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Individual differences in defensive stress-responses: the potential relevance for psychopathology

Hannah CM Niermann[1,2], Bernd Figner[1,2] and Karin Roelofs[1,2]

Alterations in primary freeze and fight-or-flight reactions in animals have been associated with increased vulnerability to develop anxious or aggressive symptomatology. Despite the potential relevance of these primary defensive responses for human stress-coping, they are still largely unexplored in humans. The present paper reviews recent evidence suggesting that individual differences in primary defensive stress responses in humans are associated with individual differences in anxiety and aggression. In addition, we discuss (neuro)endocrine systems that may underlie increased freezing and flight behavior in anxiety and increased fight tendencies in aggression-related disorders. We conclude with a research agenda for the study of human defensive stress-responses as potential behavioral markers for stress-related disorders, including anxiety and aggression.

Addresses
[1] Behavioural Science Institute, Radboud University, The Netherlands
[2] Donders Institute for Brain, Cognition and Behaviour, Radboud University, The Netherlands

Corresponding author: Niermann, Hannah CM (h.niermann@psych.ru.nl)

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The defensive cascade of freeze and fight-or-flight (FFF) responses plays a crucial role in the way various species cope with threat. Freezing – a stage of attentive immobility – is characterized by bodily immobility and heart rate deceleration [1,2] which together allow for an active preparation for optimal fight-or-flight actions (*i.e.*, attacking or avoiding the predator) [3••,4•,5]. Individual differences in animals' freezing responses remain relatively stable throughout development [6,7]. Research in rodents and primates suggests that increased freezing and flight behavior is associated with heightened stress susceptibility, increased activity in stress hormones (*i.e.*, norepinephrine and cortisol; corticosterone in rodents) and stress-related brain systems, as well as with maladaptive stress coping later in life [6,8–10]. Therefore, increased freezing and flight reactions are considered an anxious intermediate phenotype that – similar to the stable anxious temperament of behavioral inhibition in humans [11] – constitutes an important risk factor for the development of anxiety-related phenotypes [8]. While anxiety is associated with high cortisol and low testosterone concentrations [12–14], aggressive phenotypes have been associated with high testosterone and low cortisol concentrations in animals [13–16]. So, in animal research FFF responses and their association with symptomatology are well established, in particular with anxiety-related symptoms. However, despite their potential relevance for human stress-coping and psychopathology, individual differences in FFF tendencies remain largely unexplored in human studies (see also Box 1) [17••]. The importance of investigating human FFF tendencies has recently been highlighted in the clinical context, where defensive stress-responses were consistently observed in traumatized patients [3••] and where freezing during trauma exposure appeared to be predictive of the development of posttraumatic stress disorder [18].

Accordingly, the aim of the present paper is to review the literature on FFF tendencies in humans and their association with anxiety and aggression-related symptomatology. In addition, we discuss alterations in the steroid hormones cortisol and testosterone that are associated with both anxiety and aggression-related symptoms as well as with altered FFF tendencies. Finally, we describe future perspectives, and end with a research agenda to advance insights into this emerging field of human defensive stress-responses.

Freeze-fight-flight (FFF)

The expression of FFF tendencies is shaped by both the sympathetic and parasympathetic branches of the autonomic nervous system (ANS). During threat exposure both the sympathetic and parasympathetic branches of the ANS are activated. However, while sympathetic dominance facilitates active fight-or-flight responses, parasympathetic dominance facilitates freezing responses by serving as a 'break' on the activated system [28]. Freezing is most likely to occur when the threat is still at a distance [28,29]. It is thought to optimize the animal's attentional processes serving the selection and preparation of appropriate sympathetically dominated fight-or-flight responses to cope with threat [5,30••,31]. Also in humans, exposure to threat cues – like aversive pictures or threat of shock – has been associated not only with sympathetic activity such as pupil dilation and skin

Box 1 The value of assessing freeze-fight-flight (FFF) tendencies in humans.

Traditionally, FFF tendencies were measured using self-report questionnaires. Additional objective quantification of human FFF tendencies may be promising for further advancement of insights in human adaptive and maladaptive stress-responses:

- FFF responses to stress and their neuroendocrine mechanisms can be conceptualized as intermediate phenotypes that help to bridge the gap between genotypes and observable phenotypes [8]. In general, intermediate phenotypes are more stable and more heritable than subjective self-reports [19]. Although stability and heritability of FFF tendencies still need to be determined for humans, they have been demonstrated for several nonhuman species [6,7,20,21].
- FFF responses to stress reflect dynamic changes to the environment and have been shown to be sensitive to contextual changes and life events [22,23].
- Psychophysiological and behavioral indices of FFF tendencies can be measured continuously by time scales ranging from milliseconds to hours, thus allowing the study of the temporal dynamics of freezing and fight-or-flight reactions at various time scales (HCM Niermann *et al.*, Submitted) [24].
- FFF tendencies capture unique variance in explaining social and affective behaviors. For example, a behavioral measure of avoidance was more sensitive in differentiating highly anxious individuals from non-anxious controls than a self-report measure [25,26], and was better in predicting clinical treatment outcomes in anxiety patients compared to self-reported pre-treatment anxiety levels [27].
- FFF tendencies provide behavioral markers for the etiology of social and affective symptoms in humans and nonhuman animals [3••,8,18].
- Being able to objectively quantify FFF tendencies in humans gives us the opportunity to benefit more directly from insights from animal research where FFF tendencies are major outcome measures [17••], as well as to facilitate animal-to-human translational research.

conductance [32,33], but also with heart rate deceleration (which is one of the clearest indications of parasympathetic dominance over sympathetic activity) [24,32,33]. Recent attempts to relate heart rate deceleration to posturographic measures in humans showed that heart rate deceleration in response to aversive pictures was indeed associated with reductions in body sway measured by using a stabilometric force platform [22,34,35•].

Using similar posturographic and electrocardiographic methods, increased freezing was shown in participants who were more anxious or had been previously traumatized [22,35•]. In addition, a prospective study in adolescents with a history of insecure infant-parent attachment showed increased freezing at age 14 [36]. Another recent study suggested that aggressive behavior is associated with a specific freezing pattern: highly aggressive participants (compared to participants with lower levels of aggression) initially showed less freezing in response to threatening opponents, whereas they showed more signs of freezing shortly before they needed to initiate a fight response [24]. Individual differences in bodily freezing have also been shown to be related to instrumental approach-avoidance decisions [37,38]: healthy individuals with stronger freezing responses to angry faces showed an avoidance bias during subsequent but unrelated instrumental approach-avoidance actions [37]. Interestingly, aggressive delinquents with psychopathic traits showed no such avoidance bias during instrumental actions, implying reduced transfer of automatic FFF tendencies to instrumental actions [38]. Together, these findings suggest that humans, just like animals, show threat-induced freezing behavior, that freezing affects more complex instrumental behavior, and that individual differences exist corresponding to decreased freezing in aggression and increased freezing in anxiety.

Not only parasympathetically dominated freezing, but also sympathetically dominated fight-or-flight reactions have been differentially associated with anxiety and aggression. Flight reactions can be seen as active avoidance behavior aiming at preventing or minimizing contact with an acute threatening cue or situation [39,40]. Avoidance behavior has been recognized as one of the most important maintenance factors in anxiety [41], hampering fear extinction and even enhancing fear [41–44]. A useful tool for objectively and implicitly assessing active avoidance tendencies in humans is the approach-avoidance task. In this task, participants either approach or avoid appetitive and threatening stimuli (*e.g.*, happy and angry faces, respectively), using full body movements, or manually using a handle or a joystick. Typically, participants are faster to approach (than avoid) appetitive stimuli and faster to avoid (than approach) threatening ones. Highly socially anxious individuals avoided emotional faces more strongly than neutral ones [25,45]. In a related study using the same paradigm, higher vigilance ratings in patients with posttraumatic stress disorder were associated with stronger avoidance tendencies to trauma-related stimuli [46]. Interestingly, on similar approach-avoidance tasks, patients diagnosed with psychopathy showed an absence of avoidance behavior to socially threatening stimuli [47] and participants high on reactive aggression displayed an approach tendency to fighting scenes [48]. Together, these studies suggest that anxiety is associated with increased freezing and flight tendencies, whereas aggression is associated with facilitated approach actions that may signal fight tendencies [47,48].

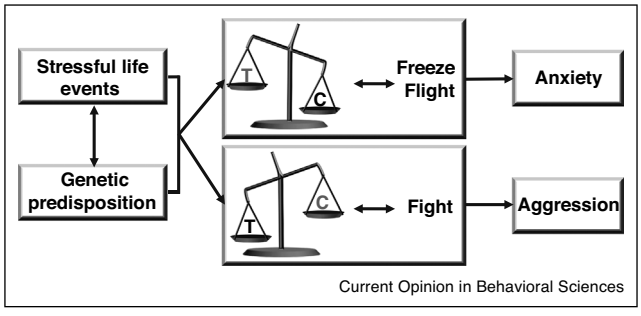
Steroid regulation of anxiety and aggression

Activity in the hypothalamic–pituitary–gonadal (HPG)-axis and the hypothalamic–pituitary–adrenal (HPA)-axis and their respective steroid hormones testosterone and cortisol (corticosterone in rodents) are important for the regulation of social and emotional behavior in humans and nonhuman animal species [13,49,50]. These endocrine axes have been shown to have mutually antagonistic properties in animals [51]. Whereas socially submissive (fearful and avoidant) behavior is typically associated with elevated cortisol and low testosterone concentrations

[12–14], socially dominant and aggressive behavior has been widely associated with elevated testosterone and low cortisol concentrations in animals [13–16]. In humans, individuals with high social anxiety also show high reactive cortisol levels and low basal testosterone concentrations [52–54], whereas aggressive and dominant individuals show high basal testosterone and low basal cortisol levels [55,56]. Hence, both anxiety and aggression-related disorders seem to be featured by an HPA–HPG imbalance, though in opposite directions (See Figure 1 for a schematic representation).

Recent theories have added yet another factor contributing to the HPA–HPG imbalance in aggression: according to the triple imbalance theory of reactive aggression [57], the effects of a high testosterone–cortisol ratio that biases the amygdala towards threat approach, are amplified by reduced serotonin transmission. Low serotonin transmission is thought to be associated with reduced frontal control over the amygdala, thereby increasing the risk for aggressive outbursts [57,58••]. Although this theory still needs to be tested in humans, there is indeed evidence from genetic and pharmacological studies in humans and animals suggesting that the relation between steroid hormone function and social behavior varies as a function of serotonin [59–61]. Accordingly, one could argue that in anxiety disorders – also associated with reduced serotonin transmission – reduced frontal control over the amygdala could similarly amplify pre-potent action tendencies. In the case of anxiety, however, reduced serotonin transmission would not amplify approach, but rather avoidance behavior, thereby enhancing effects resulting from high cortisol and low testosterone levels on the amygdala [62•]. Indeed the short allele of the serotonergic transporter gene (5-HTTLPR gene) – a polymorphism that codes for reduced serotonin transporter availability and reduced serotonin reuptake – has been associated with reduced frontal–amygdala coupling during exposure to emotional faces [63,64•], and with increased risk of developing social and affective psychopathologies – such as anxiety – particularly after experiencing stressful life events [65]. Based on these observations, we propose that it may be worthwhile for future studies to investigate serotonin-steroid hormone interactions, not only in relation to aggression, but also in anxiety disorders.

Figure 1



Schematic presentation of the proposed relations between steroid hormones, defensive stress-responses, and social and affective psychopathology (*i.e.*, anxiety and aggression): stressful life events can interact throughout life with genetic factors to determine activity of the hypothalamus–pituitary–adrenal-axis and the hypothalamus–pituitary–gonadal-axis, resulting in the release of cortisol (C) and testosterone (T), respectively. An imbalance in these steroid hormones as well as genetic and environmental factors may affect the expression of primary defensive freeze-fight-flight (FFF) responses to stress, alterations which may contribute to social and affective psychopathologies. More specifically, a steroid imbalance of high cortisol and low testosterone may affect the expression of defensive freezing and flight tendencies, potentially serving as an intermediate phenotype for anxiety, whereas a steroid imbalance in the opposite direction – low cortisol and high testosterone – may affect the expression of fight tendencies, potentially serving as an intermediate phenotype for aggression.

Steroid regulation of freeze-fight-flight (FFF)

The HPA-axis plays a crucial role in the expression of defensive freezing, which is supported by various pharmacological studies in rodents. Stimulation as well as blockage of the HPA-axis has been shown to respectively increase or decrease rodents' freezing behavior [66–68]. Furthermore, removal of the adrenal glands disrupted the activity of the rodents' HPA-axis and freezing responses, while daily administration of corticosterone restored adaptive freezing responses to threat in these same rodents [69]. Although the association between freezing and HPA-axis activity is well established in nonhuman animal species [66–69], this association remains largely unexplored in humans. The few existing studies are suggestive of similar associations in humans, though: Children's increased freezing behavior in response to a low-threat situation (a stranger approaches the child) has been associated with both increased basal and reactive cortisol levels [70••]. In a related study, decreased levels of basal endogenous cortisol were recently found to be associated with a specific freezing pattern in response to a standardized stress induction procedure, such that individuals with lower levels of basal cortisol showed reduced *immediate* stress-induced freezing as well as reduced freezing *recovery* approximately 1 hour after acute stress (HCM Niermann *et al.*, Submitted). Interestingly, reduced freezing recovery also acted as a mediator in an indirect path going from lower basal cortisol via reduced freezing recovery to increased levels of internalizing symptoms. This suggests that reduced freezing recovery might serve as a potential marker for the etiology of internalizing symptoms (HCM Niermann *et al.*, Submitted).

Not only freezing but also avoidance and flight behavior have been associated with HPA-axis activity. Stress-induced cortisol as well as cortisol administration enhanced avoidance behavior towards angry faces on an approach-avoidance task in highly socially anxious

and avoidant individuals [54,71]. In contrast, testosterone administration diminished threat avoidance tendencies towards angry faces and promoted relative threat approach tendencies towards angry faces in healthy controls and in patients with social anxiety disorder [72,73]. Recent fMRI studies – using similar approach-avoidance tasks with happy and angry faces – have shown that the control over approach-avoidance tendencies crucially involves the anterior prefrontal cortex (aPFC) and its connections with the amygdala [64•,74]: the aPFC is particularly active when people have to override their action tendencies (*i.e.*, in affect-incongruent conditions where they are instructed to approach an angry face or to avoid a happy face) [75]. Furthermore, elevated endogenous testosterone levels have been associated with reduced negative functional connectivity between the aPFC and the amygdala when individuals had to control their action tendencies (again, in affect-incongruent conditions of the approach-avoidance task) [50]. The dynamics of this prefrontal–amygdala crosstalk may be distorted in individuals with social psychopathologies. For example, individuals with psychopathy (compared to healthy controls) showed reduced aPFC activity and less aPFC–amydala coupling when controlling approach-avoidance actions. This pattern was predominantly observed in psychopaths with elevated levels of endogenous testosterone [76]. This set of studies is consistent with the idea that a pattern of high testosterone and low cortisol is associated with aggression-related phenotype (*i.e.*, approach or fight behavior), whereas the opposite pattern – low testosterone and high cortisol – is associated with anxiety-related phenotype (*i.e.*, avoidance or flight behavior).

Interestingly, similar reductions in aPFC–amygdala connectivity during approach-avoidance control have been observed in short allele carriers (s-carriers) of the 5-HTTLPR gene [64•]. When s-carriers had to exert control over their approach-avoidance action tendencies, they showed increased amygdala activity in response to emotional faces [64•], replicating previous studies [65,77,78]. Most importantly, dynamic causal modeling analyses indicated a decreased pattern of down-regulation of the amygdala by the aPFC in s-carriers [64•]. In line with the differential susceptibility hypothesis [79], such an intermediate phenotype may form a risk marker for poor control over approach-avoidance actions when the system is challenged by adverse events: Several prospective longitudinal studies have indicated that s-carriers have an increased risk of developing psychopathology following the experience of adversity [80–82]. In addition, both human and animal research shows that stressful life events can have long-lasting effects on the activity of the HPA and HPG-axes [83–86]. Together, these results suggest that gene–environment interactions may result in altered primary defensive responses and the associated neuroendocrine patterns. These altered primary defensive stress responses may in turn affect the risk of developing psychopathologies (see Figure 1 for a tentative model). Such a gene–environment interaction model has often been proposed to explain complex symptoms such as aggression and anxiety, with mixed results [87–90]. On the basis of this review, we propose to apply such a model to the intermediate phenotypes of primary defensive reactions (FFF tendencies), which are less complex and easier to objectively quantify compared to complex symptomatologies. In addition, we propose to investigate primary FFF tendencies in interaction with steroid hormones.

Future perspectives

There is a great need for prospective longitudinal research to investigate whether altered primary FFF tendencies may indeed serve as an important risk marker for psychopathology. These longitudinal investigations should focus particularly on vulnerable groups (*e.g.*, police officers, firefighters, and adolescents) who have an increased vulnerability for developing stress-related behavioral problems such as anxiety and aggression.

Moreover, future research should investigate moderating (*e.g.*, genetic) and mediating (*e.g.*, epigenetic) factors that may explain the association between defensive stress-responses and psychopathology. Our schematic presentation of some proposed relations (Figure 1) should not be considered comprehensive, but is purely illustrative, to guide future research towards factors that may contribute to altered primary FFF responses. Individual differences in serotonin-related genes might be a potential candidate [63,64•,80–82] worth exploring, because the neurotransmitter serotonin plays a crucial role in the regulation of social and emotional processes [65]. However, catecholaminergic neurotransmitter systems (*e.g.*, dopamine [91] and norepinephrine [92]) as well as other hormones and peptides (*e.g.*, oxytocin [93••]) also play an important role in social emotional and stress-systems and in the shift to fight and flight behavior [93••]. Future research should therefore investigate their role in FFF tendencies as well.

Future research is also needed to disentangle the complex interplay between the HPA-axis and the HPG-axis in relation to serotonin and anxiety/aggression. Although known sex differences exist regarding the activity of the HPA and HPG-axes and the occurrence of anxiety and aggression, we suggest that the proposed relations (Figure 1) can guide future research towards factors that may be involved in the expression of FFF responses in both sexes.

Finally, more research is needed to define and clarify the precise role of freezing in the defensive cascade in exposure to threat. Freezing is both classified as a stage of action preparation and of risk assessment, potentially being important for adequate decision making in

response to threat [24,30••]. As a result, freezing can be qualified as an adaptive response. However, it has recently been suggested that freezing-responses may be impaired, for example, in clinical populations with posttraumatic stress disorder (I Fragkaki *et al.*, Submitted) [94] and that immediate stress-induced freezing is not related to internalizing symptoms but instead, reduced freezing *recovery* is (HCM Niermann *et al.*, Submitted). Future research is needed to clarify and specify the dynamics of adaptive freezing responses.

Conclusion

The reviewed literature suggests that FFF responses combined with (neuro)endocrine stress-responses are promising markers for the etiology of various social and affective psychopathologies. Altered FFF responses may represent a transdiagnostic intermediate phenotype underlying several stress-related symptomatologies. Increased freezing and flight behavior, as well as elevated cortisol and reduced testosterone concentrations characterize anxiety-related symptomatology [18,25,26,35•,45,46,52–54,62•,71]. In contrast, increased fight tendencies, as well as elevated testosterone and reduced cortisol concentrations are associated with aggression-related disorders [24,38,47,48,55–57,58••,76]. However, to date, we have only just started to investigate the role of these defensive stress-responses in human psychopathology.

Conflict of interest statement

Nothing declared.

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