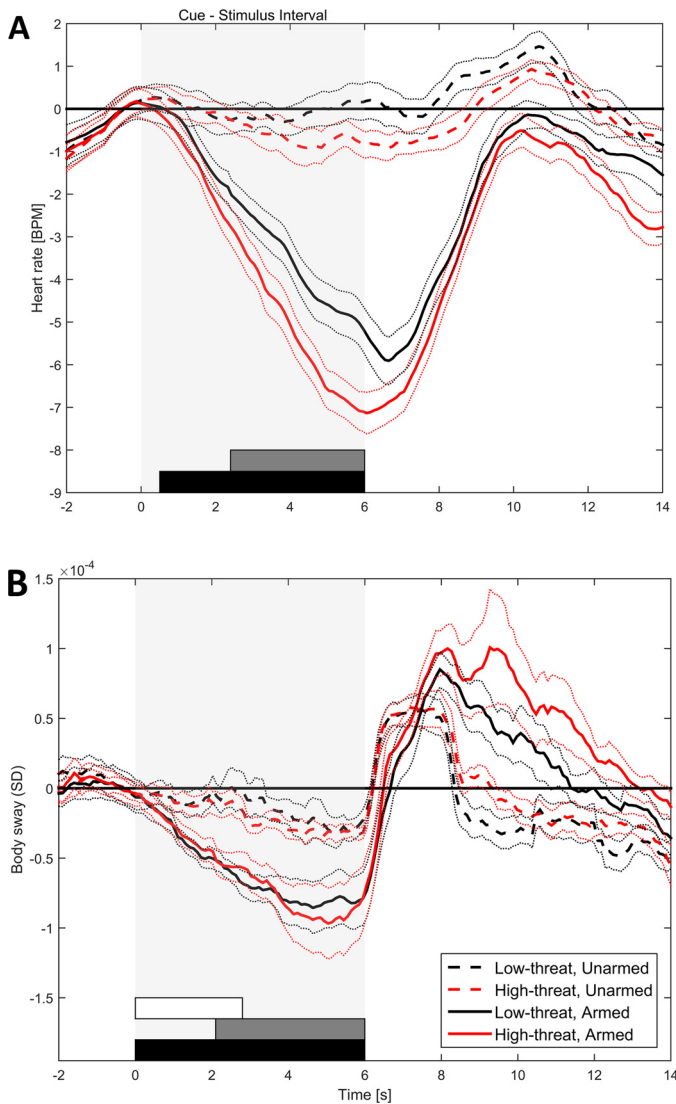


Fig. 2. Measures of freeze during the cue-stimulus interval.

Note Time course of freezing measures for the four preparatory conditions: Threat versus Safe opponent, and Armed versus Unarmed participant. The subplots show heart rate (A) and 1–7 Hz body sway (B). The shaded area indicates the start and earliest end of the cue-stimulus interval: At time 0, the opponent appeared, and the opponent acted between 6 and 6.5 s. The black, gray and white bars indicate intervals in which contiguous time windows with significant differences were found. In subplot A, the black interval contained effects of armed versus unarmed, and the gray interval contained effects of threat. In subplot B, the black interval contained effects of armed versus unarmed; in the gray interval more aggressive participants showed enhanced freezing on the armed versus unarmed contrast; and in the white interval more aggressive participants showed reduced freezing on the threat versus safe contrast.

Body sway in the 1–7 Hz range (Fig. 2B) showed a far clearer preparatory decrease for the Armed versus Unarmed condition. Repeated measures analysis of mean body sway over the whole preparation interval showed a significant effect of being Armed ($F(1, 29) = 8.06$, $p = 0.008$).

In the sliding windows analyses, significant differences of the Armed factor were found in all time windows covering the interval up to 5.3 s ($p < 0.05$). No main effect of Threat or Threat \times Armed interaction was found, nor did exploratory analyses show simple effects of threat within the Armed or Unarmed condition separately. However, more aggressive participants showed less Threat-evoked body sway reduction early in the cue-stimulus interval, up to 2.8 s ($r > 0.37$, $p < 0.05$); only the window around 0.9 s did not show a significant effect. Further, later in the cue-stimulus

interval, the reduction in body sway in the Armed versus Unarmed condition was stronger in more aggressive participants (time windows from 2.1 s to 5.3 s and 4.4–6 s post-cue, $r < -0.37$, $p < 0.05$).

2.4. Behavioral performance

Mean accuracy was 0.808 (SD = 0.03) for Safe & Phone trials, 0.758 (0.03) for Threat & Phone trials, 0.407 (0.02) for Safe & Gun trials, and 0.528 (0.03) for Threat & Gun trials (recall that the titration procedure was aimed at a 0.5 accuracy over all Gun trials). On Gun trials, responses were more likely to be accurate (i.e., the participant shot fast enough) on Threat than on Safe trials ($t(29) = 2.86$, $p = 0.008$). No effect of threat on reaction time was found (Safe trials: 329 ms, SD = 10; Threat trials: 322 ms, SD = 7). As the distribution of the RTs was clearly non-normal from visual inspection and descriptive measures (Safe: skewness 2.595, kurtosis 9.970; Threat: skewness 1.963, kurtosis 4.481) the Wilcoxon signed rank test was also performed, also showing a non-significant threat effect ($p = 0.765$).

3. Discussion

To our knowledge very few previous studies have addressed the relationships between threat, response availability, and human freezing measures during active preparation [but see 19], in particular not the time course of heart rate and body sway effects over a purely anticipatory preparation interval containing no stimulus events. Freezing was therefore studied from the perspective of its evolutionary role as active preparation, using body sway and heart rate measurements during a novel shooting task in which subject were confronted with potential attackers, one of which had the potential to cause an electric shock. Self-report measures indicated that the threat manipulation was effective, and threat evoked increased preparatory heart rate deceleration, but no differential effect of threat was found on body sway. Most importantly, pronounced bradycardia and reductions in body sway were found during a preparatory period when subjects had the ability to respond to a possible attack versus when they were helpless. Furthermore, effects on body sway were found to be related to individual differences in aggression. More aggressive participants showed stronger reductions in body sway towards the end of the preparation period when armed versus unarmed.

The results show that the measures typically used to operationalize freezing in humans – bradycardia and reduced body sway – are both strongly related to preparatory state. This is in accordance with an active view of freezing, in which the animal is not waiting passively or only orienting, but is maintaining a state of preparation for action. Note that the hypothesis that freezing has an active preparation component does not exclude the possibility that freezing can occur when no response is objectively possible (e.g., in a passive viewing task). Intuitively, when we experience aversive stimuli that do not convey any real physical or social threat, we may nevertheless prepare for action, despite there being no rational reason to do so. Such reactions may reflect potentially dysfunctional automatic preparation, in which contextual information does not fully inhibit unnecessary preparation.

In further support of the view of freezing as active rather than passive, the results involving individual differences could have painted a more passive picture, with stronger freeze in the unarmed condition for anxious participants. However, interestingly, aggressive participants showed less postural freeze for threat compared to safe opponents relatively early in the cue-stimulus interval. This effect could be tentatively interpreted as a reflection of an orienting aspect or phase of freezing, as opposed to the active preparation that increases towards the end of the interval. In that case, the data

would suggest that aggression involves reduced threat-evoked orienting and enhanced reactivity to the availability of a response [30,cf.31]. Clearly further research is needed to disentangle such possibilities and deal with the reverse inference problem of having the same measure being sensitive to different underlying processes under different conditions and times [cf.32].

The current results are also interesting from the perspective of parasympathetic and sympathetic contributions to freezing. During threat exposure, the balance between activation of the sympathetic and parasympathetic branches of the autonomic nervous system shapes the expression of defensive behavior [1]. Whereas sympathetic dominance facilitates active fight-or-flight reaction, parasympathetic activity underlies freezing [1]. A defining characteristic of freezing in both humans and animals is a transient, parasympathetically driven, deceleration of heart rate or fear bradycardia [13,17,33]. Fear bradycardia is thought to support a state of reduced body motion and thereby decreases the chance of being detected by predators [33]. However, although freezing-related bradycardia indicates dominance of the parasympathetic nervous system over the sympathetic nervous system [1,e.g.,13,16,33,34], this may involve overruling rather than inhibition of sympathetic activation. That is, an adaptive need to both reduce body movements and be prepared for action could involve a pattern of concurrent activation of the sympathetic and parasympathetic nervous system, yet with parasympathetic dominance. Indeed, in a previous imaging study on freezing in a passive viewing context, both bradycardia (indicating parasympathetic activation) and pupil dilation (indicating sympathetic activation) occurred following threatening stimuli [16]. In the same study, the viewing of threatening stimuli was shown to be associated with activation of the periaqueductal grey, which was correlated with bradycardia at a trial-to-trial level. Such results, together with the current findings, suggest that while freezing can be described as a parasympathetic “brake on the system,” this does not necessarily imply a general inhibition of concurrent sympathetic activation, nor the absence of active action preparation. Preparatory and restraining processes may occur in parallel during freezing.

A further general theoretical question of interest to further research is the relationship between concepts related to freeze such as fright [35,36], subjective fear, the orienting response [37], and motor preparation. There are some points to be made on the interpretation of the current results in terms of freezing as opposed to, e.g., orienting. First, note that freezing and threat have indeed been associated with heart rate deceleration in previous studies in human fear responses [16,18,38]. Second, the locus of heart rate effects in the present study involved an increase over the course of a relatively long period of anticipation and was sensitive to our response-contingent threat manipulation, suggesting a preparatory process rather than an orienting response. Third and most critically, orienting is subject to habituation and freezing is typically not. The current effects were found after training blocks of hundreds of trials in which the cues were presented hundreds of times. Since the process being measured appears not to strongly dissipate with habituation, it seems unlikely to involve the orienting response [cf. 39]. However, it is evident that such relationships are complex and subject to different theoretical viewpoints on freezing. Future research is needed to further explore the distinction, or overlap, between freezing and processes such as attentional orienting and action preparation.

The current results suggest that studying freezing while manipulating preparatory context is a promising line of research, and that it may be particularly important to consider physiological state during pure anticipation versus responses to changes in stimuli, which may also be an important component of the defensive response. However, we note some limitations of the current study. An aim of the exploratory individual difference analyses in the current study

was to further understand the nature of freezing. The current study was however limited to a student sample that was relatively small. Future research with more varied samples would provide a wider range of individual differences: Both in terms of cognitive ability and clinical or subclinical symptoms. Interesting results might also be found in professional groups such as the police or military [cf. 40]. An individual difference that was not assessed in the current study was familiarity with video games. However, games usually do not involve threat of real electric shocks and the participants underwent an extensive training period on the specific task before the assessment started, so this variation seems unlikely to strongly influence the results. The current study was relatively fast-paced, which increased the available number of trials but limited the time available for baseline duration. While the focus of the current study was on the 1–7 Hz range, future studies aimed at the slow, sub –1 Hz part of the sway spectrum should consider using longer intervals.

A question the current study was not designed to address, but that is of significant theoretical and practical interest, is how freezing transitions to action. Even if freezing is indeed active, it appears that there must nevertheless be some “trigger” to break out of the immobility. Some individuals could hypothetically become locked into the preparatory state, leading to a paradoxical relationship between preparatory freezing and a failure to act. Neuroimaging or psychophysiological study of this transition could provide information on this aspect of freezing. Previous work suggests that freezing is related to activation of the periaqueductal grey and its connectivity to the amygdala [16], so that transitional processes would be expected to involve control over these regions. Insights from such studies would not only be theoretically interesting, but could suggest clinical interventions or training possibilities. An interesting direction for future research would be the use of preparation for reward rather than aversive stimuli, raising the complex question of whether mechanisms involved in freezing under threat may be common to those involved in avoidance of the loss of reward.

In conclusion, freezing may not reflect a state of helpless anticipation, but to the contrary, a state of active preparation of a defensive response to a triggering stimulus. The experimental manipulation of active versus passive action preparation thus seems likely to be an essential factor in the study of freezing and decision making under threat.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.neulet.2016.03.027>.

References

- [1] M.S. Fanselow, Neural organization of the defensive behavior system responsible for fear, *Psychon. Bull. Rev.* 1 (1994) 429–438, <http://dx.doi.org/10.3758/BF03210947>.
- [2] R.J. Blanchard, D.C. Blanchard, J. Rodgers, S.M. Weiss, The characterization and modelling of antipredator defensive behavior, *Neurosci. Biobehav. Rev.* 14 (1990) 463–472 (accessed 24.06.13). <http://www.ncbi.nlm.nih.gov/pubmed/2287483>.
- [3] M.M. Bradley, M. Codispoti, B.N. Cuthbert, P.J. Lang, Emotion and motivation I: defensive and appetitive reactions in picture processing, *Emotion* 1 (2001) 276–298, <http://dx.doi.org/10.1037/1528-3542.1.3.276>.

- [4] R. Misslin, The defense system of fear: behavior and neurocircuitry, *Neurophysiol. Clin.* 33 (2003) 55–66 (accessed 9.12.13). <http://www.ncbi.nlm.nih.gov/pubmed/12837573>.
- [5] R.J. Blanchard, K.J. Flannelly, D.C. Blanchard, Defensive behavior of laboratory and wild *Rattus norvegicus*, *J. Comp. Psychol.* 100 (1986) 101–107 (accessed 2.12.13). <http://www.ncbi.nlm.nih.gov/pubmed/3720282>.
- [6] D.C. Blanchard, G. Griebel, R. Pobbe, R.J. Blanchard, Risk assessment as an evolved threat detection and analysis process, *Neurosci. Biobehav. Rev.* 35 (2011) 991–998, <http://dx.doi.org/10.1016/j.neubiorev.2010.10.016>.
- [7] R.N. Leaton, G.S. Borszcz, Potentiated startle: its relation to freezing and shock intensity in rats, *J. Exp. Psychol. Anim. Behav. Processes* 11 (1985) 421–428.
- [8] C.F. Plappert, P.K. Pilz, H.U. Schnitzler, Acoustic startle response and habituation in freezing and nonfreezing rats, *Behav. Neurosci.* 107 (1993) 981–987 (accessed 31.03.15). <http://www.ncbi.nlm.nih.gov/pubmed/8136073>.
- [9] D.C. Blanchard, A.L. Hynd, K.A. Minke, T. Minemoto, R.J. Blanchard, Human defensive behaviors to threat scenarios show parallels to fear- and anxiety-related defense patterns of non-human mammals, *Neurosci. Biobehav. Rev.* 25 (2001) 761–770 (accessed 2.06.13). <http://www.ncbi.nlm.nih.gov/pubmed/11801300>.
- [10] M.J. Bovin, S. Jager-Hyman, S.D. Gold, B.P. Marx, D.M. Sloan, Tonic immobility mediates the influence of peritraumatic fear and perceived inescapability on posttraumatic stress symptom severity among sexual assault survivors, *J. Trauma. Stress* 21 (2008) 402–409, <http://dx.doi.org/10.1002/jts.20354>.
- [11] L.C.L. Portugal, M.G. Pereira, R. de, C.S. Alves, G. Tavares, I. Lobo, V. Rocha-Rego, et al., Peritraumatic tonic immobility is associated with posttraumatic stress symptoms in undergraduate Brazilian students, *Rev. Bras. Psiquiatr.* 34 (2012) 60–65 (accessed 25.11.13). <http://www.ncbi.nlm.nih.gov/pubmed/22392390>.
- [12] V. Rocha-Rego, A. Fiszman, L.C.L. Portugal, M. Garcia Pereira, L. de Oliveira, M.V. Mendlowicz, et al., Is tonic immobility the core sign among conventional peritraumatic signs and symptoms listed for PTSD? *J. Affect. Disord.* 115 (2009) 269–273, <http://dx.doi.org/10.1016/j.jad.2008.09.005>.
- [13] T.M. Azevedo, E. Volchan, L.A. Imbiriba, E.C. Rodrigues, J.M. Oliveira, L.F. Oliveira, et al., A freezing-like posture to pictures of mutilation, *Psychophysiology* 42 (2005) 255–260, <http://dx.doi.org/10.1111/j.1469-8986.2005.00287.x>.
- [14] M.A. Hagenaars, J.F. Stins, K. Roelofs, Aversive life events enhance human freezing responses, *J. Exp. Psychol. Gen.* 141 (2012) 98–105, <http://dx.doi.org/10.1037/a0024211>.
- [15] J.F. Stins, P.J. Beek, Effects of affective picture viewing on postural control, *BMC Neurosci.* 8 (2007) 83, <http://dx.doi.org/10.1186/1471-2202-8-83>.
- [16] E.J. Hermans, M.J.A.G. Henckens, K. Roelofs, G. Fernández, Fear bradycardia and activation of the human periaqueductal grey, *Neuroimage* 66C (2012) 278–287, <http://dx.doi.org/10.1016/j.neuroimage.2012.10.063>.
- [17] K. Roelofs, M.A. Hagenaars, J.F. Stins, Facing freeze: social threat induces bodily freeze in humans, *Psychol. Sci.* 21 (2010) 1575–1581, <http://dx.doi.org/10.1177/0956797610384746>.
- [18] M. Lojowska, T.E. Gladwin, E.J. Hermans, K. Roelofs, Freezing promotes perception of coarse visual features, *J. Exp. Psychol. Gen.* 144 (2015) 1080–1088, <http://dx.doi.org/10.1037/xge0000117>.
- [19] A. Löw, M. Weymar, A.O. Hamm, When threat is near, get out of here: dynamics of defensive behavior during freezing and active avoidance, *Psychol. Sci.* (2015), <http://dx.doi.org/10.1177/0956797615597332>.
- [20] C. Spielberger, R. Gorsuch, R. Lushene, P. Vagg, G. Jacobs, *Manual for the State-Trait Anxiety Inventory*, Consulting Psychologists Press, Palo Alto, CA, 1983.
- [21] H.M. Ploeg, The development and validation of the Dutch form of the test anxiety inventory, *Appl. Psychol.* 33 (1984) 243–254, <http://dx.doi.org/10.1111/j.1464-0597.1984.tb01432.x>.
- [22] C. Spielberger, *Manual for the State-Trait Anger Expression Inventory (STAXI)*, Psychological Assessment Resources, Odessa, FL, 1988.
- [23] H. van der Ploeg, P. Defares, C. Spielberger, *Handleiding Bij De Zelf-Analyse Vragenlijst, ZAV, Lisse*, 1982.
- [24] M.M. Bradley, P.J. Lang, Measuring emotion: the self-assessment manikin and the semantic differential, *J. Behav. Ther. Exp. Psychiatry* 25 (1994) 49–59 (accessed 26.02.15). <http://www.ncbi.nlm.nih.gov/pubmed/7962581>.
- [25] The Mathworks, MATLAB, (2015).
- [26] S. Demura, T. Kitabayashi, Comparison of power spectrum characteristics of body sway during a static upright standing posture in healthy elderly people and young adults, *Percept. Mot. Skills* 102 (2006) 467–476, <http://dx.doi.org/10.2466/pms.102.2>.
- [27] S.D. Forman, I.D. Cohen, M. Fitzgerald, W.F. Eddy, M.A. Mintun, D.C. Noll, Improved assessment of significant activation in functional magnetic resonance imaging (fMRI): use of a cluster-size threshold, *Magn. Reson. Med.* 33 (1995) 636–647 (accessed 20.01.15). <http://www.ncbi.nlm.nih.gov/pubmed/7596267>.
- [28] R. Heller, D. Stanley, D. Yekutieli, N. Rubin, Y. Benjamini, Cluster-based analysis of fMRI data, *Neuroimage* 33 (2006) 599–608, <http://dx.doi.org/10.1016/j.neuroimage.2006.04.233>.
- [29] C.-W. Woo, A. Krishnan, T.D. Wager, Cluster-extent based thresholding in fMRI analyses: pitfalls and recommendations, *Neuroimage* 91 (2014) 412–419, <http://dx.doi.org/10.1016/j.neuroimage.2013.12.058>.
- [30] A. Raine, Autonomic nervous system factors underlying disinhibited, antisocial, and violent behavior. Biosocial perspectives and treatment implications, *Ann. N. Y. Acad. Sci.* 794 (1996) 46–59 (accessed 1.04.15) <http://www.ncbi.nlm.nih.gov/pubmed/8853591>.
- [31] A. Raine, P.H. Venables, M. Williams, High autonomic arousal and electrodermal orienting at age 15 years as protective factors against criminal behavior at age 29 years, *Am. J. Psychiatry* 152 (1995) 1595–1600 (accessed 1.04.15). <http://www.ncbi.nlm.nih.gov/pubmed/7485621>.
- [32] R.A. Poldrack, Can cognitive processes be inferred from neuroimaging data? *Trends Cognit. Sci.* 10 (2006) 59–63 (accessed 20.11.13). <http://www.sciencedirect.com/science/article/pii/S13646661305003360>.
- [33] B.A. Campbell, G. Wood, T. McBride, Origins of orienting and defensive responses: an evolutionary perspective, in: P. Lang, R. Simons, M. Balaban (Eds.), *Atten. Orienting Sens. Motiv. Process*, Erlbaum, Hillsdale, NJ, 1997, pp. 41–67.
- [34] P.J. Lang, M.M. Bradley, Emotion and the motivational brain, *Biol. Psychol.* 84 (2010) 437–450, <http://dx.doi.org/10.1016/j.biopsycho.2009.10.007>.
- [35] H.S. Bracha, Freeze, flight, fight, fright, faint: adaptationist perspectives on the acute stress response spectrum, *CNS Spectr.* 9 (2004) 679–685 (accessed 11.04.13). <http://www.ncbi.nlm.nih.gov/pubmed/15337864>.
- [36] H.S. Bracha, T.C. Ralston, J.M. Matsukawa, A.E. Williams, A.S. Bracha, Does fight or flight need updating? *Psychosomatics* 45 (2004) 448–449, <http://dx.doi.org/10.1176/appi.psy.45.5.448>.
- [37] B.A. Campbell, G. Wood, T. McBride, Origins of orienting and defensive responses: an evolutionary perspective, in: P.J. Lang, R.F. Simons, M. Balaban (Eds.), *Atten. Orienting Sens. Motiv. Process.*, Lawrence Erlbaum Associates, Inc., New York, 1997, pp. 41–67.
- [38] M.A. Hagenaars, M. Oitzl, K. Roelofs, Updating freeze: aligning animal and human research, *Neurosci. Biobehav. Rev.* 47 (2014) 165–176, <http://dx.doi.org/10.1016/j.neubiorev.2014.07.021>.
- [39] R.J. Barry, G.Z. Steiner, F.M. De Blasio, Event-related EEG time-frequency analysis and the orienting reflex to auditory stimuli, *Psychophysiology* 49 (2012) 744–755, <http://dx.doi.org/10.1111/j.1469-8986.2012.01367.x>.
- [40] A. Nieuwenhuys, G.J.P. Savelsbergh, R.R.D. Oudejans, Shoot or do not shoot? Why police officers are more inclined to shoot when they are anxious, *Emotion* 12 (2012) 827–833, <http://dx.doi.org/10.1037/a0025699>.